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# Antioxidant, and Antimicrobial Activities of Phenolic and Flavonoid Rich Medicinal Plants (*Fritillaria zagrica* and *Tulipa kurdica*) Bulbs Collected in Kurdistan Region of Iraq

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**A B S T R A C T**

*Fritillaria zagrica* and *Tulipa kurdica* had been used as traditional herbal remedies since antiquity to treat human diseases in the Kurdistan region of Iraq. This is the first report and conceive to analyze these two medicinal plants based. Methanol, 80% ethanol and aqueous extracts of two medicinal plants (*Fritillaria zagrcia* and *Tulipa kurdica*) were evaluated for their antibacterial activity and antifungal activities. We have quantified the total phenolic (TPC) and flavonoid (TFC) contents and their relation to antioxidants (ABTS) [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) and 2,2′-diphenyl-1 picryl hydrazyl (DPPH)scavenging free radicals in a dose dependent method. Results showed that *F. zagrica* and *T. kurdica* bulb extractions by using different solvents exhibited strong Antimicrobial activities against selected bacterial and fungal strains except *C. guilliermondii ATCC 6260*. Aqueous bulb extract of *F. zagrica* and *T. kurdica* showed better antibacterial activity compared to other solvent extracts. Methanol *F. zagrica* bulb extracts contain significantly higher phenolic content than *T. kurdica*. The TPC and TFC contents were varied between the plant bulb extracts and the DPPH and ABTS scavenging activities were exhibited to be significantly correlated with the amount of TPC and TFC.

## 1. INTRODUCTION

Kurdistan is a closed and stored pool as a buried treasury industrial pharmacy. There are a large number of medicinal plants used in folk medicine by the traditional herbalists to treat varieties of human ailments. Among them, *Fritillaria zagrica* and *Tulipa kurdica* had been priced for their local traditional medicinal resources.

*Fritillaria zagrica Stapf.* is a species very closely allied to *F. tulipifolia* and *F. armena*. Their flowers dark lurid untesselated, unchequered with a thick bloom outside, purple glaucous outside and at the tips of the petals there is always a bright yellow spot (Weathers, 1911, Ruksans, 2007). *F. zagrica Stapf.* belongs to the *Fritillaria*, which is a genus of over 160 species of bulbous plants within the monocot family Liliaceae, native to temperate regions of the Northern Hemisphere (Liu et al., 2012, Hao et al., 2015, Kiani et al., 2015). The species of the *Fritillaria* genus are distributed especially in the Mediterranean regions and eastern parts of Anatolia and Thrace, the Black Sea, and Central Anatolia in Turkey, and in Iran, Iraq, Syria, Afghanistan, Russia, Lebanon, Palestine, Jordan, Pakistan, China, Japan, Greece, Bulgaria, Italy, Spain, Portugal, North Africa, and California and Oregon in the USA (Tekşen and Aytaç, 2011). *Fritillaria* L.

has been commonly used in traditional Chinese medicine for thousands of years, contain many pharmaceutically active constituents (Hao et al., 2013).

Many *Fritillaria* species have long been exploited as the natural source of the widely used traditional medicine 'bulbus Fritillaria' (*i.e.*, dry bulbs or a decoction of *Fritillaria* species; 'Beimu' in Chinese) that has long been used as one of the most important antitussive, expectorant, and antihypertensive drugs and treat tumors, hemoptysis and deficiency of milk in traditional Chinese medicine (Li et al., 2006, Li et al., 2013, Matsuo et al., 2013).

Similarly, *T. kurdica* belongs to *Tulipa L.* (Liliaceae), which is a genus of about 100 species of bulbous monocots (Bryan, 2002, Bryan, 2005, Zonneveld, 2009) naturally occurring from southwestern Europe and North Africa, the Middle East to Central Asia (Christenhusz et al., 2013). *T. kurdica* closely to *T. humilis* which has stems 4-6 inch and flowers brick red or orange red with the basal blotch black, late spring (Bryan, 2005). From a medicinal and industrial viewpoint, *Tulipa* and *Fritillaria* are two of the most important genera in Liliaceae family due to their relatively rich pharmacological history (Li et al., 2006).

Likewise, the bulbs of *F. zagrica* and *T. kurdica* have been traditionally used in clinic as herbal remedies by Kurdish people who are living in the mountains of Kurdistan Region-Iraq, due to their positive potent therapeutic effectivities during lesion treating, wound healing, preventing and reducing the pain and removing the excessive tumor mass (lump) beneath the skin of breast, armpit and underarm with discharging abscess.

The medicinal values of the plants depend on the presence of certain chemical substances called secondary metabolites, involving alkaloids, tannins, flavonoids, terpenes and phenolic compounds that are involved in antioxidant and free radical scavenging (Saniewski and Horbowicz, 2003, Sobia, 2011). Phenolic compounds are secondary plant metabolites that are found naturally in all plant materials, including plant based food products. These compounds are thought to be an integral part of human and animal diets. They represent the most important group of natural antioxidants (Huyut et al., 2017). Furthermore, as a result of high medicinal plants application in treating many diseases, now the plants are also useful as novel antimicrobial agents (Khan et al., 2018).

In particular, reactive oxygen species (ROS), such as hydroxyl radicals, superoxide anions, and hydrogen peroxide, are frequently generated spontaneously in the living cell during metabolism and play an important role in cell signalization (Liu et al., 2012). However, excessive amount of ROS can induce oxidative stress, resulting in significant damage to cell structures and macromolecules, including proteins, lipids, and nucleic acids. As it is well known, ROS also contributes in development of cancer, inflammation, diabetes, aging, inflammation and cardiovascular disease (Marvibaigi et al., 2016, Ismail et al., 2017).

In this context, the antibacterial and antioxidant properties of various medicinal plants are being investigated throughout the world because of the toxicological concerns associated with the synthetic antioxidants and preservatives (Baba and Malik, 2014).

However, to the best of our knowledge, there is no scientific work on antimicrobial and antioxidant activities of *F. zagrica and T. kurdica* medicinal plants. Therefore, the aim of this study to determine the total phenolic-, flavonoid- contents and to elucidate the antioxidant properties and antimicrobial activities of bulb extracts of both *F. zagrica*

*and T. kurdica* medicinal plants grown in Kurdistan region- Iraq.

## 2. MATERIALS AND METHODS

### 2.1. MATERIALS

#### 2.1.1 Plant Material Collection:

*Fritillaria zagrica* and *T. kurdica* were collected in April 2014 from Zine-Asterokan mountain near to Karokh mountain and Weza village near Choman district - Erbil / Kurdistan region from northern Iraq, respectively. The plants were classified and identified by botanists Prof. Dr. Abdul Hussain Al Khayat and Mrs. Bnar Khalid Bakr according to the most common classification key. The voucher specimens, accession number (0007627) for *F. zagrica* and (0007628) for *T. kurdica* plants were deposited at the Herbarium of Department of Biology, College of Science, Salahaddin University, Kurdistan-Iraq. The plants raw materials (bulbs) were washed and air-dried under shade at room temperature (20-25$^{o}$C). After drying, the bulbs' plants were grounded into fine powder using a laboratory grinding mill and sieved with 710 um sieve, to provide homogeneous powder for analyzing. Powdered materials were stored in bottles in a dark room temperature and then used.

#### 2.1. .2 Preparation of Freeze-Dried Bulb Extracts

The powdered *F. zagrica* and *T. kurdica* plants bulb materials (100 g) were carried out and macerated with 1 L of extracting solvents (99.9 % absolute methanol and 80% ethanol) and water in the beakers covered by aluminum foil and incubated in Ultrasonic bath at room temperature for two hours. The solvents were drained out after 4 h and replaced with fresh one. This procedure was repeated thrice. The extracts were then separated from the sample residues by filtration through Whatman No.1 filter papers. The resultant bulb extracts were concentrated in a rotary evaporator in a water bath at 40 °C until the crudes solid extracts were obtained, which were then freeze-dried for completing solvents removal. Finally, the obtained bulb extracts were weighed, their yields were calculated, and stored at -20 °C in sealed tubes until used for further analysis. The methanol, 80 % ethanol and water extracts' yields were 7.76, 2.97, 2.60 % for *F. zagrica* bulb extracts while the methanol, 80 % ethanol and water yields were 7.40, 4.70, 3.57 % for *T. kurdica* bulb extracts respectively.

### 2.2 Chemicals and Microorganisms

All chemicals and reagents used in the study including solvents were of analytical grade. Methanol and Ethanol were obtained from Score Scientific SDN BHD. 1,1-Diphenyl- 2-picryl hydrazine (DPPH), 2, 2'-azino bis-(3-ethyl benzo thiazoline-6-sulphonic acid) (ABTS), quercetin, catechin, Folin-Ciocalteu's phenol reagent, Difco™ nutrient broth, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman- 2-carboxylic acid (Trolox), sodium nitrite, sodium hydroxide (NaOH), sodium carbonate ($Na_2CO_3$), aluminum chloride ($AlCl_3$), potassium persulfate ($K_2S_2O_8$), ethylenediaminetetraacetic acid (EDTA), and ascorbic acid were purchased from Sigma Aldrich Chemicals (USA). Dimethyl sulfoxide (DMSO) was obtained from Fisher Scientific (USA).

The standard bacterial strains of the Gram negative bacteria *Escherichia coli* (*ATCC 25922*), *Pseudomonas aeruginosa* (*ATCC 27853*), *Acinetobacter baumannii* (*ATCC 19606*) and of the Gram positive bacteria *Staphylococcus aureus* (*ATCC 25923*) and *Bacillus subtilis* (*ATCC 6633*) and pathogenic fungal strains, *Candida tropicalis* (*ATCC 13803*), *Candida albicans* (*ATCC*

10231), *Candida guilliermondii* (*ATCC 6260*) were used and obtained from the Department of Biology, College of Science, Salahaddin University, Erbil/ Kurdistan-Iraq. A standard antibiotic (amikacin) was used as positive control.

### 2.3 Assay of Antibacterial Activity against Pathogenic Bacterial Strains

The antibacterial activities have been checked against Gram-negative *E. coli* (*ATCC 25922*), *P. aeruginosa* (*ATCC 27853*), *A. baumannii* (*ATCC 19606*) and Gram-positive *S. aureus* by using a modified Kirby-Bauer disk diffusion method. Aliquots of bacterial suspension (100 uL) were spread on Difco™ nutrient agar containing testing microorganism with optical density of 0.7 at 595 nm. Filter paper disks (8 mm) saturated with 20 uL of the prepared bulb extracts *F. zagrica* and *T. kurdica* (10 mg/ mL) in different solvents were placed on nutrient agar seeded with tested bacteria. The plates were incubated for 24 h at 37 ºC, and then the zones of growth inhibition were measured (Sharma et al., 2018). All the tests were done in triplicate and mean ± S.D. were calculated.

### 2.4 Assay of Antifungal Activity against Pathogenic Fungi

Sensitivity of *Candida spp.* were tested against bulb extracts of *F. zagrica* and *T. kurdica* (1 mg/mL) in methanol, 80% ethanol and aqueous solution by using filter paper disc diffusion method (agar disc diffusion method) the filter paper disc prepared by using ordinary office two-hole puncture, paper discs with approximate diameter of 6mm. were punched out one by one from a sheet of filter paper, the disks placed in vials, sterilized by oven and allowed to cool. Then sterilized discs (6 mm) were soaked in known concentration (1 mg/mL) of plant extracts; another filter paper disc was soaked with sterilized distilled water (SDW) used as negative control and DMSO as a solvent control, 50-100 discs were placed in small sterile air tight labeled containers and then allowed to dry for 2 hours. The sterile discs were placed in petri dishes. For antifungal activity, yeast suspension prepared from 24h colony by using phosphate buffer saline (PBS) in compare with standard control with concentration $41.5*10^6$ cell/mL of yeast suspension. Then 0.1 ml of yeast suspension was spread over sabouraud glucose agar medium (SGA), then incubated at $37^oC$ for 24-48h. Zones of inhibition were obtained by measurement of the radius from the center of the disc to the edge of the x inhibition of growth. Measurements were made from both sides of the slope and their average accepted (Al-Refai, 2006, Kamel et al., 2014).

### 2.5 Determination of Total Phenolic Content

The total phenolic content (TPC) quantification of *F. zagrica* and *T. kurdica* plants bulb extracts were achieved by Folin-Ciocalteu colorimetric method with slight modifications (Hatami et al., 2014, Marvibaigi et al., 2016). Briefly, 100 µl of extracts (1mg/ml diluted in distilled water) were mixed with 100 µl of 0.2 N Folin-Ciocalteu reagents. After 5 min with intermittent shaking, 80 µl of 7.5% sodium carbonate ($Na_2CO_3$) solution was added and incubated for 2h at room temperature. The absorbance (of the resulting blue color) was measured at 765 nm against the blank. The calibration curve was prepared using the standard gallic acid solution. All TPC determinations were carried out three times (n = 3) and the results were expressed in mg of Gallic acid equivalent (GAE/100 g of sample) (Marvibaigi et al., 2016, Ismail et al., 2017) as shown in figure 1.

### 2.6 Determination of Total Flavonoid Content (TFC)

The total flavonoid content estimation of *F. zagrica* and *T. kurdica* plants bulb extracts were measured spectrophotometrically by aluminum chloride colorimetric method, which is based on the formation of a flavonoid–aluminum complex with slightly modifications (Lin and Tang, 2007, Marvibaigi et al., 2016). Briefly, 200 µl of extract (1 mg/mL) was mixed with 12 µl 5% $NaNO_2$ and 12 µl 10% $AlCl_3$. After 5 min incubation at ambient room temperature, 80 µl of NaOH was added and re-incubated for 30 min. The absorbance of samples was read at 515 nm. The calibration curve was prepared using standard catechin solution. The TFC of extracts were expressed in mg of g catechin equivalent (CE/ 100 g of sample) as illustrated in figure 2. The analysis was performed in triplicate and mean values were reported.

### 2.7. Antioxidant Assay

#### 2.7.1. DPPH Free Radical Scavenging Activity Assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl radical), is a dark-colored crystalline powder composed of stable free-radical molecules. The antioxidant activities were determined when using DPPH as a free radical because the use of $DPPH^{\bullet}$ provides an easy and rapid way to evaluate the antiradical activities of antioxidants (Brand-Williams et al., 1995). Hence DPPH has major application in laboratory research most notably in anti-oxidant assays (Bhandari et al., 2017). The antioxidant activity of *F. zagrica* and *T. kurdica* plants bulb extracts, on the basis of the scavenging activity of the stable DPPH free radical ($DPPH^{\bullet}$) in prepared 96 well plates, were performed following a previously described method with slight modifications (Iqbal et al., 2006, Akter et al., 2010). For each determination, the stock solution (1mg/ml) was diluted to a dilution series (156 µg-1000 µg/ml) with DMSO. An aliquot of each extract working concentration (20 µl) was mixed with methanolic solution of DPPH (200 µl, 0.06 mM). The mixtures were shaken vigorously and incubated at room temperature in the dark for 30 min. A control sample was placed under the same conditions. A color change from violet to yellow occurred during the reaction time. Sample absorbance was read at 517 nm using UV-VIS spectrophotometer and the percentage of free radical scavenging potential of the different extracts against $DPPH^{\bullet}$ was determined using the following equation:

DPPH radical scavenging activity (%) = [(Abs control- Abs sample) /Abs control] x100

DPPH solution was used as a control and ascorbic acid and quercetin were used as references standard. The percentage of DPPH scavenging versus concentration of samples was plotted. The antioxidant activity was expressed as median effective concentration (EC50) where the concentration caused 50% reduction of DPPH. All determinations were assayed in triplicate (Brand-Williams et al., 1995, Akter et al., 2010, Ismail et al., 2017).

#### 2.7.2. ABTS Free Radical Scavenging Activity Assay

The free radical scavenging capacity of *F. zagrica* and *T. kurdica* plants bulb extracts were also measured using TEAC (Trolox equivalent antioxidant capacity) method. The TEAC value is based on the ability of the antioxidant to scavenge the blue-green coloured $ABTS^{\bullet+}$ radical cation relative to the $ABTS^{\bullet+}$ radical cation scavenging ability of the water-soluble vitamin E analogue, Trolox (Re et al., 1999, Gliszczyńska-Świgło, 2006). ABTS stock solution was dissolved in water to

a 7 mM concentration. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and the mixture was allowed to stand in dark at room temperature for 12-16 h. Prior to use, ABTS radical cation was diluted in methanol until the absorbance at 0.7 ± 0.02 units. ABTS solution (300 µl) was added to 30 µl sample and incubated for 6 min at room temperature. The absorbance was read at 734 nm. Ascorbic acid was chosen as positive control. The radical-scavenging activity was expressed as inhibition percentage, and calculated using the following formula.

$$\text{ABTS Radical scavenging activity} = \frac{Ac - As}{Ac} \; x \; 100$$

where *Ac* is the absorbance of reaction without samples and *As* is the absorbance of tested extract. The antioxidant activity was as median effective concentration (EC50) where the concentration caused 50% reduction of ABTS (Gliszczynskaswiglo, 2006, Ismail et al., 2017).

### 2.8. Statistical Analysis

Results of all analysis are presented as means of triplicate ± standard deviation (SD) and/or standard error (n = 3). Median effective concentrations (EC50) were statistically analyzed by GraphPad Prism 6 software. Non-linear regressions of log(agonist) vs. response with variable slope (four parameters) was selected for EC50 and LogEC50 estimation. The bottom and top constrain were 0% and 100% respectively. Additionally, statistical analysis was performed using GraphPad Prism 6 software with two-way ANOVA and Tukey's multiple comparisons post hoc test and Dunnett's multiple comparisons test. The results were considered statistically significant at P value < 0.05. Furthermore, Pearson correlation coefficient (r) was used find the relationships between the total phenolic and flavonoid contents and various antioxidant assays. The P value < 0.05 was considered significant.

## 3. RESULTS AND DISCUSSION

### 3.1. Extraction Yield

Extracts of bulbs of both *F. zagrica* and *T. kurdica* were obtained following a sequential solvent extraction procedure. Different organic solvents of increasing polarity including ethanol, methanol, and water were used to determine if any of these solvents could selectively extract compounds with antioxidant and antimicrobial activities. The yield percentages of *F. zagrica* and *T. kurdica* bulb extracts were expressed in terms of mass percentages of samples as mentioned in Table 1 and Figure 3. Extraction yield was calculated using following formula:

Extraction yield (%) = (weight of the freeze-dried extract x 100) / (weight of the original sample).

Results showed that the methanol bulb extracts of both *F. zagrica* and *T. kurdica* provide highest yields of extracts when compared to 80 % ethanol and aqueous extracts. The higher yield of methanol extract might be owed to the fact that methanol possesses high vapor pressure. Although the solubility of bio-active components and the rate of mass transfer are different, the results validated that nature and polarity of solvent affect the percentage yield of the extract. This was in agreement with the study by Marvibaigi, et al. (2016), who reported a high yield of mistletoe (*Scurrula ferruginea*) extracts when using polar solvents (Marvibaigi et al., 2016). The lowest percentage yield was exhibited for bulb aqueous extract of *F. zagrica*, while the highest yield was observed in the bulb

methanol extract of *F. zagrica*. In addition to the lowest percentage yield was recorded for bulb aqueous extract of *T. kurdica*, while the highest yield was revealed in the bulb methanol extract of *T. kurdica*. Large variations were showed between extraction yields of methanol, 80 % ethanol and aqueous extracts for both medicinal plants. Also using 80% water-ethanol solvent observed higher yield than using water alone to bulb extracts of both *F. zagrica* and *T. kurdica* plants. The results of other studies showed that the highest extraction yields with aqueous solutions might be ascribed to an increase of polarity of the solvents by adding water having a high dielectric constant (Kim et al., 2004, Kallel et al., 2014), which corroborate results of the present study. Hence, the sequence for increasing extraction yields were methanol extract > 80% ethanol extract > water extract.

### 3.2. Assay of Antimicrobial Activity against Pathogenic Organisms

This is the first report on the antibacterial screening and antifungal activity of *F. zagrica* and *T. kurdica* growing from Kurdistan Region-Iraq. Plants and their secondary metabolites have shown great potential as antibacterial and antifungal source. The first step towards this goal is the *in-vitro* antimicrobial activity. The antibacterial and antifungal assay of bulb extracts of *F. zagrica* and *T. kurdica* were performed against some pathogenic bacterial and fungal strains. The results in Table 2 and Figure 4 elucidated that *F. zagrica* and *T. kurdica* bulb extractions by using different solvents had strong antibacterial activities against all using bacterial strains (Gram positive and Gram negative bacteria) according to positive standard control (amikacin).

Maximum zones of inhibition of *F. zagrica* aqueous extracts (15.0, 26.33, 25.33 mm) were noticed against each of *E. coli ATCC 25922, A. baumannii ATCC 19606* and *S. aureus ATCC 25923,* respectively. While maximum zones of inhibition of *P. aeruginosa ATCC 27853* (24.0 mm) and *B. subtilis ATCC 6633* (27.67 mm) were found for each of *F. zagrica* methanol extract and *F. zagrica* ethanol extracts, respectively.

*Tulipa kurdica* aqueous extracts were showed maximum zones of inhibition (25.0, 31.0, 26.67 mm) against each of *E. coli ATCC 25922, P. aeruginosa ATCC 27853* and *S. aureus ATCC 25923* respectively, whereas ethanol extracts of *T. kurdica* were exhibited maximum inhibition zones against *A. baumannii ATCC 19606* (27.0 mm) and *B. subtilis ATCC 6633* (30.0 mm). The difference in antibacterial activity of different bulb extracts may be due to difference in solubility of bioactive compounds in various solvents and variations in cell wall structure of tested bacteria. The mode of action of different bioactive compounds varies which represent the ability of these compounds to kill or inhibit bacteria (Howell, 2007).

Aqueous extract of *F. zagrica* and *T. kurdica* showed better antibacterial activity compared to other solvent extracts. The findings of the present study are in agreement with previous investigation, which concluded that water extract exhibited higher antibacterial activity than ethanol extract of leaves and bark of *Cassia alata*, when tested against *C. albicans* and *S. aureus* (Somchit et al., 2003, Chen et al., 2018). The broad antibacterial activities of the Garlic (*Allium sativum* L.) and *Sida rhombifolia* Linn extracts can probably be attributed to the presence of various bio-actives components such as the phenolic (Kallel et al., 2014) alkaloid and flavonoid compounds (Dzoyem et al., 2010). As previously mention in the present that polar solvents are frequently

worked for the recovery of polyphenols from a plant matrix.

*Fritillaria zagrica* and *T. kurdica* methanol, 80% ethanol and aqueous extracts like many other plants showed antibacterial activity. Kumaraswamy *et al.* (2008) revealed that methanol, ethanol and aqueous *Betula utilis* D extracts had significant activity against some human pathogenic bacteria. Also Moon *et al.* (2011) showed that methanol and ethanol bulb extracts from *F. unibracteata* dose-dependently have inhibitory effect and increased antimicrobial activity, the strongest inhibition showed at the highest concentration of 20 mg/mL (Moon et al., 2011). Johnson and his colleagues (2011) screened five important medicinal plants, and the results observed that the maximum of Aloevera plant was to be exposed against *S. aureus* and *E. coli*, while *Lanatacamara* inactive against bacterial strains (Johnson et al., 2011). However, the aqueous fraction of the *Pongamia pinnata* had more active as compared to alcoholic extract against *E. coli*.

In other side Shigetomi et al. (2013) demonstrated that the natural products 1-tuliposide B and the lactonized aglycon (±)-tulipaline B in bulb *Tulipa* are potent inhibitors of MurA, which may partly explain the known antibacterial activity of these compounds (Shigetomi et al., 2013) and recently, a potent antibacterial activity of 6-tuliposide B has been reported (Shigetomi et al., 2010) . Also Tuliposides in bulb *Tulipa* had been reported to reveal antimicrobial activities and the formation of tulipalins played a key role in antimicrobial action (Shigetomi et al., 2011, Lim, 2014).

The results Table 3 and Figure 5 demonstrated the antifungal activities of *F. zagrica* and *T. kurdica* bulb extractions by using different solvents. *F. zagrica* methanol extracts were observed the maximum inhibition zones (18.0, 15.0, 9.0 mm) against *C. tropicalis ATCC 13803, C. albicans ATCC 10231* and *C. guilliermondii ATCC 6260*, respectively.

The *T. kurdica* bulb (methanol, 80% ethanol and aqueous) extracts showed antifungal activities against *C. tropicalis ATCC 13803* with the maximum inhibition zone (16 mm) of 80% ethanol extract. The *T. kurdica* 80% ethanol and aqueous, extracts were exhibited antifungal activities against *C. albicans ATCC 10231*. While The *T. kurdica* bulb (methanol, 80% ethanol and aqueous) extracts had no antifungal effect against *C. guilliermondii ATCC 6260.*

In parallel to our finding, previous studies were showed that *T. kurdica* bulb extracts have potent Tuliposides and tulipalins compounds, which exhibited their antifungal activity (Shigetomi et al., 2011). Bergman et al. (1967) isolated and identified α- methylene- butyrolactone (tulipan A), a fungitoxic substance from tulips bulb. Accordant with Moon et al. (2011) who were concluded that methanol and ethanol bulb extracts of *F. unibracteata* have potent anti-microbial activity (for example *C. albicans*) for the supportive treatment on respiratory diseases, food poisoning, and gastroenteritis diseases.

### 3.3. Total Phenolic and Flavonoid Content

Table 4 and 5 summarize the TPC and TFC in methanol, 80% ethanol and water bulb extracts of both *F. zagrica* and *T. kurdica* medicinal plants. The TPC values varied widely, ranging from 21.83 mg GAE/100 g samples to 153.7 mg GAE/100 g samples. MF exhibited the highest phenolic content at 153.7 mg GAE/100 g sample followed by EF (150.4 mg GAE/100 g sample), AF (139.4 mg GAE/100 g sample), MT (32.61 mg GAE/100 g sample), AT (29.16 mg GAE/100 g sample)

and ET (21.83 mg GAE/100 g sample). In another hand, the TFC detected varied from 80.12 CE/100 g sample to 365.5 mg CE/100 g sample. The highest flavonoid content was in MF at 365.5 mg CE/100 g sample followed by EF (179.2 mg CE/100 g sample), MT (130.3 mg CE/100 g sample), AF (114.4 mg CE/100 g sample) ET (99.49 mg CE/100 g sample) and AT (80.12 mg CE/100 g sample). TPC and TFC of *F. zagrica* and *T. kurdica* were statistically different from each other ($P < 0.05$), these two phytochemical compounds in methanol, 80% ethanol and aqueous extracts of both plants were statistically different from each other ($P < 0.05$) as shown in Figure 6 and 7.

Furthermore, results of the current study represented that different plant extracts contained different levels of TPC and TFC. *F. zagrica* contain significantly higher phenolic content than *T. kurdica*. The total phenol and flavonoid contents of the extracts were very near to those most medicinal plants. Results of the current study were in agreement with the previous studies (Marvibaigi et al., 2016), which presented that methanolic extracts of *Scurrula ferruginea* were rich in phenolic and flavonoid compounds. The presence of large amounts of phenolic compounds in the methanol, 80% ethanol and aqueous extracts may contribute to the antioxidant activities and the ability to adsorb and scavenge free radicals (Kumar et al., 2014).

### 3.4. DPPH and ABTS Free Radicals Scavenging Activities and Their Correlation to Phenolic and Flavonoid Contents

DPPH is a stable free radical, when antioxidant reacts with $DPPH^{\bullet}$ the electron is paired off and the DPPH solution is decolorized and $DPPH^{\bullet}$ provides an easy and rapid way to evaluate the antiradical activities of antioxidants. For that reason, the antioxidant activities were determined using DPPH as a free radical (Brand-Williams et al., 1995). The medicinal plants showing strong scavenging capacity on DPPH•, which is possibly due to the hydrogen donating ability of the polyphenolic (phenolic and flavonoid) compounds in the extracts (Mazhar, 2014, Bhagat et al., 2011).

The antioxidant activities of the *F. zagrica* and *T. kurdica* bulb extracts were assessed by different *in vitro* tests; DPPH and ABTS radical scavenging activities as presented in Figure 8. The EC50 calculated for DPPH (Table 6) were (2.006 2.661 µg/ml, 2.961 µg/ml, 9.241 µg/ml, 11.88 µg/ml and 21.77 µg $ml^{-1}$) µg/ml for MF, EF, AF, ET, MT and AT respectively. Meanwhile, a similar trend was mentioned (Table 6) in ABTS in which the EC50 for EF, AF, MT, MF, ET, and AT were (0.1535, 0.1611, 0.4435, 1.147, 1.532 and 2.493 µg $ml^{-1}$) respectively. Scavenging of DPPH radicals is mechanized by the donation of hydrogen atom to the unpaired electron of nitrogen bridge causing the purple color turn to yellowish. Meanwhile, the $ABTS^{+}$ radical cation undergoes the reduction process by hydrogen donating antioxidant and can be spectrophotometrically measured. It is that the phenolic and flavonoid content significantly influence the antioxidant activities (Othman et al., 2014, Ismail et al., 2017).

This original method quantified scavenging capacity by measuring a test compound at different concentrations and calculating the compound concentration required to reduce the initial DPPH concentration by 50% at steady-state (EC50). A lower EC50 value is associated with a stronger DPPH and ABTS radical scavenging capacity under the same testing conditions. Higher antioxidant activity is related to lower EC50 value (Liangli, 2008). Based on DPPH

and ABTS radical scavenging activity analysis, all bulb extracts of *F. zagrica* and *T. kurdica* represented antioxidant activity. The lowest EC50 value of DPPH was gained from methanol bulb extracts (MF) followed by ethanol (EF) and aqueous (AF) bulb extracts of *F. zagrica* while the lowest EC50 value was obtained from ethanol (ET) bulb extracts followed by methanol (MT) and aqueous (AT) bulb extracts of *T. kurdica*. The lowest EC50 value of DPPH in methanol bulb extract may due to the fact that methanol is better solvent than the others in extracting phenolic compounds from the extracts due to their polarity and good solubility for phenolic components from different plant materials such as walnut green husk, cacao bean husk and wild rice hulls (Kallel et al., 2014). Thus the samples with higher total phenols content showed the higher antioxidant properties (lower EC50 values). The results of present study indicated that increased concentrations resulted in enhancing the scavenging capacity of bulb extract samples. Broad variation of antioxidant activity may be possibly attributable to the presence of wide range of biologically active components like phenols, flavonols, carotenoids and some other compounds (Marvibaigi et al., 2014). Additionally, Liu et al (2012) indicated that a water-soluble polysaccharide (FUP-1) was obtained from *Fritillaria ussuriensis* Maxim, exerts antioxidant activity not only through its own radical-scavenging activity but also by boosting the enzymatic and non-enzymatic antioxidant defense system of the host.

From the observation, the extracted compound from the 70% ethanol and methanol gave higher antioxidant activity compared to the absolute solvent and Turkmen et al. (2006) reported the same findings where they found 50% and 80% of solvent mixture exhibited considerably higher DPPH radicals scavenging activity compared to the pure solvent (Turkmen *et al.*, 2006).

Besides, the (aqueous) bulb extract illustrated low antioxidant potential (higher EC50 values of DPPH and ABTS) and also low content of total phenols as compared to the other bulb extracts produced with methanol and 80 % ethanol solvents. Similar results have been reported during the extraction of antioxidant compounds from other raw materials such as mango peel and seed (Dorta et al., 2012), grape by-products (Lapornik et al., 2005).

In the present study Table 7 represents the correlation coefficients of the possible correlation between the phenolic and flavonoid contents of *F. zagrica* and *T. kurdica* bulb extracts and their antioxidant activities (DPPH and ABTS). It also observes the correlation between different methods used. The TPC and TFC showed a significant and positive linear correlation ($p<0.05$) with different antioxidant activity assays (DPPH and ABTS activities). These results suggested that the antioxidant activity is more closely related to TPC than TFC. Our findings exhibited a strong (higher) positive correlation between TPC and antioxidant activity assays ($R^2$ = 0.8261 and 0.5861 of DPPH and ABTS, respectively) and also proved that the phenolic compounds were the major contributors to the antioxidant capacity of the *F. zagrica* and *T. kurdica* bulb extracts. Similarly, Turkmen et al. (2006) who declared that the results of black mate tea showed that solvent with different polarity had significant effect on polyphenol content and antioxidant activity and a high correlation between polyphenol content and antioxidant activity of tea extracts was observed (Turkmen et al., 2006).

Moreover, Pearson correlation analysis of the results revealed a significant and positive correlation between different antioxidant

assays ($p<0.05$). The highest correlation was elucidated between ABTS and DPPH ($r = 0.7601$), whereas ABTS that could scavenge $ABTS^{\bullet+}$ was also able to scavenge $DPPH^{\bullet}$. Furthermore, the strong correlation between DPPH and ABTS methods suggests that the antioxidants in the extracts react similarly with both assays. The findings of the present study are in agreement with previous investigation of Ismail et al. (2017) who reported that radical scavenging of DPPH and ABTS were positively correlated to the phenolic and flavonoid content of tested herbal plants, which include *Andrographis paniculata* (leaves), *Cinnamon zeylanicum* (bark), *Curcuma xanthorrhiza* (rhizome), *Eugenia polyantha* (leaves) and *Orthosiphon stamineus* (whole plant). Based on our knowledge there is no report on TPC and TFC and antioxidant activities of *F. zagrica* and *T. kurdica* bulb extracts.

## 4. CONCLUSIONS

The present study demonstrated and proven that *F. zagrica* and *T. kurdica* bulb extracts in different solvents were exhibited strong antibacterial activities against all tested bacterial strains (Gram positive and Gram negative bacteria), and fungal strains except *C. guilliermondii ATCC 6260*. Aqueous bulb extract of *F. zagrica* and *T. kurdica* showed highest antibacterial activity compared to other solvent extracts.

Additionally, the phenolic and flavonoid content were varied between the tested bulb extracts and the DPPH and ABTS scavenging activities were found to be significantly correlated with the amount of TPC and TFC. Moreover, methanol *F. zagrica* bulb extracts contain significantly higher phenolic content than *T. kurdica*. Also demonstrated that the phenolic compounds were the major contributors to the antioxidant capacity of the *F. zagrica* and *T. kurdica* bulb extracts.

The strong antioxidant abilities of *F. zagrica* and *T. kurdica* along with their traditional use in the treatment of various ailments be suggesting their power potential as natural antioxidants and providing the scientific rationale to obtain pure compounds and then develop new therapeutic drugs against breast cancer with accomplishing cytotoxicity and anticancer screening for further detailed analysis in the future.

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## Conflict of Interest (1)

**Table (1)** Percentage yield of two medicinal bulb extracts in different solvents

| | Yields (%) | | |
|---|---|---|---|
| **Bulb Extract** | **Methanol** | **80%Ethanol** | **Aqueous** |
| ***F. zagrica*** | 7.76 ± 0.18* | 2.97 ± 0.15 | 2.60 ± 0.08 |
| ***T. kurdica*** | 7.40 ± 1.1* | 4.70 ± 0.18 | 3.57 ± 0.2 |

All results are means of three replicates determinations ± SD (n = 3).

***** Represents significant difference at $p < 0.05$.

**Table (2)** Antibacterial screening test [zone of inhibition (mm)] of *F. zagrica* and *T. kurdica* bulb extracts

(10mg/mL) in different solvents against some bacterial pathogenic strains.

| Bulb Extract | Inhibition zones (mm) | | | | |
|---|---|---|---|---|---|
| | *Gram (-ve) pathogenic bacteria* | | | *Gram (+ve) pathogenic bacteria* | |
| | ***E. coli ATCC 25922*** | ***P. aeruginosa ATCC 27853*** | ***A. baumannii ATCC 19606*** | ***S. aureus ATCC 25923*** | ***B. subtilis ATCC 6633*** |
| **MF** | 12.33 ± 0.33[a] | 24.00 ± 0.58 | 19.00 ± 1.16[a] | 19.33 ± 0.33 | 24.67 ± 0.33[a] |
| **EF** | 12.33 ± 0.67[a] | 13.00 ± 0.58 | 20.67 ± 0.67[a] | 15.33 ± 0.88[a] | 27.67 ± 1.45[a] |
| **AF** | 15.00 ± 0.58[a] | 19.67 ± 0.88[a] | 26.33 ± 0.88 | 25.33 ± 0.88 | 25.00 ± 0.58[a] |
| **MT** | 20.00 ± 1.16 | 10.00 ± 1.16 | 15.00 ± 0.58[a] | 15.33 ± 0.88[a] | 25.00 ± 0.58[a] |
| **ET** | 22.00 ± 1.16 | 25.67 ± 0.67 | 27.00 ±1.00 | 21.67 ± 0.88 | 30.00 ± 0.58 |
| **AT** | 25.00 ± 0.58 | 31.00 ± 1.00 | 23.67 ± 0.88 | 26.67 ± 1.20 | 28.67 ± 0.33 |
| **PC** | 14.67 ± 0.33 | 19.67 ± 0.88 | 18.00 ± 0.58 | 14.00 ± 0.58 | 24.67 ± 0.88 |

Data are means of three replicates (n = 3) ± standard error.

[a] Not statistically different from positive control (PC).

**Table (3)** Antifungal activities [zone of inhibition (mm)] of *F. zagrica* and *T. kurdica* bulb extracts against some pathogenic fungal organisms (1 mg/mL).

| Fungal Pathogenic Organism | *F. zagrica* | | | *T. kurdica* | | |
|---|---|---|---|---|---|---|
| | **MF** | **EF** | **AF** | **MT** | **ET** | **AT** |
| ***C. tropicalis ATCC 13803*** | 18 | 14 | 15 | 12 | 16 | 11 |
| ***C. albicans ATCC 10231*** | 15 | 14 | 13 | 0 | 10 | 11 |
| ***C. guilliermondii ATCC 6260*** | 9 | 8 | 6 | 0 | 0 | 0 |

Data analysis by Two-way ANOVA ($p$<0.05). There are significant differences among *Candida* spp. ($p$ value = 0.0002) and among bulb extracts of both medicinal plants ($p$ value = 0.0153).

(0): No Antifungal inhibitory activity.

**Table (4)** Total phenolic content of two medicinal bulb extracts in different solvents

| Bulb Extract | TPC (mg GAE/100 g sample) | | |
|---|---|---|---|
| | **Methanol** | **80%Ethanol** | **Aqueous** |
| ***F. zagrica*** | 153.7 ± 1.415$^{ax*}$ | 150.4 ± 0.65$^{bx}$ | 139.4 ± 0.50$^{cx}$ |
| ***T. kurdica*** | 32.61 ± 0.18$^{ay}$ | 21.83 ± 0.53$^{by}$ | 29.16 ± 0.44$^{cy}$ |

All results are means of three replicates determinations ± SD (n = 3).

* Represents significant difference at $p < 0.05$.

**Table (5)** Total Flavonoid content of two medicinal bulb extracts in different solvents

| TFC (CE/100 g of sample) | | | |
|---|---|---|---|
| **Bulb Extract** | **Methanol** | **80% Ethanol** | **Aqueous** |
| ***F. zagrica*** | 365.5 ± 20.01 | 179.2 ± 12.11 | 114.4 ± 2.48 |
| ***T. kurdica*** | 130.3 ± 1.24 | 99.49 ±13.46 | 80.12 ± 1.42 |

All results are means of three replicates determinations ± SD (n = 3).

***** Represents significant difference at $p < 0.05$.

**Table (6)** Mean of TPC, TFC and EC50 of DPPH and ABTS radical scavenging activity.

| Bulb Extract | Total phenolic content (mg GAE/100g sample) | Total flavonoid content (mg CE/100g sample) | EC50 - DPPH RSA (µg/mL) | EC50 – ABTS RSA (µg/mL) |
|---|---|---|---|---|
| **MF** | 153.7 ± 1.415 | 365.5 ± 20.01 | 2.006 | 1.147 |
| **MT** | 32.61 ± 0.18 | 130.3 ± 1.24 | 11.88 | 0.4435 |
| **EF** | 150.4 ± 0.65 | 179.2 ± 12.11 | 2.661 | 0.1535 |
| **ET** | 21.83 ± 0.53 | 99.49 ±13.46 | 9.241 | 1.532 |
| **AF** | 139.4 ± 0.50 | 114.4 ± 2.48 | 2.961 | 0.1611 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **AT** | 29.16 0.44 | ± | 80.12 1.42 | ± | 21.77 | 2.493 |

Each value in the table is represented as mean (n = 3).

**Table (7)** Pearson correlation coefficients between antioxidant activities and TPC/ TFC of *F. zagrica* and *T. kurdica* bulb extracts (at significance P < 0.05).

| **Assays** | **Correlation coefficients ($R^2$)** | | | |
|---|---|---|---|---|
| | **TPC** | **TFC** | **DPPH** | **ABTS** |
| **TPC** | 1.0000 | 0.6528* | 0.8261** | 0.5861* |
| **TFC** | 0.6528* | 1.0000 | 0.5923* | 0.1697 |
| **DPPH** | 0.8261** | 0.5923* | 1.0000 | 0.7601* |
| **ABTS** | 0.5861* | 0.1697 | 0.7601* | 1.0000 |

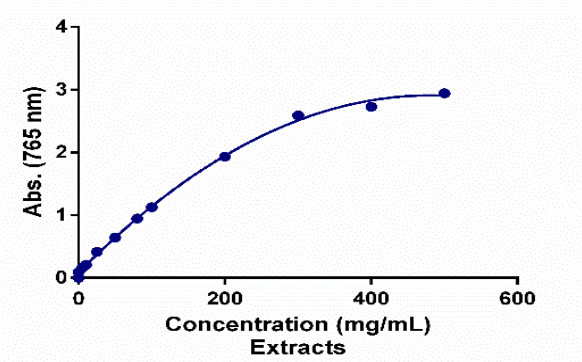


**Figure 1:** Standard curve graph of Total phenolic content (TPC).

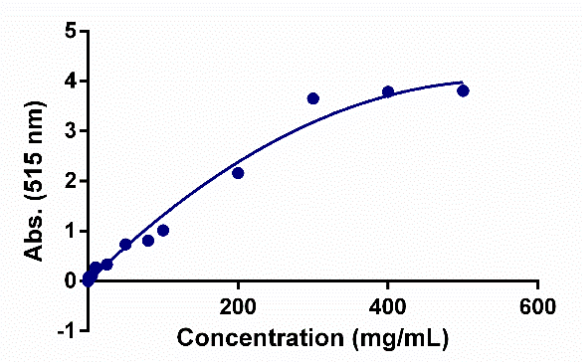


**Figure 2:** Standard curve graph of Total Flavonoid Content (TFC).

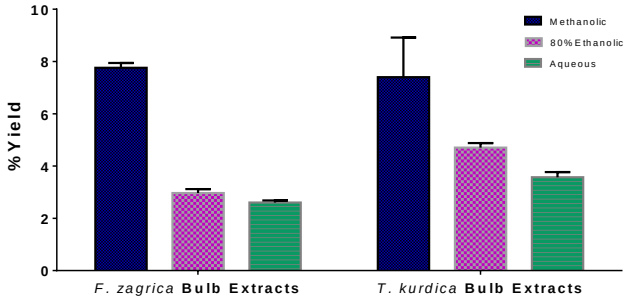


**Figure 3:** Percentage of *F. zagrica* and *T. kurdica* bulb extraction yields obtained by using different solvents.

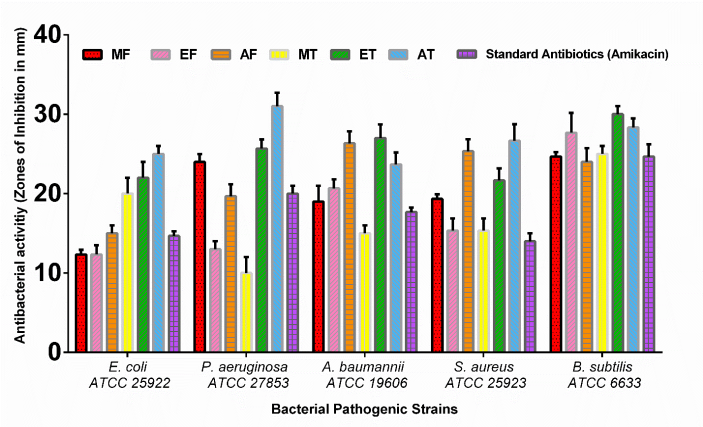


**Figure 4:** Antibacterial screening test of *F. zagrica* and *T. kurdica* bulb extracts in different solvents against some bacterial pathogenic strains.

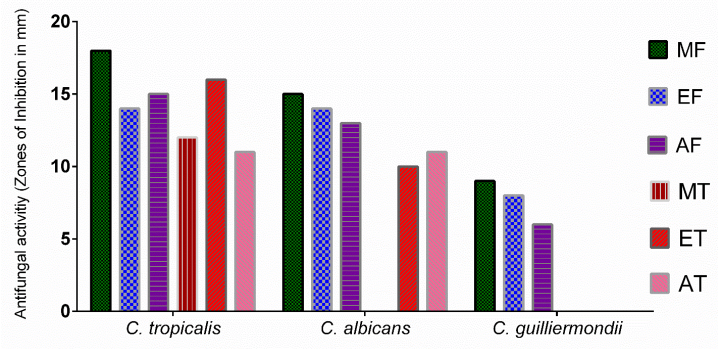


**Figure 5:** Antifungal activities of *F. zagrica* and *T. kurdica* bulb extracts against some pathogenic fungal organisms (1 mg/mL).

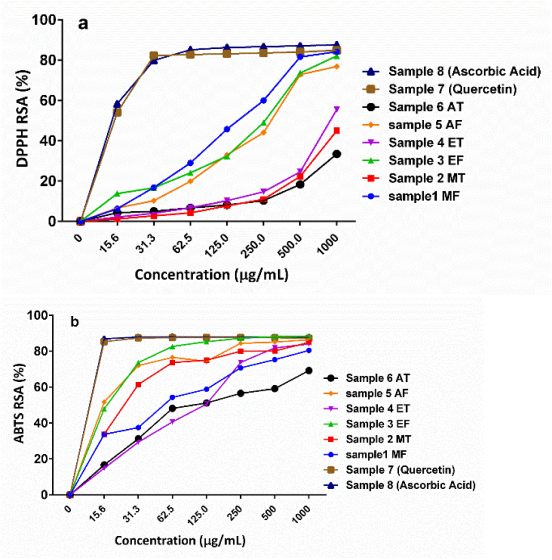


**Figure 8:** Antioxidant activities of MF, MT, EF, ET, AF and AT of *F. zagrica* and *T. kurdica* at various concentrations ranging from 15.60 to 1000 mg/ml. a) DPPH• radical scavenging activities. b) ABTS radical scavenging activities. Ascorbic acid and quercetin were used as standard (Positive controls). Results were expressed in means ± SEM (n = 3).

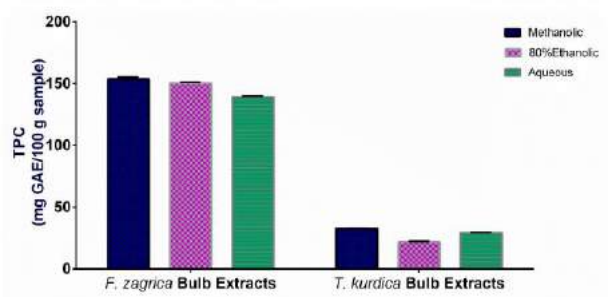


**Figure 6:** Total phenolic content (TPC) of *F. zagrica* and *T. kurdica* bulb extractions obtained by using different solvents.

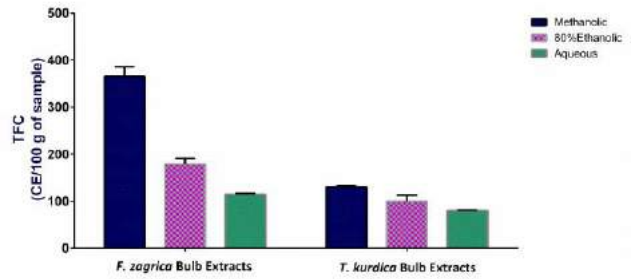


**Figure 7:** Total flavonoid content of *F. zagrica* and *T. kurdica* bulb extractions obtained by using different solvents.

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# Assessing Shekh Turab Water Resources for Irrigation Purposes by Using Water Quality Index

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**A B S T R A C T**

An Attempt was made to analyze the water quality of ShekhTurab stream for irrigation purpose. Water samples were collected from 5 sampling sites with two replications for each during July and November 2016. Studied samples were analyzed for electrical conductivity (EC), sodium adsorption ratio (SAR), sodium ($Na^{+1}$), chloride ($Cl^{-1}$) and bicarbonate ($HCO_3^{-1}$) contents. The results showed that the values of (IWQI) ranged from (41.11) to (53.65) and all sites were fall within the 4$^{th}$category ‘‘high restriction’’, it is clear that the plants have moderate resistance to salts might be grown in July and should be avoided its use for irrigation under normal conditions and only plants with high salt tolerance in November. Based on the obtained results, it is recommended to avoid grow salts sensitive plants to increase agricultural productivity in the study area.

## 1. INTRODUCTION

Water as a renewable source is the key element of sustainable development. Nowadays, and in the future water in the world, and particularly in Iraq becomes critical. In recent decades with the increase in the population around the world, water demand in different sectors (agricultural, industrial, and domestic water) may exert an increasing pressure on the groundwater resources(Gheyi, 2000).Agricultural lands and irrigation will be intensified, particularly in arid and semiarid regions in order to feed an estimated world population of 8.5 billion by 2025. However, approximately 10% of soils of these regions will lose their fertility due to potential irrigation induced problems such as sodicity, water logging and salinity and new areas need to be converted to irrigation, intensifying the problem (Gheyi, 2000).According to Shalhevet and Kamburov (1976) and Talukder *et al*.(1998) irrigation water quality mainly means the total quantity of dissolved salts and its ionic content depending on the water source and time of water sampling of water is becoming the main problem as well as water quality. The use of low water quality may have a significant impact on the agricultural productivity, especially in arid and semiarid regions due to the accumulation of salts in soil which affect water quality and fertility and permeability of soil (Shalhevet and Kamburov, 1976). Water quality indices (WQI) aims to give a single value to the water quality of a source reducing great amount of parameters into a simpler expression enabling easy interpretation of

monitoring data( Horton,1965).The (WQI) was first proposed by Horton (1965). Much of the work has been done on the water quality indices of several rivers and lakes in Iraq, Kurdistan and for various purposes such as drinking or agriculture uses by various workers (Abdul Hameed *et al.* (2010b); Abdul Hameed *et al.* (2010a); Shekha and Al-Abaychi (2010); Toma (2012) and Toma *et al.* (2013) Toma (2012) and Toma *et al.* (2013).Abdul Jabbar 2010 developed IWQI was applied to assess the irrigation water quality of Tigris, Euphrates and Shatt Al Arab rivers in Iraq based on observed water quality data. Then Meireleset al. (2010) classifies surface water quality in the Acarau Basin, in the North of the state of Ceara, Brazil for irrigation use. Mohammed (2011) used Irrigation Water Quality Index (IWQI) to classify Tigris River within Salahaddin Province in Iraq. The objective of the present study is to assess and classified the water quality of ShekhTurab stream Erbil Province for irrigation purpose by the applied model of (IWQI) developed by (11).

## 2. MATERIALS AND METHODS

### 2.1. Study area

Erbil province (Figure1) is of Kurdistan Region and located in the north east of Iraq. It is bounded to the north east and south east by the Greater and Lasser Zab respectively. Boundaries extend from longitudinal 43° 00′ 15″ E to 450 14- E and from latitude$35^{0}27^{-}$ N to$37^{0}$ $24^{-}$ N. The climate of the area is characterized by a wide diurnal and annual range of temperature (Zohary, 1950).The climate most closely related to Irano-Turanian type. The annual average rainfall for Erbil city estimated to be 440mm (Razoska, 1980).Details of geology, pedology and limnology of the area may give in (Mauloo*det al.*, 1980). Spring and autumn surface water samples were taken from five selected site in ShekhTurab stream located 20km east of Erbil city monthly over a period of two period of July and November-2016 period(July and November-2016) respectively ( Figure 1).

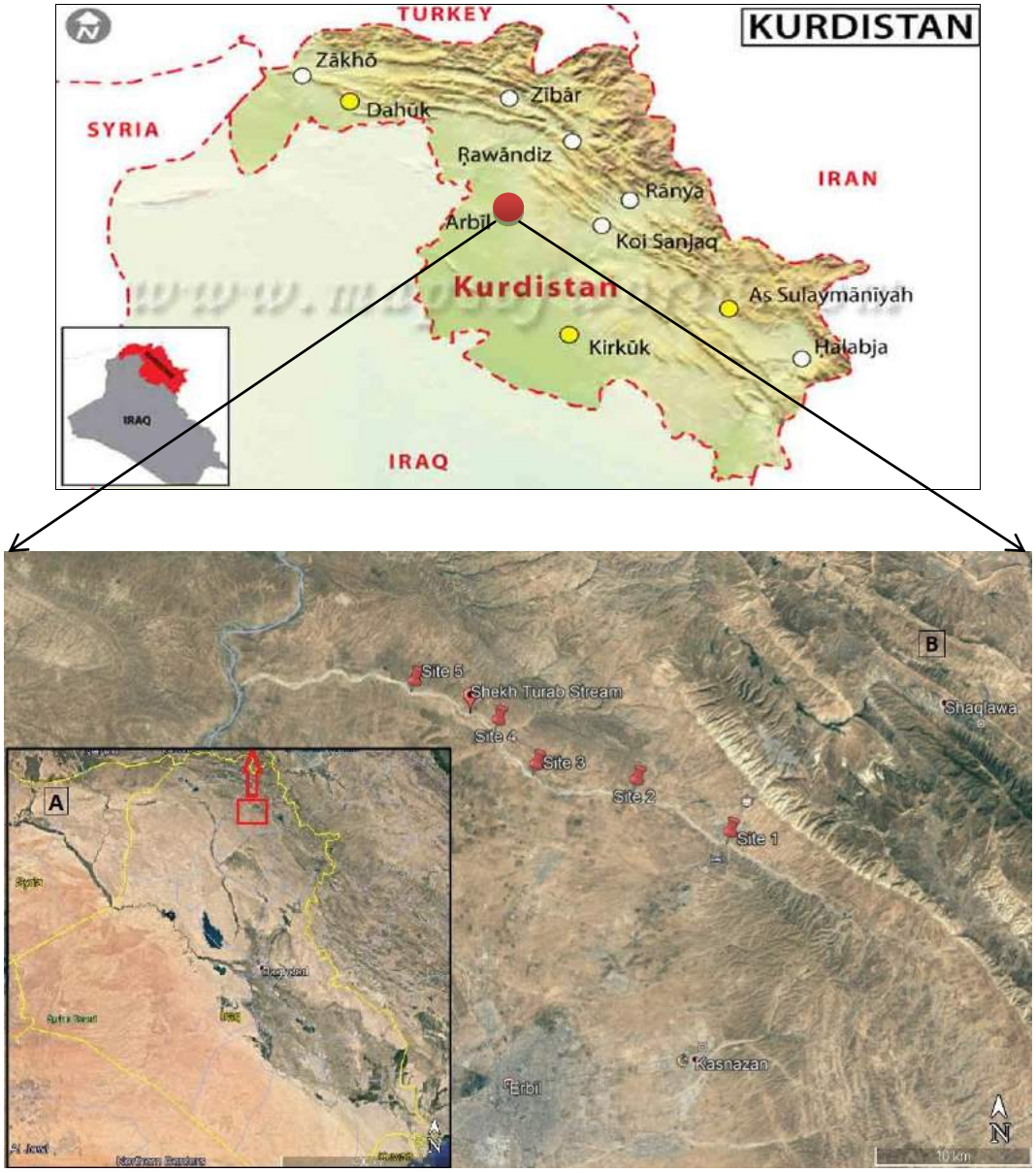


**Figure (1):- A- Map of Iraq within Erbil province**

**B- Map of Erbil province and studied sites indicated with red pins (ShekhTurab Stream)**

Studied stream is one of the branches of Greater Zab river, in which located in ShekhTurab village; it is about 75 km far away from Erbil City. The estimate terrain elevation above sea level is 393 m. The water depth is between 30 to 80 cm and width about 8 m. stream water mainly use for irrigation purposes, and sand mining is common along the stream(Maulood *et al.*, 1980).

## 2.2. Sample Collection and Analysis

The electrical conductivity was measured by using (pH-EC-TDS meter, HI 9812, Hanna instrument), SAR, $Na^{+1}$, $Ca^{2+}$, $Mg^{2+}$, $Cl^{-1}$ and $HCO_3^{-1}$ were measured according to(APHA, 2005).

### 2.3. The Model of Irrigation Water Quality Index (IWQI)

The model of (IWQI) developed by Meireles*et al.* (2010) was applied on the observed data according to the following steps:-

**Step1:** Identified parameters were considered more relevant to the irrigation use; EC, $Na^{+1}$, $HCO3^{-1}$, $Cl^{-1}$, SAR°.

**Step2:** The values of quality measurement (Quality rating) (Qi) for each parameter were calculated using the equation (1), based on the tolerance limits shown in table (1), and the observed water quality results. Table (1) was consecrated according to irrigation water quality parameters proposed by (UCCC) and by the criteria established by Ayers and Westcot (1985).

$$\mathbf{Qi = Qi\ max - \left[\frac{(Xij - Xinf) * qiamp)}{Xamp}\right]} \quad \mathbf{(1)}$$

Where Qimax is the maximum value of quality rating scale (qi) for the class of table (1) ; Xij is the observed value for the parameter; Xinf is the corresponding value to the lower limit of the class to which the parameter belongs; Qiamp is class amplitude; Xamp is class amplitude to which the parameter belongs .In order to evaluate sample, of the last class of each parameter, the upper limit was considered to be the highest value determined in the physical-chemical and chemical analysis of the water samples.

**Step 3:** The weight of each parameter has been assigned according to its relative importance in the overall quality of irrigation water, as shown in table (2):

**Step 4:** The water quality index was calculated as:

**IWQI=**

$$\sum_{i=1}^{n} \mathbf{Qi\ wi} \quad (2)$$

IWQI is dimensionless parameter ranging from 0 to 100; (Qi) is the Quality rating of the ith parameter, a number from 0 to 100 (wi) is the normalized weight of the $i^{th}$ parameter. Division in classes based on the recommended water quality index was based on present water quality indices, and classes were indicated the potential risk of salinity problems, reducing the osmotic potential of soil, as well as toxicity to plants as observed in the classifications presented by Holand and Amorim (1997). Restrictions to water use classes were characterized as shown in table (3).

**Table 1: Parameter limiting values for (Qi) calculation (AyersandWestcot, 1985)**

| **(qi)** | **EC(Meq/L)** | **SARº(Meq/L)$^{1/2}$** | **$Na^{+1}$** | **$Cl^{-1}$** | **$HCO3^{-1}$** |
|---|---|---|---|---|---|
| | | | **Meq/L** | | |
| **85-100** | 0.20≤ EC<0.75 | 0.20≤ SARº < 3 | 2≤Na< 3 | 1≤Cl< 4 | 1≤ HCO3< 1.5 |
| **60-85** | 0.75≤EC< 1.50 | 3≤ SARº < 6 | 3≤Na< 6 | 4≤Cl< 7 | 1.5≤ HCO3< 4.5 |
| **35-60** | 1.50≤ EC< 3 | 6≤ SARº < 12 | 6≤Na< 9 | 7≤Cl< 10 | 4.5≤ HCO3< 8.5 |
| **0-35** | EC< 2 or EC≥ 3 | SARº< 2 or SARº ≥ 12 | Na< 2 or Na ≥ 9 | Cl< 1 or Cl ≥ 10 | HCO3 < 1 or HCO3 ≥ 8.5 |

**Table 2: Weights for the (IWQI) parameters (Meireles*et al.*, 2010)**

| **Parameters** | **Wi** |
|---|---|
| EC | 0.211 |
| Na+1 | 0.202 |
| HCO3-1 | 0.202 |
| Cl-1 | 0.194 |
| SARº | 0.184 |
| Total | 1 |

**Table 3: (IWQI) Characteristics (Holanda and Amorim, 1997)**

| **IWQI** | **Water use restriction** | **Recommendation** | |
|---|---|---|---|
| | | **Soil** | **Plant** |
| 85-100 | No restriction (NR) | Could be used for the majority of soil types and it has the lowest probability of salt accumulation which may cause salinity and sodicity problems, being recommended leaching within irrigation practice, except for soils with extremely for soils with extremely low permeability | No toxicity risk for most plants |
| 70-85 | Low restriction(LR) | Recommended for irrigation is soils with low texture and moderate permeability, being recommended salt leaching. Soils sodicity in heavy texture soils may occur, being recommended to avoid its use in soils with high clay | Avoid low salt resistant plants. |
| 55-70 | Moderate restriction(MR) | Recommended for the soil irrigated with moderate to high permeability levels, being suggested moderate leaching of salts. | Plants that have moderate resistance to salts might be grown. |
| 40-55 | High restriction (HR) | May be used in soils with high Permeability without compact layers. High frequency irrigation schedule should be adopted for water with EC above 2000 dS m-1 and SAR above 7.0 | Plants with moderate to high resistance to salts may be grown with special salinity control techniques, except water with |

| | | | |
|---|---|---|---|
| | | | low Na, Cl and $HCO_3$concentrations. |
| 0-40 | Severe restriction (SR) | Should be avoided its use for irrigation under normal conditions. In special cases, may be used occasionally. Water with low salt levels and high SAR require gypsum application. In high saline content water soils must have high permeability, and excess water should be applied to avoid salt accumulation. | Only plants with high salt tolerance, except for waters with extremely low values of Na, Cl and HCO3 |

## 3. RESULTS AND DISCUSSION

The values of the physical and chemical measurements of ShekhTurab stream samples which have taken in two seasons, one in winter and the other in autumn-2016 for five stations with two replications for each site, are presented in table (4).

**Table (4). Average measured values of the parameters of IWQI for ShekhTurab spring water sampled at different sites and over two months of 2016.**

| Period | Site | Average value of the measured parameters | | | | | |
|---|---|---|---|---|---|---|---|
| | | **EC($dSm^{-1}$)** | **$Na^+$( meq/l)** | **$Ca^{2+} + Mg^{2+}$ (meq/l)** | **SAR(meq/l)** | **$Cl^-$(meq/l)** | **$HCO_3^-$(meq/l)** |
| **Jul-16** | 1 | 1.20 | 1.28 | 7.80 | 0.92 | 1.17 | 3.56 |
| | 2 | 1.16 | 1.26 | 7.80 | 0.91 | 1.14 | 3.49 |
| | 3 | 1.18 | 1.34 | 7.68 | 0.97 | 1.03 | 3.55 |
| | 4 | 1.16 | 1.33 | 7.20 | 0.99 | 1.11 | 3.42 |
| | 5 | 1.13 | 1.34 | 7.18 | 1.00 | 1.14 | 3.42 |
| **Nov-16** | 1 | 1.00 | 1.54 | 7.80 | 1.39 | 0.81 | 2.55 |
| | 2 | 0.99 | 1.56 | 7.80 | 1.38 | 0.90 | 2.50 |
| | 3 | 0.97 | 1.60 | 7.68 | 1.35 | 0.83 | 2.38 |
| | 4 | 1.02 | 1.63 | 7.20 | 1.45 | 0.97 | 2.39 |
| | 5 | 0.98 | 1.60 | 7.18 | 1.43 | 0.86 | 2.26 |

### 3.1. Assessment of Individual Hazard Groups

#### 3.1.1. Salinity Hazard

Concentrations of Electrical conductivity (EC) in collected samples from five sites are presented in table (4). It can be seen that EC values are observed to cover a wide range from (0.97 dS/m) which measured at site (3) in (Nov 2016) to (1.20 dS/m) which measured at site (1) in (Jul 2016), this may be attributed to the decreased discharge and increased of evaporation and lower one in (Nov 2016) of the due to rainfall that cause dilution then decrease the amount of salt in water samples. Results of the electrical conductivity throughout this survey come in accordance with the known conductivity values of Iraqi inland waters (Maulood *et al.*, 1980).The gradual reduction in conductivity with time may be due to the uptake of the ions by organisms for their metabolism. Similar observation has been reported by (Mustapha and Osmotosho, 2005). The variation in electrical conductivity of the water depend on the climate, seasonal variation, soil source, geological origin the content of the ionic salts such as calcium, magnesium etc. (Bartram and Balance,1996 ; Wetzel, 1983).

#### 3. 1.2. Infiltration Hazard

The most common water quality factor that influence the normal rate of infiltration of water is the relative concentrations of sodium, magnesium and calcium ions in water that is also known as the sodium adsorption ratio (SAR). (SAR) the value of irrigation water quantifies the relative proportions of sodium ($Na^{1+}$) to calcium ($Ca^{2+}$) plus magnesium ($Mg^{2+}$) and is computed as:

$$\mathbf{SAR}=\frac{Na+1}{\sqrt{\frac{[(Mg^{2+})+(Ca^{2+})]}{2}}} \quad \text{.... (3)}$$

The calculated values of sodium adsorption ratio (SAR) are recorded in table (4). It can be seen that the minimum value of SAR was observed in site2 (0.91 meq/l) (July 2016) and the maximum value of SAR was observed in site (4) (1.45 meq/l) (Nov. 2016). The increase in the value of (SAR) comes as a result of an increase in the sodium content relative to calcium and magnesium, and this increase can contribute to reduce the infiltration rate to such an extent that sufficient water cannot be infiltrated to supply the crop adequately from one irrigation to the another (Ayers and Westcot, 1985).

#### 3.1.3. Specific Ion Toxicity

Sodium concentrations ranged from higher value (1.26 meq/l) (Jul. 2016) at site (2) to (1.63 meq/l) (Nov. 2016) at site (4) as shown in table (4). The results indicated that the sodium concentrations increases in November are approaching or exceeding (1.63 meq/l). The present results show a slight variation in the sodium distribution patterns during two different seasons. (The main reason is associated with the solubility of salts to reach the sodium balance in the whole water body. However, the highest range of sodium ion concentration were 1.54-1.63meq/l during autumn season because the main sources of sodium ion concentration in water comes from dissolution of rocks and the overlaying water (Wetzel, 1983).

Chloride concentrations were ranged from (0.81 meq/l) measured at site (1) (Nov. 2016) to (1.17 meq/l) measured at site (1) (Jul. 2016) as shown in table (4). This variation in chloride may be due to agricultural drainage water, which disposed into the ShekhTurab stream

and geological formation of the area and also chloride may enter through the household water raised to the river directly (Morteth, 2006).

### 3.1.4. Miscellaneous Effects

The concentration of bicarbonate ranged from (2.26 meq/l as $HCO_3$)at site 5 during (Nov. 2016) to (3.56meq /l as $HCO_3$)(Jul. 2016) at site (1) as shown table (4). Increase in alkalinity values may be due to decreases in the water level. Bicarbonate increases with decreases in water levels have also been reported by Lashari*et al.* (2009).Variation in alkalinity values in this survey may be related to phytoplanktonic activity such as photosynthesis and respiration processes, and also this may be attributed to the dilution phenomena through the rainfall caused reduction in alkalinity (Lashari *et al.,* 2009)

## 3.2. Discussion of Irrigation Water Quality Index

Table (5) represents calculation of (IWQI), while the (IWQI) values for ShekhTurab stream are recorded in table (6), it can be seen that there is a strong spatial and temporary variation of (IWQI) values during 2016 due to the dynamics of water quality influenced by human interventions and seasonality of flow at each site. Again it can be recognized that the (IWQI) value had observed with the minimum value (41.11in Nov) at site (4) and with the maximum value (53.65in Jul.) at site (2). Specifically, the table (6) shows that the quality and suitability of ShekhTurab for irrigation, improved more in autumn months. The improvement of the water quality may be attributed to the increased discharge of the stream water, which contributes of salts dispersion and reduces their concentrations (Holanda and Amorim, 1997). It seems that the (IWQI) value in the summer months varied from (53.06-53.65) due to increased concentrations of salts in the river at summer season(Holanda and Amorim, 1997),and the increased evaporation rate because of high temperature at summer season. The values of (IWQI) are observed to cover a wide range between (41.11-41.76) in autumn months. It should be noted from the obtained results that any of irrigation water quality parameters may play an important role in changing the value of (IWQI).

Generally ShekhTurab water was ranked ‘‘high restriction’’; good for irrigation with some restriction at all stations in 2016, this means the stream water quality is suited to irrigated soils with light texture (Holanda and Amorim, 1997), and since the nature of soil in irrigated lands on both sides of the river is clay loam (heavy texture) so the problem of soil sodicity may occurs, and this requires washing the salts from soil constantly. The farmers also must avoid growing salts sensitive plants as shown in table (6) (Brouwer, 1985).

**Table (5). Sample calculation of IWQI for Site 1 during July 2016.**

| Period | Site | Varaible | IQWI Parameters | | | | |
|---|---|---|---|---|---|---|---|
| | | | EC($dSm^{-1)}$ | SAR(meq/l) | $Na^+$(meq/l) | $Cl^-$( meq/l) | $HCO3^-$ (meq/l) |
| **Jul. 2016** | **1** | **Average measured value($x_i$)** | 1.20 | 0.92 | 1.28 | 1.17 | 3.56 |
| | | **$Q_{max}$** | 85.00 | 35.00 | 35.00 | 100.00 | 85.00 |
| | | **$Q_{ampl}$** | 25.00 | 35.00 | 35.00 | 15.00 | 25.00 |
| | | **$X_{amp}$** | 0.75 | 2.00 | 2.00 | 3.00 | 3.00 |
| | | **$X_{inf}$** | 0.75 | 0.00 | 0.00 | 1.00 | 1.50 |
| | | **$Q_i$** | 70.00 | 18.90 | 12.60 | 99.15 | 67.83 |
| | | **$W_i$** | 0.21 | 0.18 | 0.20 | 0.19 | 0.20 |
| | | | | | | | |
| | | **$Q_iW_i$** | 14.77 | 3.48 | 2.55 | 18.84 | 13.57 |
| | | **IWQI** | 53.14 | | | | |

**Table (6). Water use restrictions based on the calculated values of IWQI for ShekhTurab Spring water sampled at different sites over two months of 2016.**

| Period | Site | IWQI | Water Use Restriction |
|---|---|---|---|
| **Jul. 2016** | **1** | 53.14 | High restriction (HR) |
| | **2** | 53.65 | High restriction (HR) |
| | **3** | 53.06 | High restriction (HR) |
| | **4** | 53.28 | High restriction (HR) |

| | | | |
|---|---|---|---|
| | **5** | 53.40 | High restriction (HR) |
| **Nov. 2016** | **1** | 41.48 | High restriction (HR) |
| | **2** | 41.60 | High restriction (HR) |
| | **3** | 41.72 | High restriction (HR) |
| | **4** | 41.11 | High restriction (HR) |
| | **5** | 41.76 | High restriction (HR) |

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# Assessment of *Azadirachta indica* seed kernel extracts to restrict the rampancy of antinematicidal –resistant *Haemonchus contortus* in ovine

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**ABSTRACT**

The current study was executed to evaluate the potency of crude aqueous methanol extract (CAME) of *Azadirachta* (*A.*) *indica* seed kernels in controlling the prevalence of oxfendazole (OXF), levamisole HCL (LEV) and ivermectin (IVM)-resistant *Haemonchus* (*H.*) *contortus* in sheep. Faecal egg count reduction test (FECRT), calculated by the RESO program, revealed an emergence of resistance among the parasite populations against the aforementioned antinematicidals particularly OXF [FECR% = -56 and lower confidence interval (LCI) = -311]. The recorded FECR% for LEV and IVM was 75 and 78 whilst the LCI for both of them was 38 and 42, respectively. Egg hatch assay (EHA) also exhibited a disastrous level of resistance in *H. contortus* toward OXF. Antinematicidal activity of *A. indica* seed kernels was assessed using FECRT, EHA and adult motility test (AMT). Mean egg per gram of faeces (EPG) recorded post-treatment of sheep at low (2g $g^{-}$ $k^{1}$ BW) and high (4g $kg^{-1}$ BW) doses of *A. indica* CAME was 539±367.11 SE and 147±58.45 SE, respectively, as compared to the control group (mean EPG= 987±364.26 SE). There was no significant difference statistically (P>0.05) in FECR% (45.62 *vs* 85.14) in sheep at low and high doses of the plant. The results of AMT revealed a dose and time dependent efficacy of CAME of the assessed plant to kill antinematicidal-resistant adult worms with calculated $LC_{50}$ values of 52.20, 15.40, 3.26, 1.55 and 0.80 mg $ml^{-1}$ after 2, 4, 6, 8 and 10 hours. Accordingly, high dose (4g $kg^{-}$ $^{1}$ BW) of *A. indica* seed kernel extract could be used to treat sheep carrying antinematicidal- resistant populations of *H. contortus*.

## 1. INTRODUCTION

Small ruminants are vastly exposed to infestation with different genera and species of gastrointestinal (GI) nematodes in tropical, subtropical and even in moderate climate zones in the world (Getachew *et al.*, 2007). Categorically, the abomasal inhabitant, blood-sucking *Haemonchus* (*H.*) *contortus* is considered more dangerous to ovine and caprine as compared to other pathogenic nematodes due to its highly detrimental impacts on livestock productivity (Urquhart *et al.*, 2007; Bowman, 2009). To control nematodiasis, organized sheep farms in developing countries and comparatively industrialized countries rely regularly on broad-spectrum synthetic chemotherapeutics such as benzimidazoles, imidazothiazoles and macrocyclic lactone derivatives (Ghisi *et al.*, 2007). By virtue of these traditional

dewormers, the epidemicity of these alimentary worms has been patently contained. Further, the emergence of antinematicidal resistance (AR) was the consequence of the recurrent annual use of these conventional antinematicidals (Neveu *et al*., 2007). Additionally, these drugs can contaminate the environment and affect public health through their residues in the food chain (Waller, 2006).

It is noteworthy to mention that the prevalence of AR has emboldened researchers to adopt and promote non-chemical alternatives of orthodox drugs. In this regard, some substitutes such as biological control, genetic approaches, immunization, nutritional supplementations and grazing management have been recommended. However, these alternate sources have some drawbacks which limit their commercialization (Stear *et al*., 2007).

On the other hand, under the umbrella of non-chemotherapeutic approaches, phytotherapy is presently an interesting area of investigation and scientific validation anticipated to be a promising alternative to control parasitism in the near future (Jabbar *et al*., 2006). Medicinal plants have been exploited for thousands of years to cure human and animal ailments as part of ethno-medicine and ethno-veterinary practices (Wanzala *et al*., 2005). In recent decades, several hundred researches have been conducted to explore the active constituents of ethnobotanicals and evaluate their efficacy against ecto and endo parasites of livestock (Macedo *et al*., 2010). The present study was carried out for assessing the potency of *Azadirachta* (*A.*) *indica* seed kernels toward antinematicidal- resistant *H. contortus* in naturally infected sheep for the first time. This phytomedicine is extensively growing in the Indo-Pakistan subcontinent and it has been known for many centuries for possessing several therapeutic activities including antiparasitic properties (Dhawan and Patnaik, 1993). The major active ingredient of *A. indica* is azadirachtin which was isolated in the last century (Butterworth and Morgan, 1968). Previously, some studies have been carried out to assess this plant for its antinematicidal activity (Akhtar and Riffat, 1984; Hördegen *et al*., 2003; Costa *et al*., 2006; Costa *et al*., 2008). These studies; however, have been conducted randomly without giving attention to the efficacy of ethnobotanicals against known antinematicidal-resistant nematodes. Antinematicidal-resistant nematodes are more pathogenic and prolific, have ameliorated inhabitancy rates in the host and an enhanced prolonged existence of the free-living stages on paddock (Kelly *et al*., 1977). Therefore, crude aqueous methanol extract of this phytomedicine was evaluated utilizing *in vivo* and *in vitro* parasitological assays.

## 2. Material and Methods

Animals (n=90) aged 3-6 months with egg per gram of feces (EPG) of more than 150 eggs, that had not been dewormed for the last 8-12 weeks, were chosen for this study (Coles *et al*., 1992). FAMACHA Anaemia Guide Chart was used to facilitate the selection of animals (Macedo *et al*., 2010). The percentage of infection with *H. contortus* as compared to other nematodes was >90% in each animal (Bowman *et al*., 2003). The selected animals for the study were isolated from the flock and randomly allocated and tagged into the following six groups:

Group 1: Oxfendazole resistance detection group (n=15)

Group 2: Levamisole HCL resistance detection group (n=15)

Group 3: Ivermectin resistance detection group (n=15)

Group 4: *A. indica* low dose (2 g $kg^{-1}$ BW) treated group (n=15)

Group 5: *A. indica* high dose (4 g $kg^{-1}$ BW) treated group (n=15)

Group 6: Infected untreated group (n=15)

Each group of the experimental animals was penned alone and fed on rough fodder without administering any kind of therapy during the experiment.

**NB.** One control group was used for both detection of antinematicidal resistance and evaluation of the plant trials because we statistically dealt with one population.

**• Diagnosis of *Haemonchus contortus***

**Faecal examination**

Qualitative and quantitative faecal examinations of the animals were performed during the selection process as a preliminary detection of nematodiasis. Fecal specimens were taken from each animal individually and other steps were carried out according to Coles *et al*. (1992). Where possible, the nematode eggs were identified using the diagnostic key of Soulsby (1982).

**Coproculture**

Coprocultures were also executed to determine the involvement of various species of nematodes in overall natural worm infestations MAFF (1986) during the selection process. Fecal specimens from each group of experimental animals were pooled and cultured in glass dishes. The cultures were incubated for seven days at 27±1°C. The larvae ($L_3$) were, then, collected using the Baermann apparatus. Lugol's iodine was added to the cultures and 100 larvae were counted and identified according to MAFF (1986).

**• Detection of antinematicidal resistance**

**FECRT:** Oxfendazole (OXF) 2.265%, levamisole (LEV) 1.5% [Epla Lab. (Pvt.) Ltd.] and ivermectin (IVM) 1% [Cherished Pharmac. (Pvt.) Ltd.] were procured from Sanna Laboratories for Pharmaceuticals and Vaccines, Faisalabad-Pakistan and analyzed for their authenticity by HPLC analysis in Central Hi-Tech Laboratories, University of Agriculture- Faisalabad, Pakistan (UAF). The animals in group one, were treated with the recommended doses of OXF (5 mg $kg^{-1}$ BW), group two was treated with LEV (7.5 mg $kg^{-1}$ BW), and group three was treated with IVM (0.2 mg $kg^{-1}$ BW) while group six was left as infected (untreated control). Fecal examinations and coprocultures of the animals were performed at day 14 (post-treatment) as depicted previously. Records of post OXF, LEV and IVM treatment EPG in addition to control group EPG and composition of nematode infections were kept. The following formula was used to calculate (FECR %):

FECR% = [1-(mean EPG treatment/mean EPG control)] x 100

RESO program (CSIRO Animal Health Research Laboratory, Private Bag 1, Parkville, Vic. 3052, Australia) was utilized to compute the FECR data including arithmetic mean, variance of counts, FECR% and 95% confidence interval. According to Coles *et al*. (1992), resistance is developed if the FECR% is below 95% and the lower limit of 95% confidence interval is below 90%. If only one of the two criteria is met, resistance is suspected. Any negative values calculated from the FECR% and lower limit of confidence interval were deemed equal to zero, indicating that the resistance is highly rampant and at the catastrophic level as proposed by Gill (1996).

**EHA** was carried out following the standardized protocol that was accepted by the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P) and described in details by Coles *et al*. (1992) to diagnose resistance against OXF.

The following formula was used for the calculation of hatching inhibition (%):

Hatching inhibition (%) = P test/ P total ×100

P test: number of unhatched or embryonated eggs.

P total: number of unhatched or embryonated eggs + Larvae ($L_1$)

$LC_{50}$ values were calculated for the eggs by probit analysis. Eggs with an $LC_{50}$ value in excess of 0.1 µg ml $^{-1}$ were deemed as an indication of antinematicidal (OXF) resistance as suggested by Coles *et al*. (1992).

**• Assessment of *Azadirachta indica* extracts against *Haemonchus contortus***

**Plant extraction**

*A. indica* seed kernels were purchased from the local market of Faisalabad-Pakistan. The plant seed kernels were finely pulverized to a powder in an electric grinding machine and kept in cellophane bags. The ground plant materials were soaked in 70% aqueous methanol (the concentration adjusted by an alcohol meter) by cold maceration at room temperature with the mixture stirred twice daily. After three days, the filtrate was collected through a piece of porous cloth and the plant materials re-soaked in 70% aqueous methanol. This process was repeated three times. The combined filtrates were concentrated in a rotary evaporator at 40°C under reduced pressure. For more evaporation, vacuum- oven at 40°C was also used to prepare crude aqueous-methanol extracts (CAMEs) (Gilani *et al*., 2004). These extracts were stored at 4°C until exploited against the parasite using *in vivo* and *in vitro* techniques. The percentage yield of extracts was calculated as under:

Ratio= A/B×100

A: weight yield after extraction (g)

B: dry matter weight (g)

**FECRT:** The animals in groups 4 and 5 were treated with *A. indica* CAME at low (2 g kg$^{-1}$ BW) and high (4 g kg$^{-1}$ BW) doses, whereas

group 6 served as infected, untreated control. Fecal examinations and coprocultures of the animals were executed at day 14 (post-treatment) as mentioned above. Records of post CAME treatment EPG and composition of nematode infections were kept. FECR % was calculated by the following formula:

FECR% = [1-(mean EPG treatment/mean EPG control)] x 100

**EHA** was carried out to evaluate the inhibitory effects of different concentrations of the CAME on the hatching of the parasite eggs. The assay was conducted pursuant to the methodology described by Coles *et al*. (1992) with minor modifications by some researchers to be more suitable for the evaluation of plants (Macedo *et al*., 2010).

**AMT** was used with minor modifications to determine the effect of the plant extracts on the viability of live adult resistant *H. contortus* in sheep (Singh *et al*., 1985). The mature worms of either sex were collected from the abomasa of two experimental animals slaughtered at the end of the experiment. The collected worms were washed and suspended in PBS. Acetone (70%) and PBS (50:50 v/v) were used to dissolve the CAME. The stock solution (100 mg $ml^{-1}$) was serially diluted (two-fold serial dilution) in PBS to prepare different concentrations (100-0.048 mg $ml^{-1}$) in a 24-well flat-bottomed titration plate. The positive control well received 25 µg $ml^{-1}$ of closantel dissolved in PBS, while the negative control well contained 1 ml of 70% acetone and 1 ml of PBS. The experiment was done at room temperature (25-30°C). Ten live worms were placed in each well containing CAME, positive and negative controls. The worms were observed at 0, 2, 4, 6, 8, 10 and 24 hours for their motility, paralysis and mortality. There were three replicates for each concentration.

## 3. Results and Discussion

### 3.1. Results

**• Composition of natural nematode infections in experimental animals**

The experimental animals were predominantly infested with *H. contortus*. However, other nematode species were also concomitant such as: *Teladorsagia* spp., *Chabertia* spp., *Oesophagostomum* spp. and *Trichostrongylus* spp. According to the coproculture, *H. contortus* was the main contributor to the infection (>90%) of the experimental groups of animals (Table 1).

**• Resistance studies**

**Coproculture**

The proportions of infection with *H. contortus* and other coexisting nematode species pre-treatment and post-treatment (day 14) with OXF, LEV and IVM other than control group, after performing coproculture of pooled fecal samples of the animals in each group, are presented in table 1 above.

**FECRT:** Mean EPG reduction and FECR% on day 14 (post-treatment with OXF, LEV and IVM) analyzed by the RESO program in

addition to the calculation of lower confidence interval 95% (table 2) had indicated that the resistance was at disastrous levels in the farm, particularly in the case of OXF.

**EHA:** The $LC_{50}$ of OXF was found to be 1.86 µg $ml^{-1}$ (range 1.45-2.45), which was in excess to 0.1 µg $ml^{-1}$ proposing evolution of resistance against *H. contortus* (Coles *et al*., 1992). Correlation between ovicidal activity of different concentrations of OXF and hatching inhibition (%), through executing EHA, is exhibited in figure 1.

- **Efficacy of *Azadirachta indica* against resistant *Haemonchus contortus***

The yield of CAME from *A. indica* seed kernels was 2.34%. Post -treatment coproculture of pooled fecal samples of the animals included in the high dose group of *A. indica* revealed very poor recovery of *H. contortus* and other nematode larvae ($L_3$). In the low dose group, the recorded percentage of *H. contortus* and other nematode larvae ($L_3$) was 93% and 7%, respectively. In the control group, the percentage of *H. contortus* $L_3$ was 95% while recovered $L_3$ of other nematodes was 5% (table 1).

**FECRT:** the results of antinematicidal activity of *A. indica* CAME (low and high doses) against OXF, LEV and IVM- resistant *H. contortus* populations in the experimental animals naturally infected with the predominant parasite (>90%) as well as the comparison between impacts of low and high doses of the plant extracts on the mean of egg reduction is statistically analyzed (table 3). The results of coproculture after treatment, the group treated with high dose of *A. indica* extracts (FECR% > 80), clearly shows that very few larvae of *H. contortus* and other nematodes were recovered (table 1).

**EHA:** the procured data from the analysis of variance (ANOVA) of EHA regarding ovicidal efficacy of different concentrations of *A. indica* CAME, other than the calculation of the mean±SE of hatching inhibition (%), revealed diverse influences of different concentrations (dose-dependent ovicidal activity) (figure 2).

The calculated mean square was 3748.25, which is highly significant ($P<0.01$). The calculated $LC_{50}$ was 1.169 mg $ml^{-1}$ (range 1.047-1.303).

**AMT:** pursuant to the data procured from the adulticidal efficacy of *A. indica* CAME utilizing AMT and computed F-value from the ANOVA table, there were significant differences ($P<0.01$) between the three factors (time, concentration and mortality). The mean mortality, after exposure of live resistant *H. contortus* to different concentrations of the plant extracts, was recorded every two hours. The data is displayed in figures 3. The $LC_{50}$ values at different hours are also calculated (table 4).

### 3.2. Discussion

Resistance has developed against conventional antinematicidals (OXF, LEV and

IVM) by GI nematodes particularly *H. contortus* in most countries of the world (Bartley *et al*., 2004; Neveu *et al*., 2007) including Pakistan (Babar, 2005; Saddiqi, 2005; Hamad *et al*., 2013). Due to the absence of dependable alternate sources to synthetic chemotherapeutics (Stear *et al*., 2007) researchers, especially in the Indo-Pakistan subcontinent, Africa and South Latin America have promoted indigenous medicinal plants as an effective substitute to fight parasitism (Waller *et al*., 2001; Cala *et al*., 2012). Hence, this study was conducted to assess the antinematicidal activity of *A. indica* seed kernels against antinematicidal-resistant *H. contortus* for the first time in the world.

For OXF, the calculated FECR% was (-56) while the LCI was (-311) signifying that resistance was at the disastrous level (Gill, 1996) in the farm because even a triple dose (15 mg kg $^{-1}$ BW) was ineffective to minimize the parasitic burden in the infected sheep (local veterinarian file). $LC_{50}$ value of OXF (1.86 µg ml $^{-1}$), calculated by the probit analysis after performing EHA, also indicated a rampancy of resistance among the *H. contortus* populations. The calculated $LC_{50}$ value was higher than 0.1 µg ml $^{-1}$; an indication of OXF resistance (Coles *et al*., 1992). Accordingly, it can be concluded that the resistance level among *H. contortus* populations in sheep is more than 25%, which means that the parasite is highly resistant to OXF. In this regard, the antinematicidal resistance could not be diagnosed by traditional parasitological methods if the resistance level is below 25% among the GI nematodes (Martin *et al*., 1989). Antinematicidal resistance to LEV and IVM was detected in the farm as well. The calculated FECR% was 75 and 78; respectively while the LCI was 38 and 42 according to the RESO program. It may be concluded that the parasite is more susceptible to LEV and IVM as compared to OXF. However, even a 1.5 dose (standard dose is 7.5 mg kg$^{-1}$ BW) of LEV and a double dose (standard dose is 0.2 mg kg$^{-1}$ BW) of IVM were ineffective (local veterinarian file) due to the escalation of resistance among the parasitic populations. The main reasons for the evolution and prevalence of multiple forms of resistances in the farm are the discriminate and recurrent annual uses of these drugs. The local veterinarian file showed that OXF is used five times annually, LEV is administered four times per a year, and IVM is drenched three times per annum. Coles *et al*. (2005) have indicated the development of antinematicidal resistance even when only two to three drenches were administered annually.

CAME of *A. indica* seed kernels revealed antinematicidal efficacy against OXF, LEV and IVM -resistant *H. contortus* in sheep in all the assays (FECRT, EHA and AMT) used in this study. The main active constituent of *A. indica* seeds is azadirachtin (Butterworth and Morgan, 1968), which possibly plays a big role in killing resistant *H. contortus*. Although there was a significant difference in reduction of

EPG post-treatment with low (539±367.11 SE) and high (147± 58.45 SE) doses of the plant CAME, the FECR% was non-significant (P>0.05) in the animals treated at low (45.62) dose as compared to high (85.14) dose of CAME. The reason for the non-significant difference between low and high doses is attributed to the high calculated standard deviation and standard error. The $LC_{50}$ of CAME in EHA was calculated as 1.169 µg ml $^{-1}$ (range 1.047-1.303 µg ml $^{-1}$). In AMT, all helminths were observed dead at 10 hours post-exposure to 25 mg ml $^{-1}$ of CAME. With decreasing concentration, death of the worms also declined. In accordance with the time required to kill 100% of live resistant *H. contortus*, the plant CAME at 25 mg ml $^{-1}$ was similar to closantel (positive control) in killing the parasite; the reference drug at 25 µg ml $^{-1}$ that killed all the worms 10 hours post-exposure. In negative control [50:50 v/v of 70% acetone and phosphate buffer saline (PBS) at PH 7.2], all helminths were observed dead after 24 hours. The $LC_{50}$ of CAME in AMT was calculated as 52.20 mg ml $^{-1}$ (range 30.14-114.02 mg ml $^{-1}$) two hours post-exposure, while the calculated $LC_{50}$ value was 0.80 mg ml $^{-1}$ (range 0.59-1.08) after 10 hours of exposure. Phytomedicines have been identified as antiparasitics (Waller *et al*., 2001). The presence of multiple natural phytochemicals in plants categorizes them as wide spectrum antiparasitics. Exploitation of plants to treat different parasitic diseases is justified, particularly in the rural areas of developing countries, where ailments like parasitism (Farooq *et al*., 2012) have had an enormous negative effect on animal productivity due to the inaccessibility to allopathic drugs.

In contrast to the current study, some researchers have recorded higher antinematicidal potency of *A. indica* (Iqbal *et al*., 2006). These variations may, principally, be owing to the differences among target helminth populations, resistance status of the parasitic nematodes, and source of the plant. Other contributing factors, like the biological activity of plants which depends on the source of the plant, cropping season (Hammond *et al*., 1997), mode of calculation of the dosage, variation within species of plants, storage techniques and drying processes (Croom, 1983) should also be considered. Furthermore, tther reasons that justify this variation are that resistant parasites are more fecund, pathogenic and have increased settlement rates in parasitized hosts (Kelly *et al*., 1977).

Pursuant to the recommendations of W.A.A.V.P (second edition) edited by Wood *et al*. (1995), an anthelmintic with FECR% (98) is deemed highly effective; FECR% (80) and above is effective while FECR% less than 80 is not recommended. In accordance with the present study and W.A.A.V.P categorization, *A. indica* seed kernels are classified as an effective antinematicidal.

**Table 1: Pre-treatment and post-treatment contributing nematodes in the animals chosen for the study based on pooled faecal specimens**

| Experimental | Pre-treatment $L_3$ (%) | | Post-treatment $L_3$ (%) | |
|---|---|---|---|---|
| Groups | *H. contortus* | other nematodes | *H. contortus* | other nematodes |
| Oxfendazole | 96 | 4 | 97 | 3 |
| Levamisole | 94 | 6 | 92 | 8 |
| Ivermectin | 91 | 9 | 93 | 7 |
| *A. indica* (Low dose) | 96 | 4 | 93 | 7 |
| *A. indica* (High dose) | 92 | 8 | poorly recovered | poorly recovered |
| Control (Not-treated) | 93 | 7 | - | - |

**Table 2: Mean EPG and FECR% on day 14 post-treatment in addition to resistance status of the synthetic drugs calculated by RESO program**

| Treatment Groups | Mean EPG±SE | Confidence interval | | FECR% | Status of drug resistance |
|---|---|---|---|---|---|
| | | Upper | Lower | | |
| *Oxfendazole | 1543±432.41 | 40 | -311** | -56** | Highly resistant |
| Levamisole | 247±56.41 | 90 | 38 | 75 | Resistant |
| Ivermectin | 213±63.53 | 92 | 42 | 78 | Resistant |
| Control | 987±364.26 | - | - | - | - |

* Although, my previous paper published in Pakistan V. J. 33: 85-90, (2013) has embraced these data, but it is necessary to re-cite them in this research article.

** Negative values mean zero

**Table 3: Statistical analysis of results obtained post-treatment with low and high doses of the plant CAME**

| Treatment | Mean EPG reduction Post-treatment | ±SD | ±SE | t-value | probability | Mean FECR% |
|---|---|---|---|---|---|---|
| Low dose (2 g $kg^{-1}$ BW) | 539 | 1421.83 | 367.11 | 1.05 NS† | 0.303 | 45.62 |
| High dose (4 g $kg^{-1}$ BW) | 147 | 226.36 | 58.45 | | | 85.14 |
| Control (Mean EPG=987±364.26 SE) | | | | | | |

†Non-significant difference (P>0.05) between low and high doses

**Table 4: Calculated LC50 values for adulticidal activity of *Azadirachta indica* after performing adult motility test**

| Hours (Post-exposure) | $LC_{50}$ (mg $ml^{-1}$) | 95% Confidence interval Lower | upper |
|---|---|---|---|
| 0 hr | - | - | - |
| 2 hr | 52.20 | 30.14 | 114.02 |
| 4 hr | 15.40 | 9.99 | 20.05 |
| 6 hr | 3.26 | 2.34 | 4.60 |
| 8 hr | 1.55 | 1.09 | 2.19 |
| 10 hr | 0.80 | 0.59 | 1.08 |
| 24 hr | - | - | - |

All parasites were alive at 0 hr and dead at 24 hr

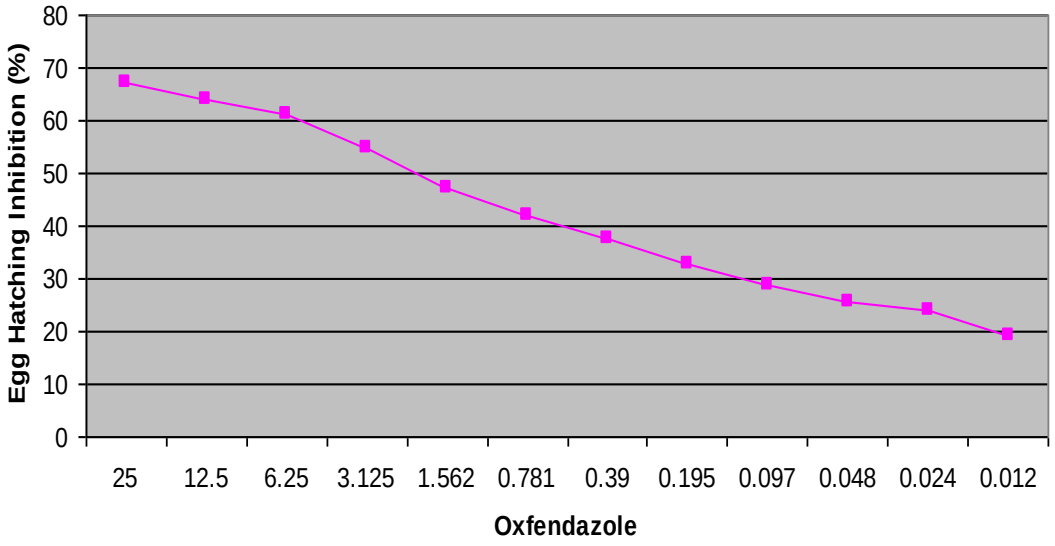


**Figure1: Correlation between ovicidal activities of different concentrations of oxfendazole (µg ml$^{-1}$) and hatching inhibition (%) through conducting egg hatch assay**

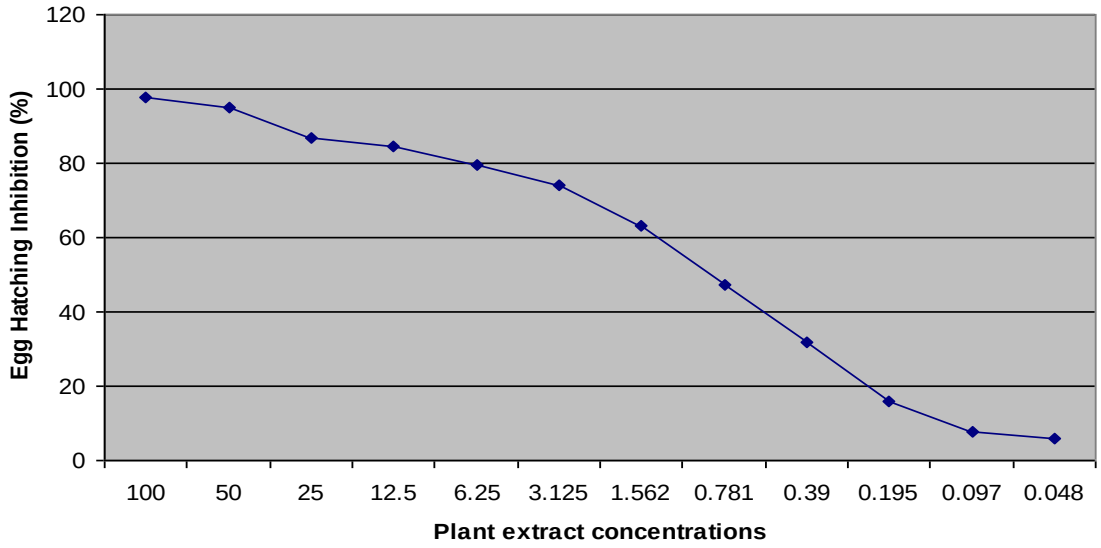


**Figure 2: Correlation between ovicidal activities of different concentrations (mg ml$^{-1}$) of *Azadirachta indica* extracts and egg hatching inhibition (%) through carrying out egg hatch assay**

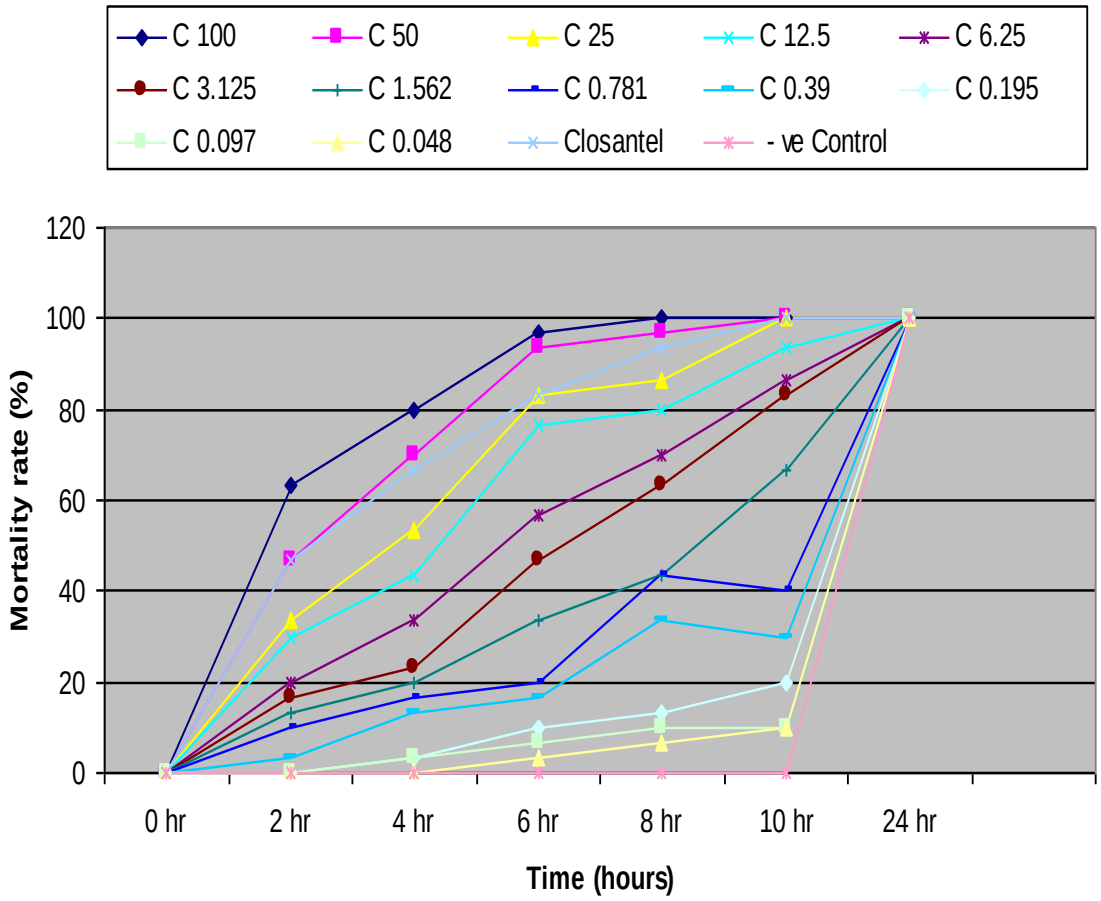


**Figure 3: Mortality (%) of resistant live adult *Haemonchus contortus* post-exposure to the CAME of *Azadirachta indica***

C= Concentration of each CAME (mg $ml^{-1}$)

Closantel concentration=25 µg $ml^{-1}$ (dissolved in PBS)

Control= I ml of 70% acetone plus 1 ml of PBS

## 4. Conclusions

In light of the results of this study, it may be concluded that resistance is prevalent among *H. contortus* populations in sheep against OXF, LEV and IVM in Angora Goat Farm. Antinematicidal resistance is at a catastrophic level in the case of OXF. The reasons behind the development and rampancy of multiple drug resistance in the farm are the indiscriminate and recurrent annual administering of antinematicidals. High doses (4g $kg^{-1}$ BW) of CAME of *A. indica* seed kernels is effective in the treatment of sheep harboring OXF, LEV and IVM-resistant *H. contortus* populations. Thus, the prevalence of multiple drug resistance could be controlled significantly using *A. indica* seed kernels.

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## Conflict of interest statement

The author of this research article attests to no conflicts of interest concerning the data incorporated in this document.

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# Soil fertility status for wheat crop production based on its soil organic matter and nitrogen contents

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**A B S T R A C T**

The study was conducted to show the role of organic carbon and total content of available nitrogen on soil suitability for wheat production. The study area located in central of Iraq, in Al-Kufa – Alnajaf province situated approximately between 32° 00' N to 32° 10' N and 44° 20' E to 44° 35' E with total area of 27664 ha. Thirty five sites were selected representing all variations within the study area and located on landsat 8 image using GPS. Soil samples were taken from each of the selected sites and analyzed in laboratory to determine some physical and chemical properties. The results revealed that most soils of the study soils are Haplosalids and to some extend the presence of Torrifluvents. Most of soils have high salt accumulation. Rating scores for soil properties were evaluated using FAO, 2007 system to determine the suitability class for each soil site .The results indicated four suitability classes for wheat production in the study area including , S3, N1 and N2 with, about 37% of the total area of the study site are not suitable for wheat production due, mainly to the effect of high salinity level and to some extent to low content of organic carbon and total available nitrogen. Also, the results demonstrated the effects of organic carbon and available nitrogen on the spatial distribution pattern of soil suitability classes for wheat production.

## Introduction

The population of the planet is growing dramatically. In order to meet the increasing demand for the food the farming community has to produce more crops mainly cereal types. Under present situations, where the land is a limiting factor, it is impossible to bring more area under cultivation, so farming community should tackle this challenge of producing more food. One approach to this point can be followed through using some conservation practices represented by land suitability evaluation systems. Further, land suitability analysis is needed for various purposes in the context of the present day agriculture. Land suitability evaluation is the prerequisites for sustainable agricultural production. It involves evaluation of the criteria ranging from soil, terrain to socio-economic, market and infrastructure (Prakash, 2003). According to the FAO general framework for land suitability evaluation (1976), the land suitability classification consists of assessing and grouping the land types in orders and classes according to their capacity. The results are intended to be used for land resource related decision making, both strategic land use planning by policy/planning institutions such as extension agencies, and specific local land allocation by the direct land users, that is, the farmers . Suitability of land is assessed considering rational cropping system, for optimizing the use of a piece of land for a specific use (FAO, 1976; Sys *et al*., 1991). The suitability is a function of crop requirements and land characteristics and it is a measure of how well the qualities of land unit match the requirements of a particular form of land use (FAO 1976). Land evaluation is a process of predicting land performance over time according to the specific types of use ( Lee and Yeh, 2009 ; Martin and Saha, 2009 ; and Sonneveld et al., 2010). Agriculture land suitability assessment is defined as the process of assessment of land performance when used for alternative kinds of agriculture (He et al., 2011; Prakash, 2003). The principle purpose of agriculture land suitability evaluation is to predict the potential and limitation of the land for crop production). Conceptually, land evaluation requires matching of the ecological and management requirements of relevant kinds of land use with land qualities, whilst taking local economic and social conditions into account. Land evaluation provides practical answers to such questions as "What other uses of land are physically possible and economically and socially relevant?", "What inputs are necessary to bring about a desired level of production?", and "What are the current land uses and what are the consequences if current management practices stay the same?. In order to reach such aims, this study was conducted to meet the following objectives: 1- To evaluate land characteristic for wheat according to FAO, 2007 system, 2- to develop regression model to predict wheat production suitability using some spectral indices and 3- To show the impact of soil organic carbon and available nitrogen on the spatial distribution pattern of wheat suitability classes in the study area.

## Materials and Methods

*Study Site*

The study area located in central of Iraq, in Al-Kufa – Al Nagaf province lying between latitude 32° 00' N to 32° 10' N and longitude 44° 20' E to 44° 35' E with total area of 27664 ha ( Figure 1 ).

Land of the study area are used for some crops production including wheat, barley and corn.

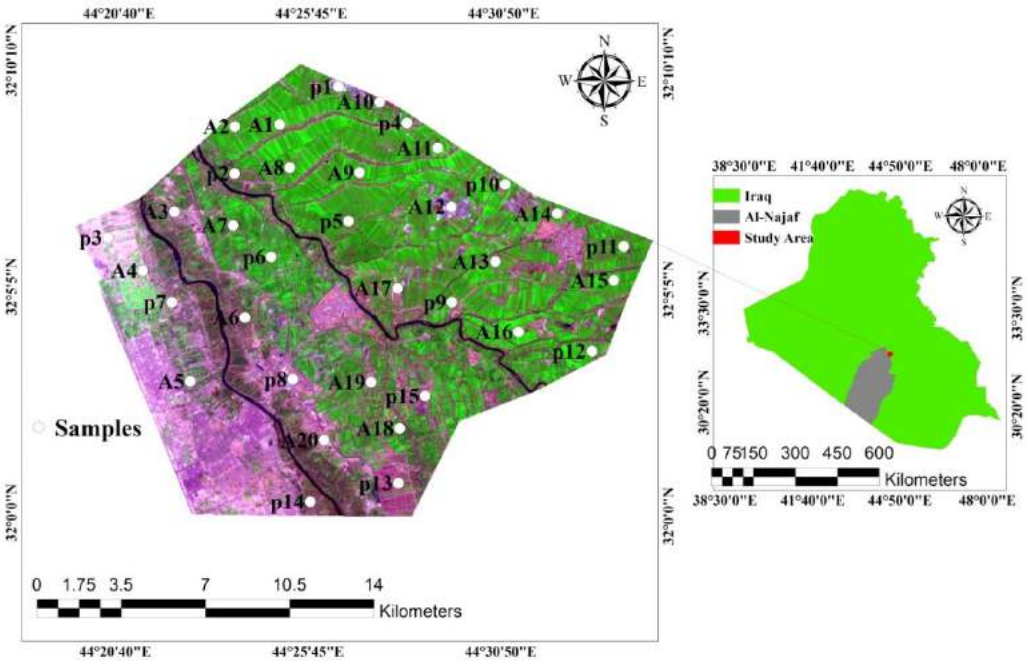


**Fig.1.** Location of the study area and sampling sites.

*Field Work*

Thirty five locations in the study area were selected to represent all variations in local conditions ( Figure 1 ) . Soil samples were taken from fifteen pedons and twenty auger holes, and from all horizons or layers and described morphologically in the field according to Soil Survey Division Staff, 1993. Some physical , chemical and fertility properties for all soil samples were determined in laboratory using the common analysis methods mentioned by Bouyoucos (1962 ) ; Page et al.(1982) ; Hesse (1971); Papanicolaou, (1976) and Jackson (1958). Soils were classified according to Soil Survey Staff, (2012).

*Satellite and ancillary data*

The Landsat 8 OLI image (acquisition date: 1 Mar 2015) obtained from the USGS EROS centre was used in this study. The OLI image consists of eleven spectral bands with a spatial resolution of 30 meters for bands 1 to 7 and 9. The resolution for band 8 (panchromatic) is 15 meters. The time of acquisition corresponded closely to the field trips, and occurred while the main cereal crop (wheat) was in the growing stages. Atmospheric correction for Landsat image was done using the FLAASH model (Perkins

et al. 2005) to correct both additive and multiplicative atmospheric effects. The corrected image was used to produce the following indices using ENVI 5 (Table 1) .

**Table 1:** Formulae of the vegetation indices.

| Index | Full name | Formula | References |
|---|---|---|---|
| SAVI | Soil-Adjusted Vegetation Index | $\frac{(1+L)(\rho_{NIR}-\rho_R)}{(\rho_{NIR}+\rho_R+L)}$ Low vegetation, L = 1, intermediate, 0.5, and high 0.25 | Huete (1988) |
| EVI | Enhanced Vegetation Index | $G=\frac{(\rho_{NIR}-\rho_R)}{(\rho_{NIR}+C1*\rho_R-C2*\rho_B+L)}$ $\rho_B$ = reflectance of blue band, G = 2.5, C1 = 6, C2 =7.5 and L = 1 | Huete et al. (1997) |
| GDVI | Generalized Difference Vegetation Index | $\frac{(\rho^n{}_{NIR}-\rho^n{}_R)}{(\rho^n{}_{NIR}+\rho^n{}_R)}$ n is power number, an integer of the values of 1, 2, 3, 4... n. | Wu (2012) |

Note: $\rho_{NIR}$ and $\rho_R$ are reflectance of the near infrared (NIR) and red (R) bands; $\rho_B$ and $\rho_{MIR}$ are respectively that of blue band and of the middle infrared band (like TM band 5)

Land suitability Evaluation:

All soil properties were evaluated for wheat production and suitability classes were determined according FAO , 2007 ( Equation 1) Table 2 ). Land suitability analysis and classes map for the study area were developed using ArcGIS .

$$\mathbf{I = A * \frac{B}{100} * \frac{C}{100} * \frac{D}{100} * \frac{E}{100} * \frac{F}{100} * \frac{G}{100} * \frac{H}{100} * \frac{J}{100} * \frac{K}{100} * \frac{L}{100} * \frac{M}{100}} \quad \text{.....[1]}$$

where :

I = suitability rating score
A = soil depth (cm)
B = soil texture
C = rock fragment
D = calcium Carbonate
E = CEC
F = PH
G = O.C.
H = EC
J = ESP
K = Slope %
L = Drainage
M= Gypsum

**Table 2** . Land Suitability classes for wheat production ( FAO, 2007 ).

| Index | Definition | Symbol |
|---|---|---|
| 80-100 | Highly Suitable | S1 |
| 60-80 | Moderately Suitable | S2 |
| 40-60 | Marginally Suitable | S3 |
| 25-40 | Currently not Suitable | N1 |
| 0-25 | Permanently not Suitable | N2 |

*Statistical Prediction Model*

A Pearson correlation analysis was applied to compute the best statistical regression models for wheat suitability using some vegetation indices included EVI, SAVI, and GDVI (Table 1). The prediction accuracy was verified by comparing the predicted and the measured values of the studied soil properties using the 35 randomly selected field observations. The results of the best fitting correlation ( Equation 1 ) was used to develop map for the pattern of spatial distribution for wheat suitability classes in the study area .

Map of the spatial distribution pattern of wheat suitability classes was developed using geostatistical analysis - ordinary Kriging model , according to Webster and Oliver , 2007 and using ArcGIS 10.1 .

**Results and Discussion**

*Soils of the study area*

The results of the physical and chemical properties of the studies soils indicated that the soils of the study area don't show some evidences for the formation of illuvial subsurface( Bt) and other horizons due to the effect of the dominant soil forming factors including dry climatic , high level of ground water and young calcareous parent materials. The results of physical and chemical soil properties revealed that the dominate soil types in the study area are Haplosalids and Torrifluvents. Most of the soils are affected by salt accumulation with low content of organic matter and available Nitrogen ( Table3).

**Table 3** . Some physical and chemical properties of the study area.

| Site | pH | $dS.m^{-1}$ | $g\ .kg^{-1}$ | | | Textural name | $g.kg^{-1}$ | | | $cmol_c.Kg^{-1}$ | ESP% | $mg.kg^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ECe | Sand | Silt | clay | | Lime | Gypsum | SOC | CEC | | T.N |
| P1 | 7.23 | 28.04 | 160.28 | 505.81 | 333.91 | SiCL | 227.57 | 1.45 | 3.15 | 26.11 | 32.25 | 30.09 |
| P2 | 7.57 | 6.14 | 366.79 | 425.21 | 208.00 | L | 201.51 | 0.47 | 5.79 | 23.17 | 12.00 | 44.90 |
| P3 | 7.02 | 41.23 | 116.45 | 510.52 | 373.03 | SiCL | 241.21 | 1.93 | 1.73 | 26.67 | 35.41 | 25.08 |
| P4 | 7.44 | 13.98 | 220.21 | 497.32 | 282.47 | CL | 208.90 | 0.78 | 4.78 | 24.68 | 24.38 | 34.87 |
| P5 | 7.56 | 5.64 | 387.27 | 406.31 | 206.42 | L | 200.50 | 0.46 | 5.93 | 23.20 | 11.19 | 45.54 |
| P6 | 7.60 | 4.18 | 348.64 | 493.30 | 158.06 | SiL | 201.37 | 0.42 | 6.13 | 22.90 | 6.90 | 48.65 |
| P7 | 7.25 | 27.00 | 163.28 | 508.30 | 328.41 | SiL | 227.06 | 1.39 | 3.19 | 26.00 | 31.83 | 28.38 |
| P8 | 7.04 | 40.13 | 65.01 | 558.97 | 376.02 | SiCL | 238.49 | 1.88 | 1.92 | 26.76 | 35.36 | 25.42 |
| P9 | 7.57 | 6.08 | 487.02 | 333.81 | 179.17 | L | 201.78 | 0.44 | 5.74 | 22.93 | 10.51 | 45.95 |
| P10 | 7.60 | 3.59 | 518.27 | 333.87 | 147.86 | SL | 199.35 | 0.40 | 6.05 | 22.43 | 5.05 | 49.90 |
| P11 | 7.59 | 4.35 | 325.29 | 514.00 | 160.71 | SiL | 199.81 | 0.42 | 6.03 | 22.72 | 7.36 | 48.29 |
| P12 | 7.59 | 3.30 | 494.32 | 363.20 | 142.48 | L | 199.10 | 0.39 | 6.09 | 22.30 | 3.89 | 50.66 |
| P13 | 7.19 | 29.83 | 154.43 | 505.91 | 339.66 | SiCL | 229.53 | 1.54 | 2.97 | 26.16 | 32.74 | 27.58 |
| P14 | 7.39 | 18.58 | 216.44 | 478.85 | 304.71 | CL | 216.10 | 1.01 | 4.33 | 25.36 | 27.75 | 32.15 |
| P15 | 7.08 | 36.59 | 117.93 | 514.35 | 367.72 | SiCL | 234.58 | 1.78 | 2.37 | 26.63 | 34.66 | 26.14 |
| A1 | 7.61 | 5.43 | 367.87 | 466.82 | 165.31 | L | 203.05 | 0.37 | 5.58 | 22.70 | 9.19 | 46.87 |
| A2 | 7.61 | 5.61 | 404.28 | 427.97 | 167.75 | L | 203.14 | 0.37 | 5.56 | 22.74 | 9.54 | 46.60 |
| A3 | 7.53 | 10.18 | 284.53 | 475.72 | 239.75 | L | 208.88 | 0.57 | 4.98 | 24.24 | 20.68 | 37.96 |
| A4 | 7.57 | 8.15 | 317.07 | 455.27 | 227.67 | L | 207.15 | 0.51 | 5.15 | 23.94 | 18.53 | 39.73 |
| A5 | 7.16 | 33.18 | 168.74 | 491.01 | 340.25 | SiCL | 235.39 | 1.48 | 2.27 | 26.16 | 33.22 | 26.77 |
| A6 | 7.48 | 13.68 | 215.73 | 513.69 | 270.58 | SiL | 212.55 | 0.69 | 4.60 | 24.72 | 24.04 | 35.13 |
| A7 | 7.59 | 7.12 | 364.80 | 414.90 | 220.30 | L | 204.43 | 0.42 | 5.44 | 23.25 | 13.47 | 43.69 |
| A8 | 7.60 | 6.65 | 294.32 | 490.07 | 215.61 | L | 204.13 | 0.42 | 5.46 | 23.15 | 12.70 | 44.27 |
| A9 | 7.62 | 5.15 | 384.07 | 453.56 | 162.37 | L | 202.89 | 0.37 | 5.59 | 22.63 | 8.61 | 47.29 |
| A10 | 6.91 | 49.51 | 27.58 | 594.01 | 378.41 | CL | 252.7 | 2.07 | 0.53 | 26.72 | 36.49 | 23.57 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A11 | 7.37 | 20.95 | 215.21 | 477.08 | 307.71 | CL | 220.48 | 0.97 | 3.8 | 25.4 | 28.55 | 31.18 |
| A12 | 7.32 | 23.97 | 162.48 | 522.40 | 315.12 | SiCL | 225.62 | 1.15 | 3.28 | 25.71 | 30.5 | 29.37 |
| A13 | 7.62 | 4.71 | 426.64 | 416.13 | 157.23 | L | 202.67 | 0.36 | 5.62 | 22.5 | 7.66 | 47.98 |
| A14 | 7.64 | 3.59 | 541.75 | 317.33 | 140.93 | SL | 202.08 | 0.34 | 5.67 | 22.13 | 4.54 | 50.13 |
| A15 | 7.62 | 4.20 | 363.68 | 486.56 | 149.76 | L | 202.39 | 0.36 | 5.64 | 22.35 | 6.36 | 48.87 |
| A16 | 7.62 | 4.59 | 328.63 | 516.00 | 155.37 | SiL | 202.62 | 0.36 | 5.62 | 22.47 | 7.38 | 48.15 |
| A17 | 7.52 | 11.36 | 242.69 | 511.42 | 245.89 | SiL | 209.90 | 0.60 | 4.88 | 24.38 | 21.75 | 37.08 |
| A18 | 7.59 | 6.84 | 311.37 | 471.09 | 217.54 | L | 204.24 | 0.42 | 5.45 | 23.19 | 12.97 | 44.04 |
| A19 | 7.43 | 17.31 | 204.15 | 501.19 | 294.66 | SiCL | 215.84 | 0.81 | 4.27 | 25.05 | 26.20 | 33.25 |
| A20 | 7.16 | 34.60 | 84.44 | 574.02 | 341.54 | SiCL | 236.91 | 1.54 | 2.13 | 26.22 | 33.60 | 26.44 |

Data of the weighted soil properties ( Table 3 ) were evaluated for wheat suitability using FAO,2007 and Sys et al.1993 ( Fable 4 ). The results show that salinity and ESP are the

most limiting factors for wheat suitability, while lime, SOC and drainage show slight or moderately effects on the evaluation of wheat suitability in the study area.

**Table 4.** Rating scores of soil properties of the study area for wheat suitability

| Site | Depth | Frag. | Tex. | Lime | CEC | pH | S.O.C. | ECe | ESP% | Slope | Drain. | Gyp. | Wheat Suit. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | Value | Class |
| P1 | 100.0 | 1.00 | 1.00 | 0.90 | 1.00 | 1.0 | 0.75 | 0.25 | 0.7 | 1.00 | 0.70 | 1.00 | 8.27 | N2 |
| P2 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 0.95 | 0.40 | 0.95 | 1.00 | 1.00 | 1.00 | 29.32 | N1 |
| P3 | 100.0 | 1.00 | 1.00 | 0.90 | 1.00 | 1.0 | 0.60 | 0.25 | 0.76 | 1.00 | 0.70 | 1.00 | 7.18 | N2 |
| P4 | 100.0 | 1.00 | 1.00 | 0.90 | 1.00 | 1.0 | 0.85 | 0.25 | 0.8 | 1.00 | 1.00 | 1.00 | 15.30 | N2 |
| P5 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 0.99 | 0.60 | 0.95 | 1.00 | 1.00 | 1.00 | 45.84 | S3 |
| P6 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 1.00 | 0.65 | 0.95 | 1.00 | 1.00 | 1.00 | 50.16 | S3 |
| P7 | 100.0 | 1.00 | 0.95 | 0.90 | 1.00 | 1.0 | 0.75 | 0.25 | 0.7 | 1.00 | 0.70 | 1.00 | 7.86 | N2 |
| P8 | 100.0 | 1.00 | 1.00 | 0.90 | 1.00 | 1.0 | 0.60 | 0.25 | 0.6 | 1.00 | 0.70 | 1.00 | 5.67 | N2 |
| P9 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 0.95 | 0.40 | 0.95 | 1.00 | 1.00 | 1.00 | 29.32 | N1 |
| P10 | 100.0 | 1.00 | 0.60 | 0.90 | 0.95 | 1.0 | 1.00 | 0.85 | 0.95 | 1.00 | 1.00 | 1.00 | 41.42 | S3 |
| P11 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 1.00 | 0.80 | 0.95 | 1.00 | 1.00 | 1.00 | 61.73 | S2 |
| P12 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 1.00 | 0.85 | 0.99 | 1.00 | 1.00 | 1.00 | 68.35 | S2 |
| P13 | 100.0 | 1.00 | 1.00 | 0.90 | 1.00 | 1.0 | 0.75 | 0.25 | 0.7 | 1.00 | 0.70 | 1.00 | 8.27 | N2 |
| P14 | 100.0 | 1.00 | 1.00 | 0.90 | 1.00 | 1.0 | 0.85 | 0.25 | 0.8 | 1.00 | 1.00 | 1.00 | 15.30 | N2 |
| P15 | 100.0 | 1.00 | 1.00 | 0.90 | 1.00 | 1.0 | 0.60 | 0.25 | 0.6 | 1.00 | 0.70 | 1.00 | 5.67 | N2 |
| A1 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 0.95 | 0.60 | 0.95 | 1.00 | 1.00 | 1.00 | 43.98 | S3 |
| A2 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 0.95 | 0.60 | 0.95 | 1.00 | 1.00 | 1.00 | 43.98 | S3 |
| A3 | 100.0 | 1.00 | 0.95 | 0.90 | 1.00 | 1.0 | 0.90 | 0.25 | 0.85 | 1.00 | 1.00 | 1.00 | 16.35 | N2 |
| A4 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 0.92 | 0.35 | 0.85 | 1.00 | 1.00 | 1.00 | 22.23 | N2 |
| A5 | 100.0 | 1.00 | 1.00 | 0.90 | 1.00 | 1.0 | 0.70 | 0.25 | 0.7 | 1.00 | 0.70 | 1.00 | 7.72 | N2 |
| A6 | 100.0 | 1.00 | 0.95 | 0.90 | 1.00 | 1.0 | 0.85 | 0.25 | 0.75 | 1.00 | 1.00 | 1.00 | 13.63 | N2 |
| A7 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 0.95 | 0.40 | 0.95 | 1.00 | 1.00 | 1.00 | 29.32 | N1 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A8 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 0.95 | 0.40 | 0.95 | 1.00 | 1.00 | 1.00 | 29.32 | N1 |
| A9 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 0.95 | 0.60 | 0.98 | 1.00 | 1.00 | 1.00 | 45.37 | S3 |
| A10 | 100.0 | 1.00 | 1.00 | 0.90 | 1.00 | 1.0 | 0.95 | 0.25 | 0.4 | 1.00 | 0.70 | 1.00 | 5.99 | N2 |
| A11 | 100.0 | 1.00 | 1.00 | 0.90 | 1.00 | 1.0 | 0.78 | 0.25 | 0.65 | 1.00 | 0.70 | 1.00 | 7.99 | N2 |
| A12 | 100.0 | 1.00 | 1.00 | 0.90 | 1.00 | 1.0 | 0.75 | 0.25 | 0.6 | 1.00 | 0.70 | 1.00 | 7.09 | N2 |
| A13 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 0.95 | 0.80 | 0.95 | 1.00 | 1.00 | 1.00 | 58.64 | S3 |
| A14 | 100.0 | 1.00 | 0.60 | 0.90 | 0.95 | 1.0 | 0.95 | 0.85 | 0.95 | 1.00 | 1.00 | 1.00 | 39.35 | N1 |
| A15 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 0.95 | 0.80 | 0.95 | 1.00 | 1.00 | 1.00 | 58.64 | S3 |
| A16 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 0.95 | 0.80 | 0.95 | 1.00 | 1.00 | 1.00 | 58.64 | S3 |
| A17 | 100.0 | 1.00 | 0.95 | 0.90 | 1.00 | 1.0 | 0.90 | 0.25 | 0.85 | 1.00 | 1.00 | 1.00 | 16.35 | N2 |
| A18 | 100.0 | 1.00 | 0.95 | 0.90 | 0.95 | 1.0 | 0.95 | 0.40 | 0.95 | 1.00 | 1.00 | 1.00 | 29.32 | N1 |
| A19 | 100.0 | 1.00 | 1.00 | 0.90 | 1.00 | 1.0 | 0.85 | 0.25 | 0.8 | 1.00 | 1.00 | 1.00 | 15.30 | N2 |
| A20 | 100.0 | 1.00 | 1.00 | 0.90 | 1.00 | 1.0 | 0.60 | 0.25 | 0.6 | 1.00 | 0.70 | 1.00 | 5.67 | N2 |

FAO, ( 2011) indicated that approximately 60% of the cultivated land in central Iraq has been seriously affected by salinity and 20-30% of the abandoned soils. Many researcher found that salinity and SOC are the most limiting factor for wheat production in central and southern Iraq ( Alshafi and Muhaimeed, 2012; Muhaimeed et al., 2015 ; Muhaimeed et al., 2016 ). The results shown in Table 4 indicated that soils of the study area are moderately to not suitable for wheat production and the dominant suitability classes found are S2 , S3 , N1 and N2 .

*Prediction of Wheat suitability*

The results of Pearson correlation analysis for computing the best statistical prediction model for wheat suitability using some vegetation indices included EVI, SAVI, and GDVI, indicated very high significant positive correlation ( $R^2 = 0.95$ ) between the actual suitability values ( Figure 2 ) and the predicted suitability values calculated using equation 1. The prediction model was used to developed the map of spatial distribution of wheat suitability in the study area( Figure 3 ) .The results shown in Figure 3 revealed the presence of four suitability classes including S2 , S3 ,N1 and N2. The percentage area for classes S2 , S3 , N1 and N2 were 12.28 , 21.69, 20.36 and 36.26 respectively . Map of the spatial distribution pattern shows that the non- suitable classes( N1 and N2 ) are located with the area of salt affected soils, while the moderately suitable classes( S2 and S3) are located within the area of non- salt affected soils.

The non- suitable classes are dominant and cover more than 56 % from the total area of the study area ( Figure 3 ). The domination of non – suitable classes are due to bad management practices as well as low content of SOC and total available nitrogen.

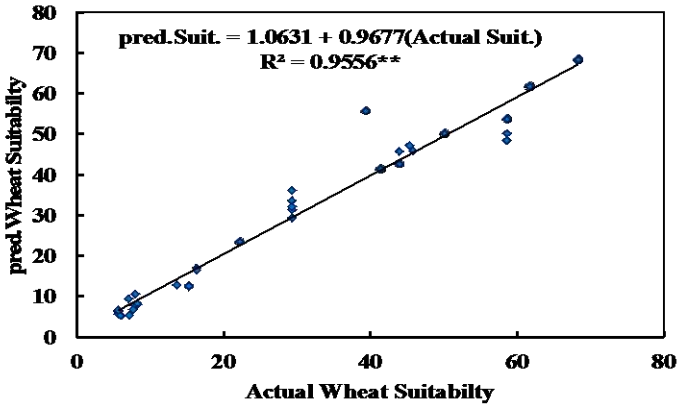


Figure 2 : The correlation between the actual and the predicted wheat suitability

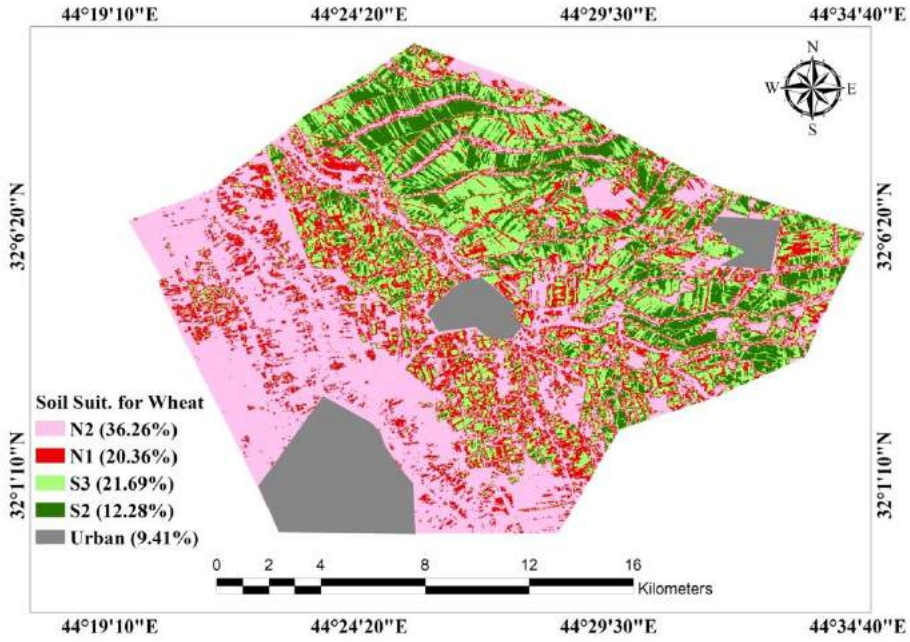


**Fig.3.** Spatial distribution of soil suitability classes for wheat production

The study area suffering from unsuitable irrigation and drainage systems as well as bad quality of irrigation water. These factors allow to increase the rate of salt accumulation in most of the soils in the study area.

Muhaimeed and Ali ,2016 indicated that land degradation which affects crop production in central Iraq is the result of complex interactions between physical, chemical, biological, socio-economical, and political issue of local and national conditions.

*Effect of SOC and TAN on Wheat suitability*

The results ( Table 3 and 4 ) show that the content of SOC and total available Nitrogen in most soils in the study area are low and have moderate effect on the degree of soil suitability for wheat production. This is reflected by the pattern of spatial distribution for SOC and TAN in the study area. Figure 4 was developed by using geostatistical analysis and ordinary Kriging model to show the relationship between spatial distribution of SOC content and wheat suitability of study area . The results ( Figure 5) revealed the presence of high positive correlation between soil suitability for wheat production and the content of SOC with $R^2$ =0.834. This results is due to the fact which indicated that soil organic matter is a major source of many nutrients including nitrogen used by crops and at any given time, a high portion of the potentially available nitrogen in the soil is in organic forms, either in plant and animal residues, in the relatively stable soil organic matter, or in living soil organisms, mainly microbes such as bacteria(Glass, 2003).

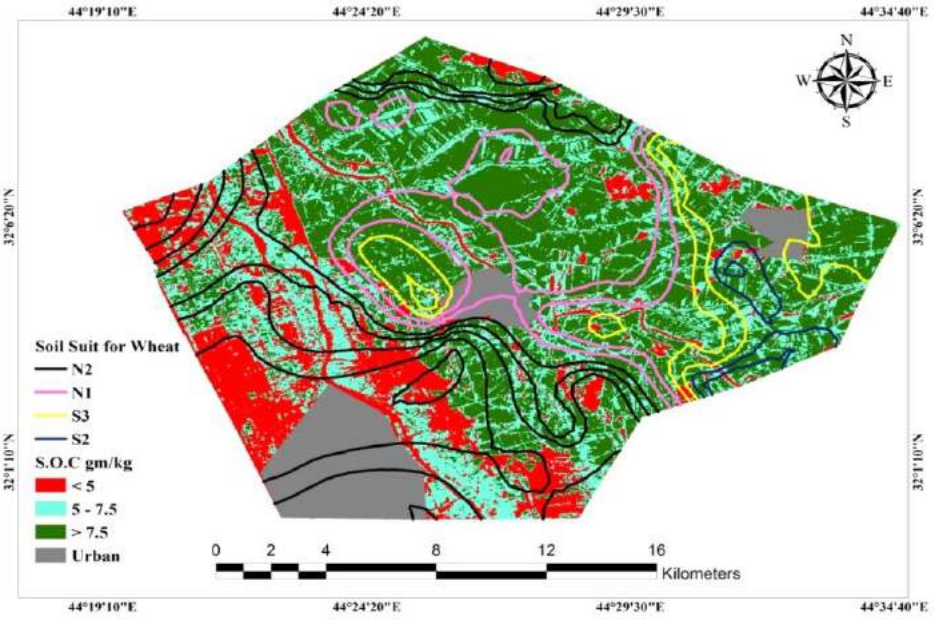


**Fig.4.** Spatial Distribution pattern for suitability classes and SOC in the study area.

Available total nitrogen in the studied soils show the same trend of correlation shown by SOC with soil suitability for wheat production. The results ( Figure 5) revealed high positive correlation between wheat suitability and TAN with $R^2$ = 0.957. This correlation was used to develop the spatial distribution map for both wheat suitability and TAN in the study area (

Figure 6 ). The results indicated a good correlation between the location of wheat suitability classes and the soil content from TAN , moderately suitable classes( S2 ,S3) located within area of moderately content of TAN, while unsuitable classes going parallel with low TAN content in the soil of the study area. This shows the importance of available nitrogen on plant growth. Nitrogen is an important nutrient in soil, a basic resource for maintaining the Earth's ecosystems, and a primary restrictive factor for crop production(Glass,2003).

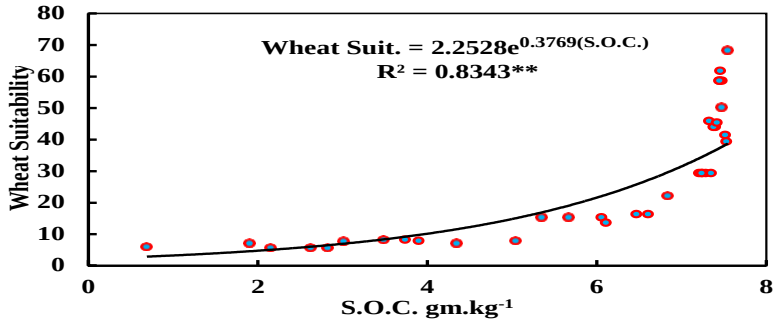


**Fig.5.** The correlation between wheat suitability score and SOC content

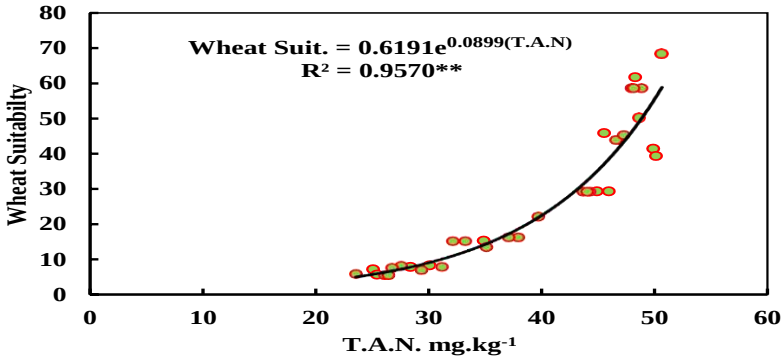


**Fig. 6 .** The correlation between wheat suitability score and TAN content

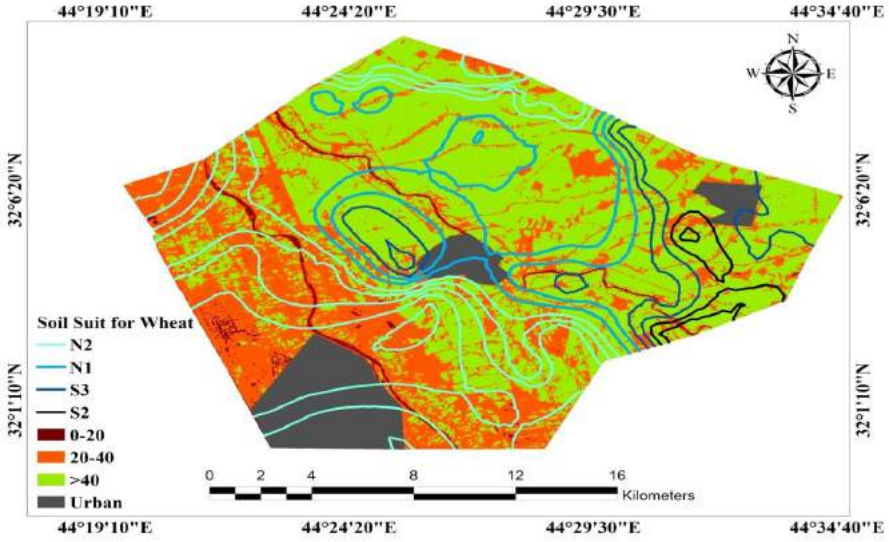


**Fig.7.** Spatial Distribution pattern for suitability classes and TAN in the study area

## Conclusions

Statistical prediction models used in this study for both SOC , TAN and soil suitability for wheat production are corresponded well with actual measured values of these attributes. Soil organic carbon and available nitrogen content showed high correlation with spatial distribution pattern for wheat suitability classes in the study area. These results revealed the important of SOC and TAN for plant growth and consequently for soil suitability for wheat production.

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# Risk Stratification and Clinical Eligibility of Invasive vs. Noninvasive Strategy in the Management of Non-ST Elevation Acute Coronary Syndrome Patients Admitted to Hawler Teaching Hospital

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**A B S T R A C T**

**Background and objectives:** The term acute coronary syndrome (ACS) is a range of acute myocardial ischemic states including unstable angina (UA), non-ST segment (NSTE), and ST-segment elevation myocardial infarction (STEMI). It is required to identify the cardiovascular events risk in those patients in order to select the beneficial therapeutic strategy during the first hours of presentation. The aim of the present study is to stratify the cardiac event risk and to determine the clinical eligibility of invasive vs. noninvasive therapeutic strategy in patients presented with UA and NSEMI as defined as NSTE-ACS.

**Methods**: In the current observational cross-sectional study, 100 consecutive patients who visited the emergency department and admitted to coronary care unit diagnosed as NSTE-ACS were recruited. Their medical and clinical information were obtained from the patient and medical records. The cardiac event risk was estimated according to the GRACE risk score (Global Registry of Acute Coronary Events) and the TIMI risk score (Thrombolysis in Myocardial Infarction). The clinical eligibility of invasive vs. noninvasive (Ischemia-guided) therapeutic strategy was determined according to the latest AHA/ACC guideline for the management of patients with NSTE-ACS in 2014.

**Results**: Of patients presented with NSTE-ACS who underwent both therapeutic strategies most of them were males (71.0%). The mean ± S.D. was 60-62 years for age. The mean ± S.D. of body mass index (BMI), chest pain duration, systolic blood pressure and heart rate were comparable in two groups. In terms of clinical characteristics hypertension (72.0%), current smoking (56.0%), dyslipidemia (48.0%), and past history of coronary artery disease (CAD) (44.0%) were the most prevalent. Patients who underwent invasive strategy had significantly more ST-depression (75.6%) as compared to those who underwent non-invasive strategy (55.9%), P=0.044. However, non-invasive group had significantly more T-wave inversion, (59.3% vs. 34.1%; P=0.013). The studied groups showed no statistically significant difference in risk of mortality according to GRACE risk score (P=0.505), TIMI risk score (P=0.057), and Killip class (P=0.252). However, the majority of non-invasive group had low risk (67.8%) while, the invasive group more common to be at intermediate risk (29.3%) for 6-month mortality. There was no statistically significant predictor of two therapeutic strategies according to the patients characteristics and risk stratification (p>0.05).

**Conclusions**: The current study showed that invasive and non-invasive (Ischemia-guided) strategies could be applicable to NSTE-ACS patients with low, intermediate, or high risk of mortality with risk stratification. It is recommended to underscore on significant and prevalent electrocardiographic findings, demographic and clinical characteristics to consider eligibility for invasive and non-invasive therapeutic strategies to patients with NSTE-ACS.

## 1. INTRODUCTION

The term acute coronary syndrome (ACS) is a range of acute myocardial ischemic states. It includes unstable angina (UA), non-ST segment myocardial infarction (Non-STEMI), and ST-segment elevation infarction (STEMI). Unstable angina defined as a clinical syndrome between stable angina and acute myocardial infarction (Grech *et al.,* 2003, Chang *et al.*, 2012).

Disruption in the atheromatous plaque is an initial stage of an acute coronary syndrome. Continuous fissuring or rupture in these plaques and subsequent exposure to materials such as lipid, foam cells, and smooth muscle result in the local generation of thrombin and fibrin deposition. Consequently, it facilitates platelet aggregation, adhesion, and intracoronary thrombus formation (Chang *et al.*, 2012).

Non-STE-ACS cover a clinical spectrum ranging from UA to non-STEMI (Fitchett *et al.*, 2006). Unstable angina and Non-STEMI are responsible for 2.5 million of hospital admissions across the world. It has been considered to be the main risk factor for morbidity and mortality in Western countries. In-hospital mortality and re-infarction account for 5-10% one month following an acute episode with a higher long-term mortality risk compared to the patients with STEMI (Bertrand *et al.,* 2000).

Unstable angina and Non-STEMI are closely associated medical conditions with similar clinical presentations making them indistinguishable. The distinction between them depends on severity sufficiency of ischemia to cause myocardial damage and release of detectable biomarkers quantities of myocyte necrosis. Cardiac troponin I and T are more specific and reliable biomarkers than creatinine kinase and its isoenzyme. The electrocardiogram (ECG) may show a normal or minor non-specific changes, ST segment depression, bundle branch block, T wave inversion, or transient ST-segment elevation and are resolved spontaneously or following giving nitrate (Braunwald *et al.*, 2002).

Preventing recurrent ACS and improving long-term outcomes are the management aims through selecting an appropriate treatment based on the risk estimation of an adverse outcome. Patients with NSTE-ACS do not adhere to the recommended treatments, and the risk stratification is not used by physicians to determine the appropriate treatment and access speed to coronary angiography (Fitchett *et al.*, 2006).

The risk stratification has its own importance as NSTE-ACS has a greater prevalence of early culprit coronary artery patency and a higher risk of recurrent ischemic events, and a wide range of therapeutic choices compared to STEMI.

Given to this wide range of therapeutic choices and the adverse events in NSTE-ACS, it is so important to choose management strategies with the greatest advantage to the patients. It is required to recognize those patients with the highest risk of cardiovascular events guiding the clinicians to select the patients who take benefit from invasive or noninvasive therapeutic interventions during the first hours of presentation.

**Aim of the study**

The aim of the present study is to stratify the cardiac event risk and to determine the clinical eligibility of invasive vs. noninvasive (Ischemia-guided) therapeutic strategy in patients presented with UA and NSEMI as defined as NSTE-ACS.

## 2. METHODS

### 2.1. Study design and sampling

In the current observational cross-sectional study, the patients were visited the emergency department of Hawler Teaching Hospital for the cardiac-based issues and consecutively screened for the eligibility criteria between 20$^{th}$ June and 25$^{th}$ December 2017.

The patients met eligibility criteria including both gender, aged 18 years and older, diagnosed with NSTE-ACS through the medical history, clinical examination, ECG and cardiac biomarker indicators, and risk factors were identified and the whole evaluation was supervised by a specialist or consultant. Pregnant women and patients with STEMI were not included in the study. Of the total 336 patients screened for the eligibility criteria, 143 were diagnosed with STEMI, 56 had no suitable health status (octogenarians and nonagenarians, frail patients, chronic kidney disease patients on replacement therapy and severely incapacitated patient with stroke), and 21 did not show their willingness to participate, 16 were pregnant, of those, 100 eligible patients were recruited in the present study.

All patients who diagnosed with NSTE-ACS at the corresponded department were managed with optimum medical therapy, including acetylsalicylic acid, P2Y12 inhibitors (mostly clopidogrel), statins, low molecular weight heparin and as appropriate with B-blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, insulin, nitrate and other anti-hypertensive or anti-ischemic medication and diuretics. Some of them were already on medications e.g. oral hypoglycemic agents (30.0%) and diuretics (22.0%).

### 2.2. Diagnostic and measurement criteria

The demographic data and clinical characteristics of the patients was obtained from the patients and their medical records. The diagnosis of NSTE-ACS was established based on the historical descriptors of symptoms such as chest pain and recurrent angina or dyspnea. The ECG was checked carefully for ST-segment depression, T-wave inversion, dynamic ST-T wave changes, and transient ST-segment elevation. Repeated sampling of cardiac biomarkers including troponins and CK-MB were performed in all patients.

### 2.3. Risk stratification

The cardiac event risk was estimated according to the GRACE risk score (Global Registry of Acute Coronary Events) and the TIMI risk score (Thrombolysis in Myocardial Infarction). The GRACE risk score is based on the age, Killip class, systolic blood pressure, ST-segment deviation, serum creatinine level, cardiac arrest, heart rate, and cardiac

biomarkers. A series of points are given to each of the above mentioned clinical variables. In this score system, patients scoring <109 points are considered for noninvasive (Ischemia-guided) therapeutic strategy and ≥109 are considered for invasive therapeutic strategy (including immediate, early or delayed), (Appendix 1) according to the latest AHA/ACC guideline for the management of patients with NSTE-ACS in 2014.

The TIMI risk score (Thrombolysis in Myocardial Infarction) is determined by the sum of the presence of 7 variables at admission; 1 point is given for each of the following variables: ≥65 y of age; ≥3 risk factors for CAD; prior coronary stenosis ≥50%; ST deviation on ECG; ≥2 angina events in prior 24 h; use of aspirin in prior 7 days and elevated cardiac biomarkers. In this score system, patients scoring 0-1 point are considered for noninvasive (Ischemia-guided) therapeutic strategy and ≥2 points are considered for invasive therapeutic strategy, (Appendix 2, 3) according to the latest AHA/ACC guideline for the management of patients with NSTE-ACS in 2014.

### 2.4. Statistical Methods

The descriptive purposes of the study were examined through the frequency distribution. A *p*-value of < 0.05 was considered as the statistically significant difference. The SPSS version 24 was used for statistical calculations. The Univariate analysis of variance was performed to determine the predictors of invasive and non-invasive strategies and chi-squared test for statistical difference between risk stratification according to GRACE and TIMI score systems.

### 2.5. Ethical considerations

The ethical clearance of the present study was obtained from the local corresponded department in Erbil Teaching Hospital in 2017. The patients have not undergone any medical intervention or invasive procedures unless consented and the written consent form was taken from all patients prior study implementation

## 3. RESULTS

Of the total patients recruited in this study, more than two third of them were males (71.0%). The mean age ± S.D. of the patients who underwent invasive strategy was 60.88 and non-invasive strategy was 62.36 years with no statistically significant difference (P=0.490). In addition, the patients mean ± S.D. in two groups were comparable in regard to body mass index (BMI) (P=0.053), chest pain duration (P=0.186), systolic blood pressure (P=0.302), and heart rate (P=0.621). In terms of clinical-based information of NSTE-ACS patients, the study revealed that hypertension (72.0%), current smoking (56.0%), dyslipidemia (48.0%), and past history of coronary artery disease (CAD) (44.0%) were the most prevalent characteristics followed by diabetes mellitus (31.0%), and renal failure (12.0%). The prevalence of family history of CAD was 22.0%. On the other hand this study showed no statistical significant difference between the patients in two groups in regard to demographic and parameters of clinical presentations (P>0.05) as shown in Table 1.

**Table 1: Demographic and clinical characteristics according to eligibility of invasive vs. non-invasive therapeutic strategy in NSTE-ACS patients**

| Demographic and clinical characteristics | NSTE-ACS patients (no. 100) | | | P-Value |
|---|---|---|---|---|
| | Invasive Strategy (Mean ± S.D.) | non-invasive Strategy (Mean ± S.D.) | | |
| **Age** (year) | 60.88 ± 10.51 | 62.36 ± 10.46 | | 0.490* |
| **Body Mass Index** (BMI) | 26.93 ± 4.29 | 25.44 ± 2.68 | | 0.053* |
| **Chest Pain Durarion** (hour) | 10.98 ± 12.67 | 14.91 ± 16.91 | | 0.186* |
| **Systolic Blood Pressure** | 151.13 ± 32.90 | 144.83 ± 23.82 | | 0.302* |
| **Heart Rate** | 80.15 ± 19.60 | 78.36 ± 14.62 | | 0.621* |
| | **Invasive Strategy no. 41 (%)** | **non-invasive Strategy no. 59 (%)** | **NSTE-ACS no. (%)** | **P-Value** |
| **Gender** | | | | 0.195** |
| Male | 32 (78.0) | 39 (66.1) | 71 (71) | |
| Female | 9 (22.0) | 20 (33.9) | 29 (29) | |
| **Smoking** | | | | 0.984** |
| Current smoker | **24 (56.1)** | **32 (56.1)** | **56 (56)** | |
| Ex-smoker | 7 (14.6) | 9 (15.8) | 16 (16) | |
| Never smoker | 12 (29.3) | 16 (28.1) | 28 (28) | |
| **Refractory Angina** | | | | 0.351** |
| Yes | 6 (14.6) | 5 (8.5) | 11 (11) | |
| No | 35 (85.4) | 54 (91.5) | 89 (89) | |
| **Shortness of Breath** | | | | 0.456** |
| Yes | 13 (31.7) | 23 (39.0) | 36 (36) | |
| No | 28 (68.3) | 36 (61.0) | 64 (64) | |
| **Diabetes Mellitus** | | | | 0.899** |
| Yes | 13 (31.7) | 18 (30.5) | 31 (31) | |
| No | 28 (68.3) | 41 (69.5) | 69 (69) | |

| | | | | |
|---|---|---|---|---|
| **Hypertension** | | | | 0.261** |
| Yes | **32 (78.0)** | **40 (67.8)** | **72 (72)** | |
| No | 9 (22.0) | 19 (32.2) | 28 (28) | |
| **Family History of CAD** | | | | 0.051** |
| Yes | 13 (31.7) | 9 (15.3) | 22 (22) | |
| No | 28 (68.3) | 50 (84.7) | 78 (78) | |
| **Dyslipidemia** | | | | 0.896** |
| Yes | **20 (48.8)** | **28 (47.5)** | **48 (48)** | |
| No | 21 (51.2) | 31 (52.5) | 52 (52) | |
| **Renal failure** | | | | 0.222** |
| Yes | 7 (17.1) | 5 (8.5) | 12 (12) | |
| No | 34 (82.9) | 54 (91.5) | 88 (91) | |
| **Past History of CAD** | | | | 0.422** |
| Yes | **20 (48.8)** | **24 (40.7)** | **44 (44)** | |
| No | 21 (51.2) | 35 (59.3) | 56 (56) | |
| **(PCI)** | | | | 0.302** |
| Yes | 3 (7.3) | 1 (1.7) | 4 (4) | |
| No | 38 (92.7) | 58 (98.3) | 96 (96) | |
| **Previous (CABG)** | | | | |
| Yes | 0 (0.0) | 0 (0.0) | 0 (0.0) | - |
| No | 41 (100) | 59 (100) | 100 (100) | - |

*Independent t-test, ** Chi-squared, and Fishers' exact tests were performed for statistical analyses.

The bold numbers show the higher prevalence. NSTE-ACS = Non ST Elevation-Acute Coronary Syndrome; CAD =

Coronary Artery Disease; CABG = Coronary Artery Bypass Graft Surgery; PCI = Percutaneous Coronary Intervention.

The patients who underwent invasive strategy had significantly more ST-depression (75.6%) as compared to those who underwent non-invasive strategy (55.9%), P=0.044. on the other hand, the non-invasive group of the study had significantly more T-wave inversion, (59.3% vs. 34.1%; P=0.013). The other Electrocardiographic and Echocardiographic features were not statistically significant between both groups of the study, as shown in Table 2.

**Table 2: Electrocardiographic and Echocardiographic findings according to eligibility of invasive vs. non-invasive therapeutic strategy in NSTE-ACS patients**

| Electrocardiographic and Echocardiographic findings | NSTE-ACS patients (no. 100) | | P-Value |
|---|---|---|---|
| | Invasive Strategy no. 41 (%) | non-invasive Strategy no. 59 (%) | |
| **ST depression** | | | **0.044*** |
| Yes | 31 (75.6) | 33 (55.9) | |
| No | 10 (24.4) | 26 (44.1) | |
| **T-wave inversion** | | | **0.013*** |
| Yes | 14 (34.1) | 35 (59.3) | |
| No | 27 (65.9) | 24 (40.7) | |
| **Dynamic ST-T changes** | | | 1.000** |
| Yes | 2 (4.9) | 2 (3.4) | |
| No | 39 (95.1) | 57 (96.6) | |
| **Ventricular fibrillation (VF)** | | | 0.066** |
| Yes | 3 (7.3) | 0 (0.0) | |
| No | 38 (92.7) | 59 (100.0) | |
| **Ventricular tachycardia (VT)** | | | 0.166** |
| Yes | 2 (4.9) | 0 (0.0) | |
| No | 39 (95.1) | 59 (100) | |
| **Cardiac Arrest** | | | 0.142** |
| Yes | 0 (0.0) | 4 (6.8) | |
| No | 41 (100) | 55 (93.2) | |
| LVEF (%) – Mean ± S.D. | 50.73 ± 11.350 | 52.44 ± 10.927 | 0.456*** |

The numbers are in frequency (percentage) except where mentioned.

*Chi-squared, ** Fishers' exact, and *** independent t-tests were performed for statistical analyses. The bold numbers show the significant difference. NSTE-ACS = Non ST Elevation-Acute Coronary Syndrome;

The differences of lab investigations were examined in Table 3. The study showed that the patients in both invasive and non-invasive groups had comparable values of serum creatinine (P=0.827), troponin (P=0.085), and estimated glomerular filtration rate (eGFR) (P=0.168) and revealed no statistical significance.

**Table 3: Laboratory findings according to eligibility of invasive vs. non-invasive therapeutic strategy in NSTE-ACS patients**

| Laboratory Findings | NSTE-ACS patients (no. 100) | | P-Value |
|---|---|---|---|
| | Invasive Strategy no. 41 (%) | non-invasive Strategy no. 59 (%) | |
| **Serum Creatinine** (mg/dL) | 1.06 ± 0.46 | 1.09 ± 0.54 | 0.827 |
| **Troponin** (ng/mL) | 1.60 ± 3.10 | 0.69 ± 1.27 | 0.085 |
| **eGFR** (ml/min) | 87.60 ± 48.44 | 75.35 ± 33.31 | 0.168 |

NSTE-ACS = Non ST Elevation-Acute Coronary Syndrome; eGFR = estimated Glomerular Filtration Rate.

Furthermore, the study did not show the patients who underwent invasive and non-invasive therapeutic strategies were more at risk of mortality according to GRACE risk score (P=0.505), TIMI risk score (P=0.057), and Killip class (P=0.252), as shown in Table 4.

**Table 4: Risk stratification by GRACE, TIMI risk scores and Killip Class according to eligibility of invasive vs. non-invasive therapeutic strategy in NSTE-ACS patients**

| Risk stratification | NSTE-ACS patients (no. 100) | | P-Value |
|---|---|---|---|
| | Invasive Strategy no. 41 (%) | non-invasive Strategy no. 59 (%) | |
| **GRACE Risk Score** | 105.40 ± 31.71 | 100.97 ± 33.43 | 0.505* |
| **TIMI Risk Score** | 4.43 ± 1.68 | 3.75 ± 1.76 | 0.057* |
| **Killip Class** | | | 0.252** |
| **1** | 27 (65.9) | 43 (72.9) | |
| **2** | 11 (26.8) | 12 (20.3) | |
| **3** | 1 (2.4) | 4 (6.8) | |
| **4** | 2 (4.9) | 0 (0.0) | |

*Independent t-test and ** Fishers' Exact test were performed for statistical analyses.

NSTE-ACS = Non ST Elevation-Acute Coronary Syndrome; GRACE = Global Registry of Acute Coronary Events; TIMI = Thrombolysis in Myocardial Infarction.

Moreover, the mortality risk in patients who underwent invasive or non-invasive strategies was examined according to GRACE risk categories in Table 5. The study did not show the one group is more at risk of mortality compared to other one (P=0.358).

**Table 5: Risk stratification by 3-class of severity (low to very high) according to eligibility of invasive vs. non-invasive therapeutic strategy in NSTE-ACS patients**

| GRACE Categories | NSTE-ACS patients (no. 100) | | | P-Value |
|---|---|---|---|---|
| | Invasive Strategy no. 41 (%) | non-invasive Strategy no. 59 (%) | NSTE-ACS no. (%) | |
| **Low Risk** (GRACE <109) | 22 (53.7) | 40 (67.8) | 62 (62) | 0.358* |
| **Moderate Risk** (GRACE 109-140) | 12 (29.3) | 12 (20.3) | 24 (24) | |
| **High Risk** (GRACE >140) | 7 (17.1) | 7 (11.9) | 14 (14) | |

*Independent t-test was performed for statistical analysis.

NSTE-ACS = Non ST Elevation-Acute Coronary Syndrome; GRACE = Global Registry of Acute Coronary Events.

Upon univariate logistic regression application on NSTE-ACS patients, the invasive or non-invasive strategy was considered as a dependent variable and its association with gender, diabetes, hypertension, dyslipidemia, age and left ventricular ejection fraction (LVEF) as predictors were examined in Table 6. The study did not show that the mentioned characteristics are the predictors of strategic types.

**Table 6: Univariate logistic regression analysis between invasive therapeutic strategy eligibility**

| Dependent Variable: Invasive Strategy Eligibility | | | | |
|---|---|---|---|---|
| **Predictors** | **Mean Square** | **F** | **Significance** | **Partial Eta Squared*** |
| **Gender** | 0.887 | 3.786 | 0.056 | 0.059 |
| **Diabetes Mellitus** | 0.004 | 0.017 | 0.898 | 0.000 |
| **Hypertension** | 0.031 | 0.130 | 0.719 | 0.002 |

| | | | | |
|---|---|---|---|---|
| **Dyslipidemia** | 0.037 | 0.159 | 0.691 | 0.003 |
| **Age** | 0.274 | 1.169 | 0.295 | 0.391 |
| **\Left ventricular ejection fraction** (LVEF) | 0.012 | 0.051 | 0.822 | 0.001 |
| * Partial eta-squared: is a measure of effect size that express the amount of variance accounted for by one or more independent variables | | | | |

## 4. Discussion

The present study conducted on NSTE-ACS patients showed that those who underwent invasive and non-invasive treatments were comparable in demographic, clinical characteristics and lab investigations. In addition, they were comparable in echocardiographic findings. The electrocardiographic changes revealed more prevalence of ST depression in invasive group and more T-wave inversion in non-invasive arm of the study. Despite these parameters, patients in both groups did not disclose a significant more risk of mortality according to the GRACE, TIMI risk scores, and Killip's class systems of stratification . The study did not show that gender, diabetes, hypertension, dyslipidemia, age and LVEF are the predictors of strategic types.

The majority of NSTE-ACS patients in this study were managed by both the internists and cardiologists that may reflect the importance of the establishment and implementation of the treatment guidelines in the form of clinical plans or algorithms to guide the clinicians for optimal therapeutic interventions. Risk assessment has been shown to be a dynamic and continuous process aiming to identify new higher risk features within the early presentation hours assisting the clinicians to implement different management strategies for NSTE-ACS patients.

According to the earlier Europe and North American registries, the treatment of patients with NSTE-ACS is not based on the current guidelines (Eagle *et al.,* 2002, Fox *et al.,* 2002, Steg *et al.,* 2002). For instance, patients with lower risk are undergoing cardiac catheterization more often at an earlier time than higher-risk patients (Bhatt *et al.,* 2004).

Clinical conditions of the patients with NSTE-ACS are quite complicated and have a high mortality risk and recurrent myocardial infarction. Therefore, it is important to manage the patients according to evidence-based strategies.

The current study identified the majority of the patients with a low risk according to the GRACE risk stratification system underwent

noninvasive management. The stratification risk is highly important to determine the patients with a severe ischemic episode, left ventricular dysfunction, and severe and/or extensive proximal coronary stenosis. In addition, simultaneous and multiple plaque rupture within several coronary arteries has been shown to be common in ACS patients (Goldstein *et al.,* 2000, Roffi *et al.,* 2016).

The study showed that 53.7% and 67.8% of the patients in invasive and non-invasive treatments, respectively had a low risk of mortality according to the GRACE system. These patients require careful and ongoing observation and further medical testing to determine that they are not at higher risk features. These patients must be considered to be low risks until confirmation by serial ECG and troponin level over a time scheduled period. Importantly, the patients with negative serial biomarkers and no ECG ST-segment change may still be located as higher risk (Sanchez *et al.,* 2004) because the sensitivity of both troponin I and T is 60% at this time and it is increased over 14 to 18 hours of presentation (Zimmerman *et al.,* 1999).

The patients with a high mortality risk were 17.1% and 11.9% in invasive and non-invasive treatment groups, respectively in the present study. These patients must be under continuous medical care to avoid the worse conditions with features of higher risk. Ongoing medical care must be continued until stabilization of higher risk features with conservative therapy as the mean age of the patients in the study is relatively high. These patients take a great benefit from a more aggressive antiplatelet and antithrombotic regimen and an early invasive strategy than the patients with a lower risk (Hoedemaker *et al.,* 2017, Tubaro *et al.,* 2017). Although we could not find any predictor in this regard, this finding could back to the patient clinical heterogeneity rather than other characteristics. Hypotension, tachycardia, elevated troponin, dynamic ST-segment changes, frequent episode of ischemia, and refractory have been documented to be high-risk predictors by clinical trials (Eagle *et al.,* 2004, Boersma *et al.,* 2000). Therefore, the decision must be taken according to the signs and symptoms of the ACS likelihood if these medical tests are not available at this period of time.

The patients with “background risk” such as known CAD, diabetes mellitus, or renal failure or having very typical ischemic cardiac symptoms, even with no high-risk changes in ECG abnormalities and lower levels of troponin must be considered for admission for further observations (Fitchett *et al.,* 2006).

## 5. Limitations of the study

The findings reported in the present study must be analyzed with caution owing to its design and sample size. The patients recruited in the present study have been selected from one geographic location precluding the study to generalize to other regions or across the country.

## 6. Conclusions

The present study showed that hypertension, current smoking, dyslipidemia, and past history of CAD were the most prevalent features among patients with NSTE-ACS. Those who underwent invasive strategy had significantly more ST-depression. However, those eligible for non-invasive strategy had significantly more T-wave inversion. There was no statistically significant difference between invasive and non-invasive strategies applied according to risk stratification with GRACE, TIMI risk scores and Killip class. The studied groups show no statistically significant difference in the risk of mortality. However, the majority of non-invasive group was at low risk as compared to invasive group who was common to be at intermediate risk for 6-month mortality. There was no statistically significant predictor of the two therapeutic strategies according to patient characteristics and risk stratification as well.

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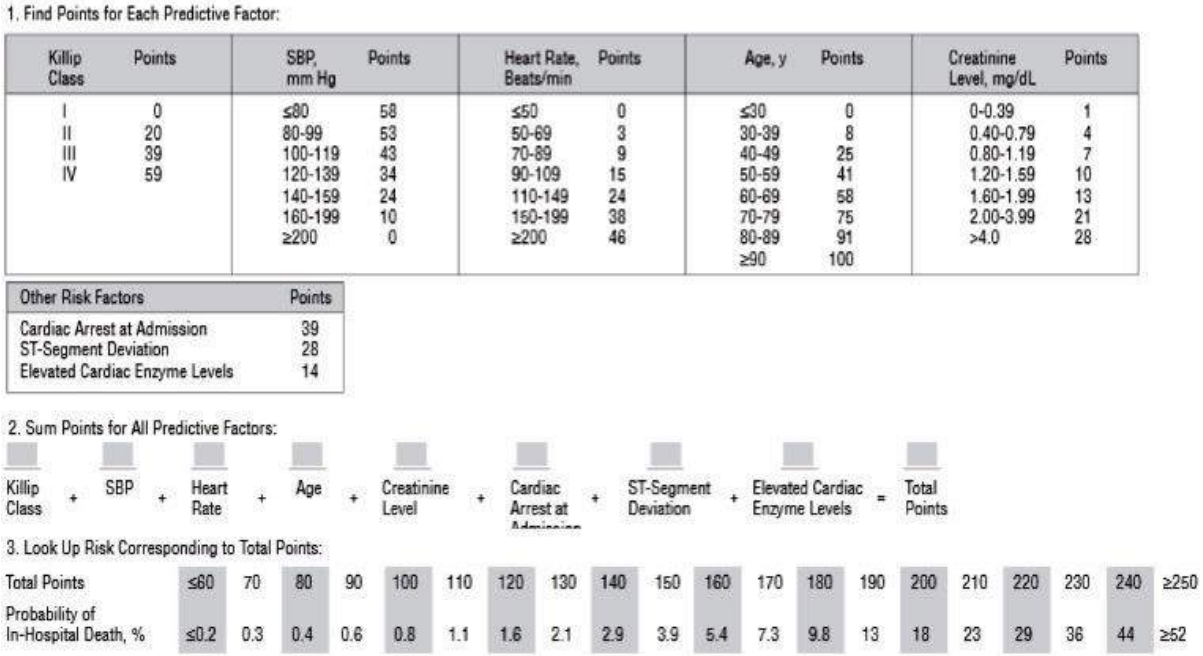
**Appendix 1**: GRACE risk score.
(AHA/ACC guideline for the management of patients with NSTE-ACS 2014).



1. Find Points for Each Predictive Factor:

| Killip Class | Points | SBP, mm Hg | Points | Heart Rate, Beats/min | Points | Age, y | Points | Creatinine Level, mg/dL | Points |
|---|---|---|---|---|---|---|---|---|---|
| I | 0 | ≤80 | 58 | ≤50 | 0 | ≤30 | 0 | 0-0.39 | 1 |
| II | 20 | 80-99 | 53 | 50-69 | 3 | 30-39 | 8 | 0.40-0.79 | 4 |
| III | 39 | 100-119 | 43 | 70-89 | 9 | 40-49 | 25 | 0.80-1.19 | 7 |
| IV | 59 | 120-139 | 34 | 90-109 | 15 | 50-59 | 41 | 1.20-1.59 | 10 |
| | | 140-159 | 24 | 110-149 | 24 | 60-69 | 58 | 1.60-1.99 | 13 |
| | | 160-199 | 10 | 150-199 | 38 | 70-79 | 75 | 2.00-3.99 | 21 |
| | | ≥200 | 0 | ≥200 | 46 | 80-89 | 91 | >4.0 | 28 |
| | | | | | | ≥90 | 100 | | |

| Other Risk Factors | Points |
|---|---|
| Cardiac Arrest at Admission | 39 |
| ST-Segment Deviation | 28 |
| Elevated Cardiac Enzyme Levels | 14 |

2. Sum Points for All Predictive Factors:

Killip Class + SBP + Heart Rate + Age + Creatinine Level + Cardiac Arrest at Admission + ST-Segment Deviation + Elevated Cardiac Enzyme Levels = Total Points

3. Look Up Risk Corresponding to Total Points:

| Total Points | ≤60 | 70 | 80 | 90 | 100 | 110 | 120 | 130 | 140 | 150 | 160 | 170 | 180 | 190 | 200 | 210 | 220 | 230 | 240 | ≥250 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Probability of In-Hospital Death, % | ≤0.2 | 0.3 | 0.4 | 0.6 | 0.8 | 1.1 | 1.6 | 2.1 | 2.9 | 3.9 | 5.4 | 7.3 | 9.8 | 13 | 18 | 23 | 29 | 36 | 44 | ≥52 |

**Appendix 2:** TIMI Risk Score* for NSTE-ACS

| TIMI Risk Score | All-Cause Mortality, New or Recurrent MI, or Severe Recurrent Ischemia Requiring Urgent Revascularization Through 14 d After Randomization, % |
|---|---|
| 0–1 | 4.7 |
| 2 | 8.3 |
| 3 | 13.2 |
| 4 | 19.9 |
| 5 | 26.2 |
| 6–7 | 40.9 |

*The TIMI risk score is determined by the sum of the presence of 7 variables at admission; 1 point is given for each of the following variables: ≥65 y of age; ≥3 risk factors for CAD; prior coronary stenosis ≥50%; ST deviation on ECG; ≥2 angina events in prior 24 h; use of aspirin in prior 7 d; and elevated cardiac biomarkers.

**Appendix 3:** Factors Associated With Appropriate Selection of Early Invasive Strategy or Ischemia-Guided Strategy in Patients with NSTE-ACS.
(2014 AHA/ACC Guideline for the management of patients with NSTE-ACS)

| | |
|---|---|
| Ischemia-guided strategy | Low-risk score (e.g., TIMI [0 or 1], GRACE [<109]) |
| | Low-risk Tn-negative female patients |
| | Patient or clinician preference in the absence of high-risk features |
| Immediate invasive (within 2 h) | Refractory angina |
| | Signs or symptoms of HF or new or worsening mitral regurgitation |
| | Hemodynamic instability |
| | Recurrent angina or ischemia at rest or with low-level activities despite intensive medical therapy |
| | Sustained VT or VF |
| Early invasive (within 24 h) | None of the above, but GRACE risk score >140 |
| | Temporal change in Tn (Section 3.4) |
| | New or presumably new ST depression |
| Delayed invasive (within 25–72 h) | None of the above but diabetes mellitus |
| | Renal insufficiency (GFR <60 mL/min/1.73 $m^2$) |
| | Reduced LV systolic function (EF <0.40) |
| | Early post-infarction angina |
| | PCI within 6 month |
| | Prior CABG |
| | GRACE risk score 109–140; TIMI score ≥2 |

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# Effect of Salicylic and Ascorbic acid on Growth, Green yield of two Broad bean Cultivars (*Vicia faba* L.)

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## ABSTRACT

The experiment was conducted in the vegetable research farm of Horticulture Department, College of Agriculture/ University of Dohuk- Iraq, interior the plan of Broad bean crop production during the growing season of 2016-2017, to investigate the effects of two Cultivars (Aguadolce & Elisa), Salicylic acid and Ascorbic acid at concentration (0, 100 & 150 $g.L^{-1}$ ) on growth, green yield and green seed yield of Broad bean (*Vicia faba*.). Results showed that the cultivars had no affect on vegetative characters. The Elisa cultivar had significant increase on some majority green yield and seed yield characters (Pod Yield g. $plant^{-1}$, Total Yield ton. $donum^{-1}$, No. of seed per pod, seed weight g and weight of 100 seed g).While Aguadolce cultivar had significant increase in (pod length cm). There were no significant effect of cultivars on (No. of pods per plant $pod.plant^{-1}$, Pods weight g, No of seeds per plant, green seed yield $g.plant^{-1}$ and total seed yield ton. $donum^{-1}$). But ascorbic acid significant increased (No. of pods per plant $pod.plant^{-1}$, No. of seed per pod, seed weight g, weight of 100 seed g Green Seed yield $g.plant^{-1}$ and total seed yield ton. $donum^{-1}$) ), While treating of broad bean plant with salicylic acid especially ($100g.L^{-1}$) led to significant increases (pods weight g, No. of branches per plant, Pods weight g, Pod Yield g. $plant^{-1}$, Total Yield ton. $donum^{-1}$, No. of seed per pod, seed weight g, weight of 100 seed g, Green Seed yield $g.plant^{-1}$ and total seed yield ton. $donum^{-1}$).

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## 1. INTRODUCTION

Legumes are the major direct source of proteins for both man and livestock, especially in poor countries where animals protein is expensive (Mohamed, 2010). Field bean (*Vicia faba* L.) is one of essential winter crops in Iraq due to its high nutritive value and high protein contents (25-42%) (Fouad *et al*.,1995). Moreover, it is a good source of nutritive minerals, such as phosphorus, potassium, calcium, sulphur and iron. Its seed produced a cheap source of protein and food of high nutritive value especially in the diet of low-income people. Its protein is a good alternative compared with expensive meat and fish protein (Chavan *et al.,* 1989).

The productivity of the plant can be increased by using some chemical compounds, including salicylic acid and ascorbic acid, ascorbic acid has an important role as an antioxidant in the plant and has a role in the protection of plants from optical oxidation (Foyer, 1993). Ascorbic acid promotes vegetative growth and emulsification and that the external addition of these compounds reduces the various stresses on the plant. It also plays an important role in controls cell growth, the elongation and division of cells, have a role in plant resistance to stress conditions such a slow and high temperature and salt stress (Smirnoff, 1996).

Salicylic acid is a plant hormone that has important physiological roles in promoting plant growth and increasing the efficiency of photosynthesis and flowering (Hayat *et al.*, 2007). The word salicylic acid (SA) was derived from Latin word Salix, meaning willow tree, it plays an important role in plant tolerance to stresses from low temperatures (Tasgin *et al*., 2003) and high heat and salinity (Khan *et al.* , 2010). Bassiouny *et al.*(2005) reported that spray the bean plants after 45 and 60 days of transplanting with ascorbic acid concentrations

of 100 or 200 or 400 mg. $L^{-1}$ , has given a significant increase in carbohydrates, protein, potassium, phosphorus and calcium in dry seeds and the number of pods $plants^{-1}$ and the number of seeds $plant^{-1}$ and weight of 100 seeds compared to untreated plants. Burguieres *et al*. (2007) reported that the treatment of pea seeds with ascorbic acid resulted in a significant increase in germination rate and weight of vegetative, plant height and root length. Azooz and Al-Fredan (2009) found that the effect of seed soaking in ascorbic acid and spraying of bean plants in the salicylic acid with different concentration (0, 10, 20) and ascorbic acid at a concentration of 100 mg $L^{-1}$, noted that treatment with ascorbic acid improved the growth of plants, seed germination and fresh weight of the vegetative and root groups. Younis *et al.* (2010) found that the treatment of ascorbic acid at a concentration of 4 ml under saline conditions led to an increase in plant growth and yield.

AL-Amri (2017) showed significant increased in plant height and leaf area due to ascorbic acid application on snap bean plants. Khan *et al.,* (2003) reported that treatment with acetylsalicylic acid ($10^{-5}$ or $10^{-4}$ ml/L) resulted in improved photosynthesis in soybean plant, increased sporulation, and dry leaf area and weight, but did not affect plant height. Hegazi and El-Shraiy (2007) found that when spraying the bean plants at 30 and 45 days with salicylic acid at concentrations of as above, the treatment with salicylic acid at a concentration of as above $ml.L^{-1}$ increased the height of the plant. Murtaza *et al.* (2007) that the application of SA at $10^{-4}ml.L^{-1}$ to pea plants significantly increased the yield and its components as compared to concentration $10^{-5}ml.L^{-1}$tratmenta.. El-Shraiy and Hegazi (2009) observed spraying the pea with acetylsalicylic acid with concentrations of 10 and 20 mg $.L^{-1}$, increased the vegetable growth of the

at 45 and 60 days after sowing, length of internodes and number of nodes at last picking, weight of pods per plant whereas Ascorbic acid 200 ppm has given best results for yield per plot and yield per hectare. The use of natural substances like (salicylic and ascorbic acid) has improved and increased plant growth. Due to the positive role of both salicylic acid and ascorbic acids on growth and yield of bean plants and the lack of research on their role in the growth and yield of the plant under the local conditions of Duhok reign. The present investigation aimed to study the effect of foliar applications with two antioxidants, i.e. ascorbic acid and salicylic acid on growth, green pods and yields of bean plant.

## 2. MATERIALS AND METHODS

150 g.L-1) in sub sub-plot and was arranged in a randomized complete block design with three replications. Each treatment included eight plants in rows. Spraying plants by two acids done three times, the first one added 45days after planting and another's one at ten day between them . A few drops of Tween 20 were added as a diffusion agent for the spray treatments. The plants were sprayed on the early morning till full wet with a 2 liter hand sprayer. The results were analyzed using the SAS, 2007 program. Means values were compared using Duncan's multiple range test at 0.05% level (AL-Rawi and Khalaf Alah, 2000). The data were taken from five plants and yield taken from ten harvesting. Data were recorded for plant high, No. of branches/plant, No. of pods per plant, pod length cm, pods weight g, pod yield g. plant$^{-1}$ , total yield ton. donum$^{-1}$, No. of seeds per pod, No. of green seeds plant$^{-1}$, fresh seed weight (mg), weight of 100 seed (g), green seed yield plant (g), total green seed yield ton. donum$^{-1}$.

## 3. RESULTS

### 3.1.Vegetative Growth Characters:-

#### 3.1.1.Plant High.

on plant length, while significant differences had been observed on plant length when sprayed with

plant, its height, number of leaves, fresh and dry weight, plant yield, pod length, number of pods per plant, number of seeds in pods. Khafaga *et al*. (2009) reported that parameters of faba bean growth (number of branches, fresh and dry weight and leaf area)were significantly increased when seeds wee soaked in salicylic acid (200 ppm) as compared with the control plants. Meanwhile, SA as foliar application significantly increase all yield and yield components (number of pods/plant, number of seeds/pod, seeds weight/plant, pods weight/plant, 100 seed weight and seed yield). Thomson *et al.* (2017) revealed that the antioxidant acetyl salicylic acid 200 ppm effectively increased the vine length of pea plant

The experiment was conducted in the Vegetable Research Farm, Horticultural Department, College of Agriculture, University of Duhok, Kurdistan region/Iraq, during the growing season of 2014-2015. To study the effect of salicylic and ascorbic acid on growth and green yield of two broad bean cultivars. The two cultivars (Elsa and Aguadolce) were planted on Nov. 14th 2015. Seed planting was achieved on both sides of ridges at 25 cm between hills and 75cm between ridges. Add urea fertilizer (46% N) at a rate of 30 kg. dunum$^{-1}$ in a ditch way after a month of cultivation. All the agricultural processes used in the production of this crop were carried out like irrigation, weeding. Irrigation was carried out according to the need of the plant. The process of weeding was carried out by hand by hand, whenever necessary. The experiment was conducted in split split plot design the two cultivars (Aguadolce and Elisa) in main plot and the two acids in sub plots the salicylic acid and ascorbic acid and three concentration (0,100 and

Data in table (1) shows that no significant differences occurred for each cultivars and acids

differences occurred between ascorbic acids and two highest concentration (100 and 150 g. $L^{-1}$ ) reached (5.32, 5.58 cm) respectively. The triple interaction between three factors revealed that significant differences occurred and the highest value was (5.67cm) between Aguadolce cultivars, ascorbic acids and 150g. $L^{-1}$ concentration.

concentrations the highest plant high (5.37cm) in 150 g. $L^{-1}$ concentration. Effect of interaction between cultivars and acids had no significant differences occurred, while significant differences found between cultivars and concentration of acids the highest value obtained between Elisa cultivars and 150 g. $L^{-1}$ concentration reached (5.52cm). The interaction between acids and concentration also significant

**Table 1: Effect of salicylic acid, ascorbic acid, cultivars and their interaction on length of broad bean(cm).**

| Cultivars | Acids | Concentration g.$L^{-1}$ | | | Cultivars * Acids | Means of Cultivars |
|---|---|---|---|---|---|---|
| | | 0 | 100 | 150 | | |
| Aguadolce | Salicylic | 4.83 ab | 5.1 ab | 4.77 ab | 4.9 a | 5.12 a |
| | Ascorbic | 5.07 ab | 5.3 ab | 5.67 a | 5.34 a | |
| Elisa | Salicylic | 4.4 3b | 5.27 ab | 5.53 a | 5.08 a | 5.21 a |
| | Ascorbic | 5.2 ab | 5.33 ab | 5.5 a | 5.34 a | |
| Means of Conc. | | 4.88 b | 5.25 ab | 5.37 a | Means of Acids | |
| Cultivars* Conc. | | 4.95 ab | 5.2 ab | 5.22 ab | | |
| | | 4.82 b | 5.3 ab | 5.52 a | 4.99 a | |
| Acids * Conc. | | 4.63 b | 5.18 ab | 5.1 5ab | 5.34 a | |
| | | 5.13 ab | 5.32 a | 5.58 a | | |

Means within a column, row and there interactions followed with the same letters are not significantly different from each other's according to Duncan multiple range test at 5% level.

### 3.1.2.Number of Branch's per plants.

100 g. $L^{-1}$concentration. The highest no. of branches ( 78.80 branch.$plant^{-1}$ ) had been observed between salicylic acid and 100 g. $L^{-1}$ concentration. The triple interaction between three factors had significant effects, the highest No. of branches per plant (83.73 branch.$plant^{-1}$) had been observed between Elisa, salicylic acid and 100 g. $L^{-1}$ concentration.

Table (2) revealed that no significant differences occurred for each cultivars and acids on the No. of branch per plants, while significant differences obtained in concentrations, the highest value (77.56 branch.$plant^{-1}$) in 100 g. $L^{-1}$concentration. The interaction between cultivars and acids had significant effect the Aguadolce cultivar sprayed with salicylic acid had the highest value (76.63 branch.$plant^{-1}$), also the interaction between cultivars and concentration had significant effect the highest value (79.40 branch.$plant^{-1}$ ) when spray Elisa cultivars with

**Table 2: Effect of salicylic acid, ascorbic acid,cultivars and their interaction on number of branch's per plant of broad bean.**

| Cultivars | Acids | Concentration g.L$^{-1}$(Sa*As) | | | Cultivars * Acids | Means of Cultivars |
|---|---|---|---|---|---|---|
| | | 0 | 50 | 100 | | |
| | | 0 | 100 | 150 | | |
| Aguadolce | Salicylic | 64.20 d | 73.87 bc | 72.53 c | 70.20 b | 73.21 a |
| | Ascorbic | 76.17 bc | 77.57 abc | 74.90 bc | 76.21 a | |
| Elisa | Salicylic | 71.63 c | 83.73 a | 74.53 bc | 76.63 a | 76.21 a |
| | Ascorbic | 74.13 bc | 75.07 bc | 80.60 ab | 76.60 a | |
| Means of Conc. | | 71.53 b | 77.56 a | 75.64 a | Means of Acids | |
| cultivars * Conc. | | 70.18 c | 75.72 ab | 73.72 bc | | |
| | | 72.88 bc | 79.40 a | 77.57 ab | 73.42 a | |
| Acids * Conc. | | 67.92 c | 78.80 a | 73.53 b | 76.41 a | |
| | | 75.15 ab | 76.32 ab | 77.75 ab | | |

Means within a column, row and there interactions followed with the same letters are not significantly different from each other's according to Duncan multiple range test at 5% level.

## 3.2.Green Yield Characters:-

### 3.2.1.No. of pods per plant

### 3.1.3.Number of pods per plant.

highest No. of pods (18.15pod.plant$^{-1}$ ) between Aguadolce and control treatment. There were briefly significant between acid and concentration, the highest value (16.78 pod.plant$^{-1}$ ) between salisylic acid and150 g.L$^{-1}$ concentration. The three interaction between treatments had significant effects the highest value (20.1 pod.plant$^{-1}$ ) between Aguadolce, ascorbic acid and control treatment.

Table (3) illustrated that no significant effect of cultivars on No. of pods per plant, while there were significant effects for each acids and concentrations on the No. of pods per plant, ascorbic acid and 150 g.L$^{-1}$ had the highest value (16.24 and 16.56 pod. plant$^{-1}$ ) respectively. The dual interaction had significant effects on No. of pods per plant, the highest No. of pods (17.23 pod.plant$^{-1}$) was observed between Aguadolce and ascorbic acid, and

**Table 3: Effect of salicylic acid, ascorbic acid, cultivars and their interaction on No. of pods per plant (pod.plant$^{-1}$) of broad bean.**

| Cultivars | Acids | Concentration g.L$^{-1}$ | | | Cultivars * Acids | Means of Cultivars |
|---|---|---|---|---|---|---|
| | | 0 | 100 | 150 | | |
| Aguadolce | Salicylic | 16.2 bc | 16.17 bc | 17.7 bc | 16.69 ab | 16.96 a |
| | Ascorbic | 20.1 a | 16.13 bc | 15.47 c | 17.23 a | |
| Elisa | Salicylic | 9.63 d | 15.83 bc | 15.87 bc | 13.78 c | 14.52 a |
| | Ascorbic | 11.37 | 17.17 | 17.23 | 15.26 | |

| | | | | |
|---|---|---|---|---|
| | d | bc | bc | bc |
| Means of Conc. | 14.33 b | 16.33 a | 16.57 a | Means of Acids |
| Cultivars *Conc. | 18.15 a | 16.15 b | 16.58 b | |
| | 10.50 c | 16.5 b | 16.55 b | 15.23 b |
| Acids * Conc. | 12.92 b | 16.00 a | 16.78 a | 16.24 a |
| | 15.73 a | 16.65 a | 16.35 a | |

Means within a column, row and there interactions followed with the same letters are not significantly different from each other's according to Duncan multiple range test at 5% level.

### 3.2.2.Pod Length (cm).

observed between Aguadolce and ascorbic acid. The highest pod length between Aguadolce and100 $g.L^{-1}$ concentration had (14.37cm). The interaction between acid and concentration had significant effects the highest value (14.58cm) between ascorbic acid and 150 $g.L^{-1}$concentration. The interaction between three factors had significant effects and the interaction between Elisa cultivar, ascorbic acid and control treatments had highest value (15.07cm).

Data in table (4) shows that significant effects between cultivars on pod length, the Aguadolce cultivar (13.78cm) was superiority over the Elisa cultivar (13.24cm). No significant differences occurred for two acids on pod length. Treated plants with concentration had significant effects on pod length, the highest pods length (14.02cm) in 150 $g.L^{-1}$ concentration. The interaction between cultivars and two acids significantly affected pod length, the highest value (14.07cm)

**Table 4: Effect of salicylic acid, ascorbic acid, cultivars and their interaction on pod length (cm)of Bbroad bean.**

| Cultivars | Acids | Concentration $g.L^{-1}$ | | | Cultivars * Acids | Means of Cultivars |
|---|---|---|---|---|---|---|
| | | 0 | 100 | 150 | | |
| Aguadolce | Salicylic | 12.73 cd | 14.63 ab | 13.1 abc | 13.49 ab | 13.78 a |
| | Ascorbic | 13.1 abc | 14.1 abc | 15.00 a | 14.07 a | |
| Elisa | Salicylic | 11.03 d | 13.07 abc | 13.8 abc | 12.63 b | 13.24 b |
| | Ascorbic | 15.07 a | 12.33 cd | 14.17 abc | 13.86 ab | |
| Means of Conc. | | 12.98 b | 13.53 ab | 14.02 a | Means of Acids | |
| Cultivars * Conc. | | 12.91 b | 14.37 a | 14.05 ab | | |
| | | 13.05 ab | 12.7 b | 13.98 ab | 13.06 a | |
| Acids * Conc. | | 11.88 b | 13.85 a | 13.45 a | 13.96 a | |
| | | 14.08 a | 13.22 a | 14.58 a | | |

Means within a column, row and there interactions followed with the same letters are not significantly different from each other's according to Duncan multiple range test at 5% level.

### 3.2.3.Pods Weight (g).

significant effects on pod weight the highest value (34.42g) between Elsa cultivar and control treatment. The interaction between acids and concentration had significant effects and the highest pods weight (28.84g) between salicylic acid and 100g.$L^{-1}$concentration compared to the lowest value (24.45g) between ascorbic acid and control treatment. The interaction between three factors significantly affected pod weight, the highest pod weight (36.40g) between Elsa cultivar, salicylic acid and control treatment, while the lowest one (20.24g) between Aguadulce cultivar, salicylic acid and control treatment.

Table (5) illustrated no significant differences between two cultivars on pod weigh of broad bean, but spraying with two acids increased pod weight significantly the salicylic acid was superior over the ascorbic acid reached (27.84 and 26.03g) respectively. The concentration treatment had significant effects and the highest value (28.27g) in 100 g.$L^{-1}$concentration compared to others. As the effect of dual interaction significantly affected on pod weight, the Elsa cultivar sprayed with salicylic acid had highest value (31.04g) while the lowest value (24.35g) obtained when Aguadolce cultivar sprayed with ascorbic acid. The interactions between cultivars and concentration had

**Table 5: Effect of salicylic acid, ascorbic acid, cultivars and their interaction on pod weight (g)of broad bean.**

| Cultivars | Acids | Concentration g.$L^{-1}$ | | | Cultivars * Acids | Means of Cultivars |
|---|---|---|---|---|---|---|
| | | 0 | 100 | 150 | | |
| Aguadolce | Salicylic | 20.24 f | 28.15 cd | 25.51 de | 24.64 c | 24.49 a |
| | Ascorbic | 16.47 g | 29.08 c | 27.51 cde | 24.35 c | |
| Elisa | Salicylic | 36.40 a | 29.52 bc | 27.21 cde | 31.04 a | 29.37 a |
| | Ascorbic | 32.44 b | 26.33 cde | 24.35 e | 27.71 b | |
| Means of Conc. | | 26.39 b | 28.27 a | 26.15 b | Means of Acids | |
| Cultivars * Conc. | | 18.36 d | 28.62 b | 26.51 bc | | |
| | | 34.42 a | 27.92 bc | 25.78 c | 27.84 a | |
| Acids * Conc. | | 28.32 a | 28.84 a | 26.36 bc | 26.03 b | |
| | | 24.45 c | 27.70 ab | 25.93 bc | | |

Means within a column, row and there interactions followed with the same letters are not significantly different from each other's according to Duncan multiple range test at 5% level.

### 3.2.4.Pod Yield ( g. $plant^{-1}$).

yield per plant, the highest value (459.63 g.$plant^{-1}$ ) in the 100 g.$L^{-1}$ concentration compared to others. The interaction between cultivars and acids had no significant effects on pod yield, while the interaction between cultivars and concentration had significant effects, the highest

Data in table (6) shows that the Elsa cultivar was superior over the Aguadolce cultivar on pod yield per plant (413.85 and 409.22g.$plant^{-1}$ ) respectively. As the effect of two acids had no significant effects on plant yield. There were significant effects of concentration on the pod

bean plants, the highest pod yield (465.83 g. plant$^{-1}$) between Aguadolce, ascorbic acid and 100 g.L$^{-1}$concentration compared to the other interaction and the lowest value was (327.90 g. plant$^{-1}$) between Aguadolce, salicylic acid and control treatments.

value (460.72 g.plant$^{-1}$ ) between Aguadolce and 100 g.L$^{-1}$concentration. The interaction between acids and concentration had significant effects the highest value (459.93and 459.32 g.plant$^{-1}$) between two acids and 100 g.L$^{-1}$ concentration respectively. The interaction between three factors significantly increased pod yield of broad

**Table 6: Effect of salicylic acid, ascorbic acid, cultivars and their interaction on Pod Yield (g. plant$^{-1}$) of broad bean.**

| Cultivars | Acids | Concentration g.L$^{-1}$ | | | Cultivars * Acids | Means of Cultivars |
|---|---|---|---|---|---|---|
| | | 0 | 100 | 150 | | |
| Aguadolce | Salicylic | 327.90 e | 454.03 ab | 451.40 ab | 411.11 a | 409.22 b |
| | Ascorbic | 330.37 e | 467.40 a | 424.23 bc | 407.33 a | |
| Elisa | Salicylic | 350.93 de | 465.83 a | 429.13 bc | 415.30 a | 413.85 a |
| | Ascorbic | 368.60 d | 451.23 ab | 417.37 c | 412.40 a | |
| Means of Conc. | | 344.45 c | 459.63 a | 430.53 b | Means of Acids | |
| Cultivars * Conc. | | 329.13 d | 460.72 a | 437.82 ab | | |
| | | 359.77 c | 458.53 a | 423.25 b | 413.21 a | |
| Acids * Conc. | | 339.42 c | 459.93 a | 440.27 ab | 409.87 a | |
| | | 349.48 c | 459.32 a | 420.80 b | | |

Means within a column, row and there interactions followed with the same letters are not significantly different from each other's according to Duncan multiple range test at 5% level.

### 3.2.5.Total Yield (ton. donum$^{-1}$).

ton.donum$^{-1}$ ) in the interaction between Aguadolce and 100 g.L$^{-1}$. The interaction between acids and conc. had significant effect the highest value (2.891 and 2.887 ton.donum$^{-1}$) between two acids and 100 g.L$^{-1}$recpectively.

The interaction between three factors significantly increased total yield of broad bean plants, the highest total yield (2.938 ton.donum$^{-1}$) had between Aguadolce, ascorbic acid and 100 g.L$^{-1}$concentration compared to the other interaction and the lowest value had (2.061

Table (6) shows that the Elsa cultivar was superior over the Aguadolce cultivar on total yield per plant (2.601and 2.572 ton.donum$^{-1}$) respectively. As the effect of two acid had no significant effect on total yield. There were significant effect of conc. on the total yield per donum and the highest value (2.889 ton.donum$^{-1}$ ) at100 g.L$^{-1}$ concentration compared to others. The interaction between cultivars and acids had no significant effect on total yield, while the interaction between cultivars and conc. had significant effect and the highest value (2.896

and control treatments.

**Table 7: Effect of salicylic acid, ascorbic acid, cultivars and their interaction on Total Yield (ton. donum$^{-1}$) of broad bean plant.**

| Cultivars | Acids | Concentration g.L$^{-1}$ | | | Cultivars * Acids | Means of Cultivars |
|---|---|---|---|---|---|---|
| | | 0 | 100 | 150 | | |
| Aguadolce | Salicylic | 2.061 e | 2.854 ab | 2.837 ab | 2.584 a | 2.572 b |
| | Ascorbic | 2.077 e | 2.938 a | 2.666 bc | 2.560 a | |
| Elisa | Salicylic | 2.206 de | 2.928 a | 2.697 bc | 2.610 a | 2.601 a |
| | Ascorbic | 2.317 d | 2.836 ab | 2.623 c | 2.592 a | |
| Means of Conc. | | 2.165 c | 2.889 a | 2.706 b | Means of Acids | |
| Cultivars * Conc. | | 2.069 d | 2.896 a | 2.752 ab | | |
| | | 2.261 c | 2.882 a | 2.660 b | 2.597 a | |
| Acids * Conc. | | 2.133 c | 2.891 a | 2.767 ab | 2.576 a | |
| | | 2.197 c | 2.887 a | 2.645 b | | |

Means within a column, row and there interactions followed with the same letters are not significantly different from each other's according to Duncan multiple range test at 5% level.

### 3.3.Green Seed Yield Characters:

### 3.3.1.Number of Seeds per Pod.

The data in table (8) illustrated that significant effects of cultivars on No. of seeds per pod, Elsa cultivar overcome to Aguadolce cultivar reached (5.87 and 4.71 seed. pod$^{-1}$) respectively. Significant effects of acids had been occurred, ascorbic acid had highest value (5.61 seed.pod$^{-1}$) compared to salicylic acid (4.97 seed.pod$^{-1}$). The effects of concentration had no significant differences. The interaction between cultivars and acids had significant effects, the highest No. of seeds (6.12 seed.pod$^{-1}$) were recorded in interaction between Elsa and ascorbic acid. The interaction between cultivars and concentration also significantly affected on the No. of seeds and highest value (6.33.seed.pod$^{-1}$) between Elsa and 150g.L$^{-1}$ concentration. There were briefly significant between acids and concentration, the highest value (5.83seed. pod$^{-1}$ ) between ascorbic acid and150 g.L$^{-1}$ concentration.

The three interaction between treatments had significant effects, the highest value (6.73 seed.pod$^{-1}$) between Elisa, ascorbic acid and 100 g.L$^{-1}$ concentration compared to other interactions and the lowest value (3.60seed.pod$^{-1}$) between Aguadolce, salicylic acid and control treatment.

**Table (8): Effect of salicylic acid, ascorbic acid, cultivars and their interaction on number of seed per pod of broad bean.**

| cultivars | Acids | Concentration g.L$^{-1}$ | | | cultivars * Acids | Means of cultivars |
|---|---|---|---|---|---|---|
| | | 0 | 100 | 150 | | |
| Aguadolce | Salicylic | 3.60 f | 4.67 e | 4.67 e | 4.31 c | 4.71 b |
| | Ascorbic | 5.40 cde | 4.93 de | 4.97 de | 5.10 b | |
| Elisa | Salicylic | 5.00 de | 5.73 ab | 6.13 abc | 5.62 ab | 5.87 a |
| | Ascorbic | 5.10 de | 6.73 a | 6.53 ab | 6.12 a | |
| Means of Conc. | | 4.78 b | 5.52 a | 5.58 a | Means of Acid | |
| Cultivars * Conc. | | 4.50 b | 4.80 b | 4.82 b | | |
| | | 5.05 b | 6.23 a | 6.33 a | 4.97 b | |
| Acids * Conc. | | 4.30 b | 5.20 a | 5.40 a | 5.61 a | |
| | | 5.25 a | 5.83 a | 5.75 a | | |

Means within a column, row and there interactions followed with the same letters are not significantly different from each other's according to Duncan multiple range test at 5% level.

### 3.3.2.Number of Seeds per Plant.

value (105.23.seed.plant$^{-1}$) was between Elsa and 150g.L$^{-1}$ concentration. There were briefly significant between acid and concentration and the highest value (97.49 seed. plant$^{-1}$ ) between ascorbic acid and150 g.L$^{-1}$ concentration and the lowest value (53.30 seed.pod$^{-1}$ ) between salicylic acid and control treatment. The interaction between three studied treatments had significant effects the highest value (115.81 seed.plant$^{-1}$) between Elisa, ascorbic acid and 100 g.L$^{-1}$concentration compared to other interaction and the lowest value (48.17seed.plant$^{-1}$) between Elsa, salicylic acid and control treatment.

Table (9) shows that no significant differences between two cultivars on No. of seeds per plant, while significant differences between two acids had been occurred, ascorbic acid had highest value (91.84 seed.plant$^{-1}$) compared to salicylic acid (75.58 seed.plant$^{-1}$). Effects of concentration significantly increased the No. of seeds per plant, two concentration had (90.44 and 92.44 seed.plant$^{-1}$) respectively compared to control treatment (68.25 seed.plant$^{-1}$). The interaction between cultivars and acids had significant differences, the highest No. of seeds (95.50 seed.plant$^{-1}$) between Elsa and ascorbic acid. The interaction between cultivars and concentration significantly affected No. of seeds the highest

**Table 9: Effect of salicylic acid, ascorbic acid, cultivars and their interaction on number of seeds per plant(seed.plant$^{-1}$) of broad bean.**

| cultivars | Acids | Concentration g.L$^{-1}$ | | | Cultivars * Acids | Means of cultivars |
|---|---|---|---|---|---|---|
| | | 0 | 100 | 150 | | |
| Aguadolce | Salicylic | 58.44 de | 75.65 cd | 82.40 c | 72.16 b | 80.17 a |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Ascorbic | 108.45 ab | 79.17 cd | 76.90 cd | 88.17 ab | |
| Elisa | Salicylic | 48.17 e | 91.12 c | 97.69 abc | 78.99 ab | 87.25 a |
| | Ascorbic | 57.92 de | 115.81 a | 112.76 ab | 95.50 a | |
| Means of Conc. | | 68.25 b | 90.44 a | 92.44 a | Means of Acids | |
| Cultivars * Conc. | | 83.45 b | 77.41 b | 79.65 b | | |
| | | 53.05 c | 103.46 a | 105.23 a | 75.58 b | |
| Acids * Conc. | | 53.30 b | 83.38 a | 90.05 a | 91.84 a | |
| | | 83.19 a | 97.49 a | 94.83 a | | |

Means within a column, row and there interactions followed with the same letters are not significantly different from each other's according to Duncan multiple range test at 5% level.

### 3.3.3.Seed Weight (g).

interaction. The interaction between cultivars and concentration significantly affected seed weight, the interaction treatment between Elisa cultivar and 100g.$L^{-1}$ had the highest seed weight which was (3.842g). Significant differences occurred from the interaction treatments between acids and concentration in seed weight, the interaction treatment between ascorbic acid and spraying with 100g.$L^{-1}$ had the highest seed weight, which was (3.787g) compared to other interaction.

The interaction treatments between three factors had significant differences, spray Elisa cultivar with 100g.$L^{-1}$ of salicylic acid had the highest weight (3.913g), while the control treatment in the same acids of Aguadolce cultivar had the lowest value (2.535g).

The Data in table (10), shows that the Elisa cultivar significantly overcomes on the Aguadolce cultivar in weight of seed, which reached (3.460g) compared to the Aguadolce cultivar which was (3.306g). The two acids revealed significant differences in seed weight, ascorbic acid produced higher seed weight (3.552mg) compared to salicylic acid (3.213g). The concentration significantly affected seed weight and the highest value (3.635g) in 100g.$L^{-1}$concentration as compared to control treatment (2.968g). Significant differences were observed between cultivars and acids in seed weight, the interaction treatment between Aguadolce cultivar and salicylic acid had the highest seed weight which was (3.575g) compared to other

**Table 10: Effect of salicylic acid, ascorbic acid, cultivars and their interaction on seed weight (g) of broad bean.**

| Cultivars | Acids | Concentration g.$L^{-1}$ | | | Cultivars * Acids | Means of Cultivars |
|---|---|---|---|---|---|---|
| | | 0 | 100 | 150 | | |
| Aguadolce | Salicylic | 2.535 e | 3.056 d | 3.520 bc | 3.037 c | 3.306 b |
| | Ascorbic | 3.535 bc | 3.802 ab | 3.387 cd | 3.575 a | |
| Elisa | Salicylic | 2.635 e | 3.913 a | 3.620 abc | 3.390 b | 3.460 a |
| | Ascorbic | 3.169 | 3.771 | 3.654 | 3.531 | |

| | | | | |
|---|---|---|---|---|
| | d | ab | abc | a |
| Means of Conc. | 2.969 b | 3.635 a | 3.545 a | Means of Acids |
| Cultivars * Conc. | 3.035 c | 3.429 b | 3.454 b | |
| | 2.902 c | 3.842 a | 3.637 ab | 3.213 b |
| Acids * Conc. | 2.585 c | 3.485 b | 3.570 ab | 3.552 a |
| | 3.352 b | 3.787 a | 3.520 b | |

Means within a column, row and there interactions followed with the same letters are not significantly different from each other's according to Duncan multiple range test at 5% level.

### 3.3.4.Weight of 100 Seed (g).

between cultivars and concentration had significantly affected weight of 100 seed, the interaction treatment between Elisa cultivar and 100g.$L^{-1}$ had the highest weight of 100 seed which was (384.18g). Significant differences occurred from the interaction treatments between acids and concentration in weight of 100 seed, the interaction treatment between ascorbic acid and spraying with 100g.$L^{-1}$ had the highest weight, which was (378.65g) compared to other interaction.

The interaction treatments between the three factors had significant differences, spray Elisa cultivar with 100g.$L^{-1}$ of salicylic acid had the highest weight (391.30g), while the control treatment in the same acids of Aguadolce cultivar had the lowest value (253.53g).

The results in table (11), display that the Elisa cultivar significantly overcomes on the Aguadolce cultivar in weight of 100 seed, which reached (346.03g) compared to the Aguadolce cultivar which was (330.61g). The two acids revealed significant differences in weight of 100 seed, the ascorbic acid produced higher weight of 100 seed (355.29g) compared to salicylic acid (321.34g). The acids concentration significantly affected weight of 100 seed and the highest value (363.55g) in 100g.$L^{-1}$as compared to control treatment (296.87g). Significant differences were observed between cultivars and acids in weight of 100 seed, the interaction treatment between Aguadolce cultivar and salicylic acid had the highest weight of 100 seed which was (357.49g) compared to other interaction. The interaction

**Table 11: Effect of salicylic acid, ascorbic acid, cultivars and their interaction on weight of 100 seeds (g) of broad bean.**

| Cultivars | Acids | Concentration g.$L^{-1}$ | | | Cultivars * Acids | Means of Cultivars |
|---|---|---|---|---|---|---|
| | | 0 | 100 | 150 | | |
| Aguadolce | Salicylic | 253.53 e | 305.6 d | 352.03 bc | 303.72 c | 330.61 b |
| | Ascorbic | 353.53 bc | 380.23 ab | 338.7 cd | 357.49 a | |
| Elisa | Salicylic | 263.53 e | 391.30 a | 362.03 abc | 338.96 b | 346.03 a |
| | Ascorbic | 316.87 d | 377.07 ab | 365.37 abc | 353.10 c | |
| Means of Conc. | | 296.87 b | 363.55 a | 354.53 a | Means of Acids | |
| Cultivars * Conc. | | 303.53 c | 342.92 b | 345.37 b | | |
| | | 290.2 c | 384.18 a | 363.70 ab | 321.34 b | |

| | | | | |
|---|---|---|---|---|
| Acids * Conc. | 258.53 c | 348.45 b | 357.03 ab | 355.29 a |
| | 335.20 b | 378.65 a | 352.03 b | |

Means within a column, row and there interactions followed with the same letters are not significantly different from each other's according to Duncan multiple range test at 5% level.

### 3.3.5.Green Seed yield (g.plant$^{-1}$).[i]

Table (12) shows no significant effect of cultivars on seed yield per plant. On the other hand, the effect of acids significantly influenced plant yield, the ascorbic acid had the highest yield (329.39g plant$^{-1}$) compared to lowest value in salicylic acid (250.99 g.plant$^{-1}$). Concentration significantly affected seed yield. The plants which received 100g.L$^{-1}$ had the highest seed yield (331.06g plant$^{-1}$) and the lowest yield was (210.46g. plant$^{-1}$) in control treatments. Significant differences were observed in yield due to cultivars with acids interaction treatments, the highest yield was recorded between Elisa cultivar and ascorbic acid (343.45g.plant$^{-1}$). The effect of interaction treatments between the cultivars and concentration also significantly affected yield, Elisa cultivar sprayed with 100 and 150 g.L$^{-1}$ improved yield and reached (395.91 and 382.24 g. plant$^{-1}$) respectively compared to other treatments. Similarly, interaction between acids and concentration significantly affected seed yield per plant, ascorbic acid with 100g.L$^{-1}$ had a highest seed yield (368.38g plant$^{-1}$) as compared to other interaction treatments. Interaction between three studies had significant effects on seed yield per plant, the maximum yield between Elisa cultivar and sprayed with 100g.L$^{-1}$ of ascorbic acid (435.06.plant$^{-1}$), while the minimum yield between Aguadolce cultivar salicylic acid and control treatment (147.86g.plant$^{-1}$).

**Table 12: Effect of salicylic acid, ascorbic acid, cultivars and their interaction on seed yield (g.plant$^{-1}$) of broad bean.**

| Cultivars | Acids | Concentration g.L$^{-1}$ | | | Cultivars * Acids | Means of Cultivars |
|---|---|---|---|---|---|---|
| | | 0 | 100 | 150 | | |
| Aguadolce | Salicylic | 147.86 e | 230.71 cd | 291.79 bc | 223.45 c | 269.39 a |
| | Ascorbic | 384.369 a | 301.70 bc | 259.90 cd | 315.32 ab | |
| Elisa | Salicylic | 126.56 e | 356.76 ab | 352.24 ab | 278.52 bc | 310.99 a |
| | Ascorbic | 183.04 de | 435.06 a | 412.25 a | 343.45 a | |
| Means of Conc. | | 210.46 b | 331.06 a | 329.05 a | Means of Acids | |
| Cultivars * Conc. | | 266.12 b | 266.20 b | 275.85 b | | |
| | | 154.80 c | 395.91 a | 382.24 a | 250.99 b | |
| Acids * Conc. | | 137.21 c | 293.74 b | 322.01 ab | 329.39 a | |
| | | 283.71 b | 368.38 a | 336.08 ab | | |

Means within a column, row and there interactions followed with the same letters are not significantly different from each other's according to Duncan multiple range test at 5% level.

### 3.3.6.Total Seed Yield (ton. donum$^{-1}$).

Table (13) displays that no significant differences between cultivars in the total seed yield. The two acids in total yield were accessed to significant level, especially ascorbic acid (2.070 ton.donum$^{-1}$) in comparison with salicylic acid (1.578 ton.donum$^{-1}$). the concentration significantly increased the total seed yield; 100 and 150 g.L-1 resulted in a higher total yield (2.081 and 2.068 ton.duonum-1) respectively as compared to lower value in control treatment (1.323 ton.donum-1). The interaction treatments between cultivars and acids significantly affected on total seed yield, the interaction between Elisa cultivars and ascorbic acid had higher total yield reached (2.159ton.donum-1) as compared to other interactions. The interaction effect between cultivars and concentrations also had significant effects the highest value (2.489 and 2.403ton.donum-1) recorded between Elisa cultivars and two concentration respectively. The interaction between acids and concentrations revealed significant effects on total green seed yield, the highest value (2.316ton.donum-1) between ascorbic acid and 100g.L-1 as compared to other interactions.

The three interaction had significant effects on total seed yield, the highest value (2.735 ton.donum$^{-1}$) between Elisa cultivar sprayed with 100g.L$^{-1}$ ascorbic acid, while the lowest value (0.929 ton.donum$^{-1}$) had between Aguadolce cultivars, salicylic acid and control treatment.

**Table 13: Effect of salicylic acid, ascorbic acid, cultivars and their interaction on total green seeds yield(ton.donum$^{-1}$)**

| Cultivars | Acids | Concentration g.L$^{-1}$ | | | Cultivars * Acids | Means of Cultivars |
|---|---|---|---|---|---|---|
| | | 0 | 100 | 150 | | |
| Aguadolce | Salicylic | 0.929 e | 1.450 cd | 1.834 bc | 1.405 c | 1.693 a |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Ascorbic | 2.416 a | 1.896 bc | 1.634 cd | 1.982 ab | |
| Elisa | Salicylic | 0.796 e | 2.243 ab | 2.214 ab | 1.751 bc | 1.955 a |
| | Ascorbic | 1.151 de | 2.735 a | 2.591 a | 2.159 a | |
| Means of Conc. | | 1.323 b | 2.081 a | 2.068 a | Means of Acids | |
| Cultivars * Conc. | | 1.673 b | 1.673 b | 1.734 b | | |
| | | 0.973 c | 2.489 a | 2.403 a | 1.578 b | |
| Acids * Conc. | | 0.862 c | 1.846 b | 2.024 ab | 2.070 a | |
| | | 1.783 b | 2.316 a | 2.112 ab | | |

Means within a column, row and there interactions followed with the same letters are not significantly different from each other's according to Duncan multiple range test at 5% level.

## 4. DISSCUTION

It is evident from the previously mentioned results in table (6, 7, 8, 10and 11) the Elisa cultivar superior to the Aguadolce cultivar in (Pod Yield g. plant$^{-1}$, Total Yield ton. donum$^{-1}$, No. of seed per pod, seed weight g and weight of 100 seed g), which is due to the genotype differences between the two cultivars and the increase in absorption of the nutrient in the soil, may be due to the differences in root system and RCEC (Root Cation Exchange Capacity) which is differing among cultivars. These results are in harmony with those of the (Salih, 2007 and Amer *et al*., 2008), and also the differences between studied cultivars in growth habit and response of each one to environmental conditions during the growing season which are controlled by genetically factors. That may be reflected on the nodulation and N-fixation consequently growth characteristics. Similar results were obtained by (Dahmardeh *et al*., (2010); El-Masry, (2010) Osman *et al.*, (2010); Darya, (2013) and Kubure *et al*., (2016)).

Spraying broad bean plants with two concentration of salicylic and ascorbic acid (100 and 150 g.L$^{-1}$) significant affected on the growth and green yield of pods and seed characteristics, these results may be due to that the role of salicylic acid to its encourage cell division which increases the level of auxin and cytokinins in plant tissues that accelerate the division of cells (Shakirova *et al*., 2003), The increase in the number of branches when treated with salicylic acid may be due to its role in increasing the levels of cytokinins that play a role in increasing cell division and breaking the capillary sovereignty (Taiz and Zeiger, 1998), and increasing the efficiency of photosynthesis by increasing the absorption of CO2 in plastids (Khan *et al.,* 2003), thus providing the materials needed to build new tissues and increase vegetative growth and thus increase the number of branches. The increase in the number of plant branches when treated with ascorbic acid may be due to the role of ascorbic acid in breaking capillary sovereignty by overcoming the inhibitory effect of auxin produced in the developing top of the stem, in addition to its role in promoting cell division and growth (Smirnoff and Wheeler , 2000). The increase in the number of pods treated with both salicylic acid and ascorbic acid may be attributed to their role in increasing the number of branches per plant (Table 2).

These two compounds (acids) also play a role in reducing the impact of ABA and increasing the production of growth-promoting plant hormones such as auxin and gibberellin (Rai *et al.,* 1986; Smirnoff and Wheeler, 2000), ABA oxidation and hyper plasticity, The increase in pod weight when treated with salicylic acid and ascorbic acid may be due to its role in increasing the processed food and going to the pods and increase seed weight (Table, 10). The increase in the number of seeds in the treatment of both salicylic acid and ascorbic acid with both concentration may be attributed to the role of these substances on reduction competition between flowers and vegetative growth on photosynthesis products and thus increased fertilization. This may also be attributed to the role of these two acids in increasing the length of the pod (Table 4) and increasing the number of seeds where the number of seeds in pod was found to be increased by the length of the pod (Salih *et al*., 1993). The increase in the weight of 100 green seeds of plants treated with both salicylic acid and ascorbic acid may be due to the role of these acids in increasing nutrients in the leaves and then mobilized to seeds. Plants of both salicylic acid and ascorbic acid may be attributed to their role in increasing the number of pod. plant$^{-1}$ (Table 3) and number of seeds in pod (Table 8) increased the superiority of salicylic acid in the weight of 100 green seeds (Table 10).The increase in the total yield of the green pod and seeds in the treatment with both salicylic acid and ascorbic acid was similar to the increase in the yield of green pod and seeds per plant (Table 6 and 12). These results is harmony with those of (Burguieres *et al*. (2007); Azooz and Al-Fredan (2009); Khafaga *et al*. (2009); Younis *et al.* (2010); AL-Amri (2017)& Thomson *et al.,* (2017) Salwa *e tal*.,(2013) ).

## 5. CONCLUSION

The obtained results revealed that the Elisa cultivar superior over the Aguadulce cultivar in more character. Foliar spraying faba bean plants with both acids was beneficial to the crop growth and yield along with green pods and seeds. Hence, it could be suggested that the Elisa cultivar is suitable with area condition and faba bean grown under the experiment and similar growing conditions and foliar sprayed with salicylic and ascorbic to produce high quantity and good quality of some characters green pods, green yield and green seed yield suitable for marketing.

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# Spectrophotometric Methods for Determination of Metronidazole in Pharmaceutical Formulations

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**A B S T R A C T**

Two accurate and reproducible spectrophotometric methods were developed for determination of metronidazole (MET). The methods are based on the reduction of metronidazole with iron metal and hydrochloric acid followed by coupling with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) reagent to form a purple colored chromogen which was easily measured spectrophotometrically at 546 nm (method A).The proposed method B involves the reduction of metronidazole in the presence of tungstate and/or molybdate in an alkaline medium and then coupling of the reaction product with FCR to form a blue colored chromogen which was measured spectrophotometrically at 725nm.The optimized experimental condition were obeyed the beer's law with a good linearity over the concentration ranges 0.3-30 and 0.5 - 40µg/ml of metronidazole with both NBD-Cl and FCR respectively.The limit of detection (LOD) and limit of quantitation (LOQ) were 0.16 and 0.56 µg/ml for method A; while for method B were 0.05 and 0.18 µg/ml, respectively. The proposed methods were applied successfully for determination of MET in pharmaceutical formulations

## 1. INTRODUCTION

( MET) is a 5-nitroimidazole derivative that used as antiprotozoal, antiamebic and antibacterial drugs (Wade et al., 1977). Metronidazole, merchandised under the brand name Flagyl among others, is an antibiotic and antiprotozoal medication. It is utilized either alone or with other antibiotic to treat pelvic fiery malady, endocarditis, and bacterial vaginitis (The American Society of Health-System Pharmacists, 2015). Chemically, metronidazole is known as 2-methyl-5-nitroimidazole-1-ethanol and it belongs to nitroimidazole group (Remington and Gennaro, 1990). MET ($C_6H_9N_3O_3$, M.Wt= 171.156g/mole) is slightly soluble in water but soluble in alcohols and acetone and has a

chemical structure as shown in Fig 1.(British Pharmacopeia, 2013).

MET has been determined by potentiometric titration using 0.1M perchloric acid as a titrant(British Pharmacopeia on CD-ROM., 2013). Other methods have also been reported for the determination of MET in pharmaceutical formulation such as electrochemical methods (Kattab et al., 2011; Liu et al., 2013; Yilmaz et al., 2013; Sarr et al., 2016) , chromatography ( Ouyang, 2010; Ashour and Kattan , 2010 ;Al-Halabi et al., 2012) , and flow injection analysis (Simoes et al., 2006).

Visible spectrophotometry remains competitive comparing with other methods for pharmaceutical analysis due to its simplicity, cost effectiveness, sensitivity, accuracy and precision. Initial reduction of the nitro group has been used as a basis for determination of metronidazole in the visible region followed by either diazotization and coupling of the resulting amine with different coupling agents (Ibrahim and Bashir, 2012; Youssef et al., 2015) or resulting amine undergoes a condensation reaction with aromatic aldehyde to form Schiff's bases (Siddappa et al., 2008). Other visible spectrophotometric methods based on different reaction schemes have been reported in literature for the determination of MET in pharmaceutical formulations (Thulasama and Venkateswarlu 2009 ; Miljkovic et al., 2014) . However, none of these methods are satisfactory, Some of these methods have narrow linear range (Saffaj et al., M., 2004), time consuming steps like heating and extraction ( Lopez et al., 1997; Bhatkar and Chodankar, 1980), used costly reagents and include an additional diazotization step (Adegoke and Umoh, 2009 ), critical dependence on pH (Alsamarrai, 2011). Hence, there is a need to develop another method in order to minimize such disadvantages.

The developed spectrophotometric methods are based on the reduction of MET with powdered iron and HCl, followed by coupling with NBD-Cl or FCR. The experimental conditions were optimized and then Beer's law limits, Sandell's sensitivity and molar absorptivity were calculated for the proposed methods. The accuracy and precision of the developed methods were evaluated and the effect of some common excipients was studied. The standard potentiometric titration method (British Pharmacopeia on CD-ROM., 2013) was accomplished for comparing the present methods statistically, and it was found that the results of both methods were not significantly different.

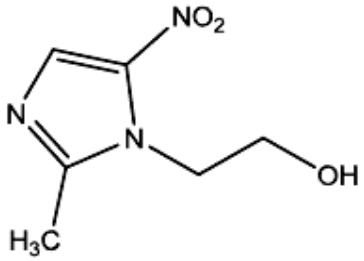



**Fig.1. Chemical structure of metronidazole (MET)..**

## 2.EXPERIMENTAL AND METHODS

### 2.1. Experimental

#### 2.1.1. Apparatus

UV-VIS spectrophotometer (Cecil CE3021-England) was used for all spectral and absorbance measurements using 1.0 cm quartz cells. Thermostatically controlled water bath (Lab. companion shaking BS-11, Korea) was used for temperature control.

#### 2.1.2. Reagents

All chemicals used in this study were of analytical grade. A pure metronidazole (MET) was obtained as gifts from (Awamedica Company for Drug Industries and Medical Applications Awa, Erbil). Folin-Ciocaleteu reagent (FCR) and 4-chloro-7-nitrobenzo-2-

oxa-1, 3-diazole (NBD-Cl) were purchased from Sigma Chemicals Co., USA. Sodium hydroxide and sodium bicarbonate were obtained from Fluka.

**2.1.3. Solutions**

- Reduced metronidazole solution (100 µg /ml) was prepared by dissolving 0.01g of MET in 10 ml deionized water followed by addition of 0.4 g of powdered iron and 5 ml of concentrated HCl.The solution was stirred for 20 min at room temperature and then filtered. The filtrate was diluted with deionized water to 100 ml in a volumetric flask and then kept in an amber-glass volumetric flask, where it was stable at least for 2 days (Al-Temur, 2015).

-Serial dilutions of reduced MET solution (100 µg /ml) with deionized water were made to cover the working range of the calibration graph.

- Folin-Ciocaleteu reagent (FCR)(1:3, v/v) was prepared by mixing accurately measured 25 ml of commercially available FCR in 75 ml of deionized water.

- Solution of 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl) (0.3%) was freshly prepared by dissolving 0.3 g of NBD-Cl in 100 ml of acetone.

- Sodium hydroxide solution (2 M) was prepared by dissolving 8 g of sodium hydroxide in deionized water and then diluted to the mark with deionized water in 100 ml volumetric flask.

- Sodium bicarbonate solution (2 M) was prepared by dissolving 16.8 g of the pure sodium carbonate in deionized water and then filtered .The filtrate was diluted to the mark with deionized water in 100 ml volumetric flask.

- interferences solutions (1000 ppm) were prepared by dissolving 100 mg of each interferences in exactly 100 ml of deionized water.

**2.2. Recommended procedures**

**2.2.1. Method A**

Different volumes of MET ranging from 0.03 to 3.0 ml (0.3-30 µg /ml) were transferred into a series of 10 ml volumetric flasks followed by adding 0.2 ml of 0.3% NBD-Cl and 1.0 ml of 2M NaOH, then completed to the mark with deionized water. After 5 min, the absorbance of the colored product was measured at 546 nm at room temperature against reagent blank.

**2.2.2. Method B**

Different aliquots of working standard of MET ranging from 0.05 to 4.0 ml (0.5-40 µg /ml) were transferred into a series of 10 ml volumetric flasks. To each flasks, 0.5 ml of 2M sodium carbonate and 0.3 ml of 1:3 FCR reagents were added, and then diluted to the mark with deionized water and kept at room temperature for 10 min to complete the reaction. The absorbance of the blue colored complex was measured at 725 nm against reagent blank.

**2.3. Assay procedure for pharmaceutical tablets**

Two different brands of metronidazole tablets were analyzed. Twenty tablets for each brand of metronidazole were powdered. An accurate quantity of powdered tablets equivalent to 0.01 g of MET was weighed and dissolved in about10 ml of deionized water, then the procedure of reduction (section 2.3) was applied. The solution was then filtered, and the residue was washed out thoroughly with 10 ml portions of deionized water (three times).The total volume of the filtrate was then made up to 100 ml with deionized water and the recommended procedure was applied for determination of MET. The absorbance of the solution was measured against the solution

blank and the amount of MET was calculated from the calibration graph.

### 2.4. Assay procedure for suspended solution

0.25 ml of the suspended solution (equivalent to 0.01 g of MET) was diluted with 10 ml of distilled water and the procedure of reduction (section 2.3) was applied. The solution was then diluted to the mark in a 100 ml volumetric flask with deionized water to get the concentration of 100 ppm MET. The recommended procedure was then applied for determination of MET. The absorbance was measured against the blank solution and the amount of MET was calculated from the calibration graph. The readings were taken in triplicate.

## 3. RESULTS AND DISCUSSION

The proposed spectrophotometric methods for determination of metronidazole were based on the reduction of the nitro group to an amino group by powdered iron in an acidic medium (HCl). The resulting amine undergoes a rapid reaction in alkaline medium with NBD-Cl /NaOH and FCR/Na2CO3 to form two colored complexes with maximum absorbances at 546 and 725 nm, respectively. NBD-Cl and FCR have been used as chromogenic reagents for the colorimetric determination of phenolic compounds and amines (primary and secondary) and for spectrophotometric determination of compounds such as acyclovi certain nitrogen compounds,salbutamol,lisinopril , paroxetine hydrochloride, hydralazine hydrochloride , pencillin, sencnidazole and ferulic acid (Basavaiah and Prammela, 2002; Ikawa et al., 2003; El-Enany et al., 2004; El-Emam et al., 2004; Onal et al., 2005; Siddappa et al., 2009; Singh and Maheshwari, 2010; Kuma et al., 2012; Jadhav et al., 2012). In the method A, the reaction is a nucleophilic Aromatic Substitution (SNAr) in which the chlorine atom of NBD-Cl reagent attacks the primary amino group of the drug in alkaline medium followed by the liberation of hydrochloric acid to form a purple colored complex that shows a maximum absorption at 546 nm (Fig. 2).

In the method B, the estimation is based on reacting of MET with Folin-Ciocaleteu reagent in an alkaline medium to form a blue colored product that shows maximum absorption at 725 nm (Fig. 2). Folin-Ciocaleteu reagent (FCR) is a mixture of phosphomolybdate & phosphotungstate. The complex formation was due to the oxidation of the drug and the reduction of FCR. The proposed reaction mechanisms based on the reported methods (Kuchekar et al., 2005; Swamy et al., 2012) are given in scheme 1.

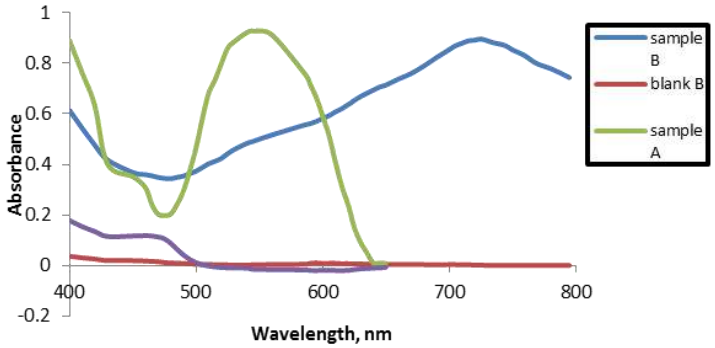
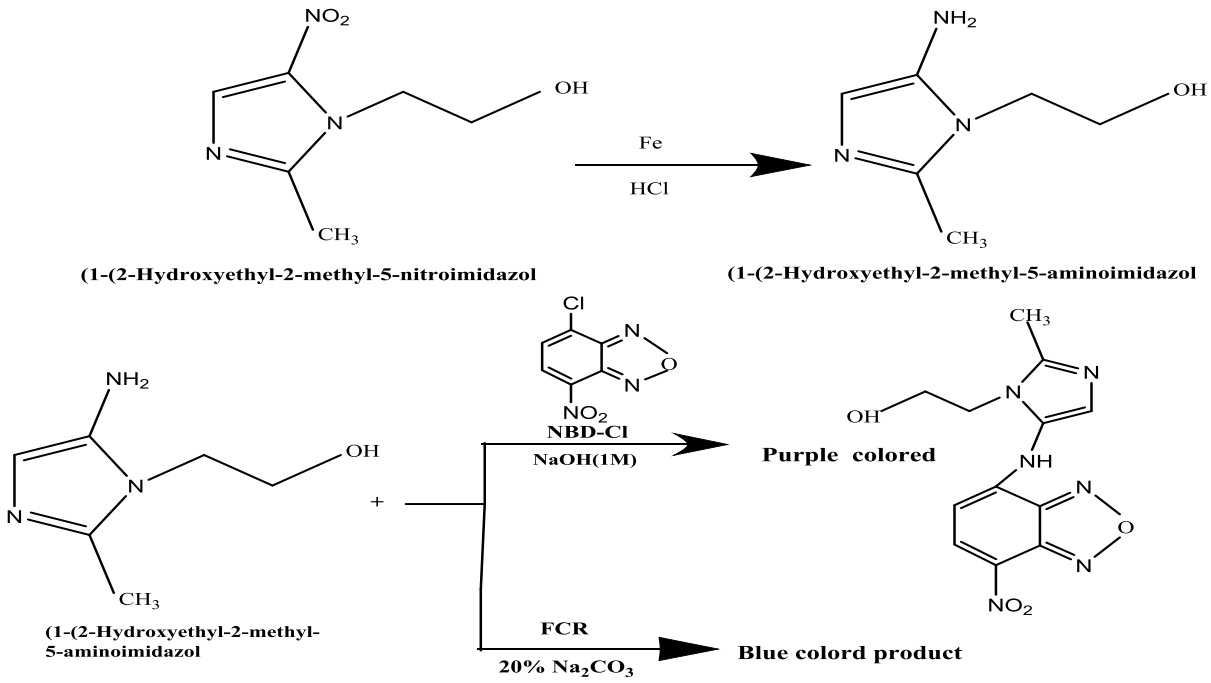



**Fig.2. Absorption spectra of MET (20 µg / ml) for methods A and B against reagent blank.**




**Scheme 1. Proposed chemical reactions of MET in basic medium with NBD-Cl and FCR**

## 3.1. Optimization of reaction variables

### 3.1.1. Effect of reagents

The influence of the concentration of NBD-Cl and FCR was studied using different volumes of 0.3% NBD-Cl or 1:3 diluted FCR solutions of the reagent on the intensity of the developed color at constant MET concentration (20 µg/ ml). It was found that 0.2 ml of NBD-Cl and 0.3 ml of FCR give the maximum absorbance; while above this volume the absorbance decreases (method B) or remains constant (method A) (Fig.3). Therefore 0.2 ml of (NBD-Cl) and 0.3 ml of (FCR) were selected as the optimum volumes and used in all subsequent measurements.

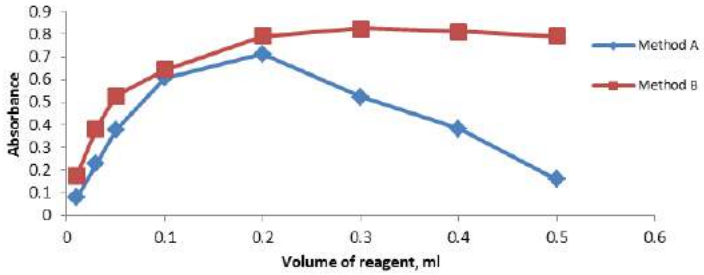



**Fig. 3. Effect of volume of 0.3% NBD-Cl and 1:3 diluted FCR on the absorbance of the final colored product in the solution of MET (20 µg/ ml).**

### 3.1.2. Effect of alkaline solution

To obtain the maximum absorbance ,the effect of concentration of different types of alkaline solution were studied .Different volumes ranged from 0.3-2.5 ml of 2M of three base solutions (sodium hydroxide, potassium hydroxide, and sodium bicarbonate) were examined. The results showed that the maximum absorbances can be obtained by

using 1 ml of 2M sodium hydroxide in method A and 0.5 ml of 2M sodium carbonate in method B (Fig. 4). Hence, these volumes were selected and used in all subsequent measurements.

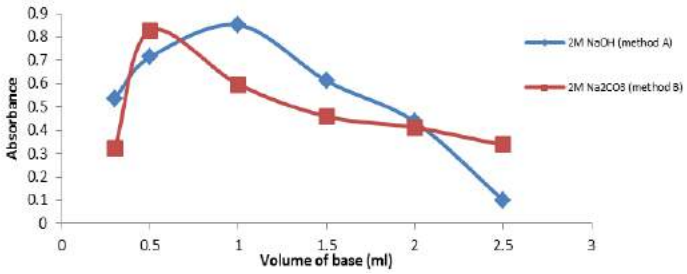



**Fig. 4. Effect of different concentrations of alkaline solutions (NaOH and $Na_2CO_3$) on the absorbance of the final reaction solution.**

### 3.1.3. Effect of temperature and time of reaction

It was found that an increasing of the temperature has a negative effect on the absorbance of the reaction solution for both methods A and B. the reason may be due to the degradation of the main produced colored product in the reaction .The effect of temperature ranged from 20-60°C and reaction times on the color intensity were studied. It was found that an increasing of the temperature has a negative effect on the absorbance of the reaction solution for both methods A and B.The reason may probably due to the degradation or instability of the main produced colored product in the reaction solution. As shown in Table 1,the maximum color development was obtained at room temperature (25°C) (optimum temperature ) after 5 min and 10 min for methods A and B ,respectively and they were used throughout the determination process for method A and B ,respectively (Table 1).Afterwards, the stability of the product in both method A and B were monitored by measuring the absorbance of the final colored solution. It was found that the final colored products are stable for up to 1 hour (method A) and 3 hours (method B) at 25°C as shown in Fig. 5.

**Table 1**
**Effect of temperature and time on the absorbance of final reaction solution.**

| Temp.° C | Time (min) with the absorbance values (method A) | | | | | Time (min) with the absorbance values (method B) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 20 | 30 | 40 | 5 | 10 | 20 | 30 | 40 |
| **20** | 0.872 | 0.869 | 0.867 | 0.866 | 0.864 | 0.887 | 0.886 | 0.883 | 0.880 | 0.878 |
| **25** | **0.915** | 0.910 | 0.908 | 0.909 | 0.906 | 0.890 | **0.892** | 0.889 | 0.884 | 0.885 |
| **30** | 0.856 | 0.854 | 0.852 | 0.848 | 0.849 | 0.829 | 0.833 | 0.830 | 0.829 | 0.827 |
| **40** | 0.811 | 0.809 | 0.807 | 0.806 | 0.803 | 0.784 | 0.787 | 0.785 | 0.783 | 0.781 |
| **50** | 0.673 | 0.671 | 0.670 | 0.669 | 0.666 | 0.711 | 0.714 | 0.712 | 0.710 | 0.710 |
| **60** | 0.522 | 0.521 | 0.519 | 0.517 | 0.515 | 0.685 | 0.689 | 0.887 | 0.885 | 0.883 |

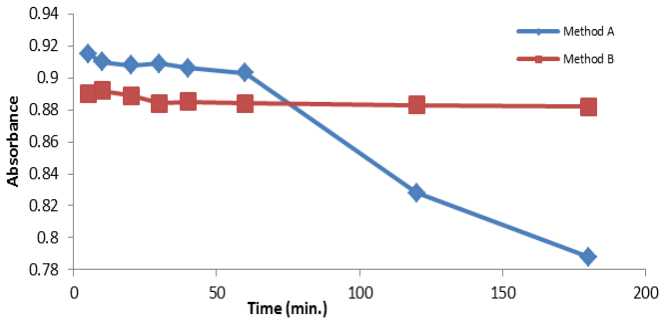



**Fig. 5. Effect of time on the stability of the final colored products in methods A and B.**

### 3.1.4. Effect of order of addition

The order of addition of reagent (R) and corresponding volume of base (B) to the sample solution (20 ppm of MET) was examined (Table 2). The results indicated that the addition orders (II) in method A and (I) in method B are the optimum as they produced the colored products with high intensity.

**Table 2**
**Effect of order of addition on the absorbances.**

| Reaction components | Order number | Absorbance (method A) | Absorbance (method B) |
|---|---|---|---|
| **MET + B+ R** | I | 0.745 | 0.896 |
| **MET + R +B** | II | 0.916 | 0.711 |
| **R + B +MET** | III | 0.577 | 0.448 |

### 3.1.5. Effect of solvents

In order to choose the best solvent for color development of the solution, different solvents were tested such as methanol, ethanol, acetone, acetonitrile and water. The results indicated that the highest absorbance and reproducible results are obtained when water is used as a solvent (Table 3). Hence deionized water was selected for dilution of NBD-Cl and FCR solutions throughout all subsequent experiments.

**Table 3**

**Effect of solvents on optical properties of the color products.**

| Solvents | Absorbance of Method A | Absorbance of Method B |
|---|---|---|
| **Methanol** | 0.301 | 0.287 |
| **Ethanol** | 0.211 | 0.118 |
| **Acetone** | Turbid | Turbid |
| **Acetonitrile** | Turbid | Turbid |
| **Water** | 0.917 | 0.898 |

## 3.2. Analytical methods validation

### 3.2.1. Analytical data

The analytical parameters such as Beer's law limits, Sandell's sensitivity and molar absorptivity were calculated for the proposed methods A and B (Table 4). Regression analysis of the Beer's law also revealed a good correlation.

**Table 4**

**Analytical parameters of methods A and B for determination of MET.**

| Parameters | The value (Method A) | The value (Method B) |
|---|---|---|
| **Color** | Purple | Blue |
| **Medium** | NaOH (2 M) | $Na_2CO_3$ (2 M) |
| **$\lambda_{max}$, nm** | 546 | 725 |
| **Beer's law range (µg / ml)** | 0.3-30 | 0.5-40 |
| **LOD (µg / ml)** | 0.16 | 0.05 |
| **LOQ ( µg/ml)** | 0.56 | 0.18 |
| **ε (l/mole. cm)** | $0.712 \times 10^4$ | $0.685 \times 10^4$ |
| **Sandell's sensitivity (µg/cm$^2$)** | 0.024 | 0.025 |
| **Regression equation : Y = bX + a*** | | |
| **Intercept (a)** | 0.1058 | 0.103 |
| **Slope (b)** | 0.0416 | 0.04 |
| **Determination coefficient ($R^2$)** | 0.9982 | 0.9967 |
| **RSD%** | 0.225 | 0.31 |
| **Recovery%** | 100.69 | 99.04 |
| **Stability (h.)** | 1 | 3 |

* Y is absorbance and X is concentration.

### 3.2.2. Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) indicate the sensitivity of the method. LOD is the lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated according to the International Union of Pure and Applied Chemistry (IUPAC) definition (IUPAC, 1978):

$LOD = 3\ \sigma/s$ and $LOQ = 10\sigma/s$

Where: σ is the standard deviation of replicate measurements under the same conditions as used for the sample analysis in the absence of the analyte; s is the sensitivity of the method i.e. the slope of the calibration curve. The results showed in Table 4 indicate the high sensitivity of the proposed methods.

### 3.2.3. Precision and accuracy

The accuracy and precision of the proposed methods were evaluated by replicate analysis (n =5) of calibration standards at three concentration levels as the RSD and recovery percentage were calculated for both methods A and B. The results illustrated in Table 5 indicate that the methods were satisfactory and have a good accuracy and precision.

**Table 5**

**Accuracy and precision of the proposed methods A & B.**

| MET taken, µg/ml | MET found, µg/ml for method A | MET found, µg/ml for method B | Recovery, % for method A | Recovery, % for method B | RSD, %* for method A | RSD, %* for method B |
|---|---|---|---|---|---|---|
| **5.0** | 5.08 | 4.79 | 101.6 | 95.8 | 0.236 | 0.46 |
| **15.0** | 15.12 | 15.28 | 100.8 | 101.8 | 0.244 | 0.24 |
| **25.0** | 24.92 | 24.88 | 99.68 | 99.52 | 0.195 | 0.23 |
| | **Average** | | 100.69 | 99.04 | 0.225 | 0.31 |

*Average of five determinations.

### 3.3. Interference study

The effects of common excipients used in the pharmaceutical preparations on the performing of the proposed analytical methods were studied. It was carried out by analyzing a synthetic sample solution containing 20 µg of MET in the presence of different amounts of excipients (starch, glucose, lactose and sodium chloride).Experimental results showed that the excipients or additives up to 500 µg has no impact on the determination of MET ( Table 6).

**Table 6**
**Effect of excipients for assay of MET.**

| Excipients | µg Excipients | Recovery% (n=3) for method A | Recovery% (n=3) for method B |
|---|---|---|---|
| **Starch** | 100 | 101.2 | 101.2 |
| | 200 | 102.8 | 100.9 |
| | 500 | 96.7 | 99.2 |
| **Glucose** | 100 | 100.7 | 99.8 |
| | 200 | 98.4 | 101.9 |
| | 500 | 97.6 | 102.5 |
| **Lactose** | 100 | ٩9.8 | 100.8 |
| | 200 | 98.1 | 101.3 |
| | 500 | 96.9 | 98.5 |
| **Sodium chloride** | 100 | 99.3 | 101.1 |
| | 200 | 98.9 | 97.8 |
| | 500 | 97.7 | 102.7 |

### 3.4. Application to the analysis of pharmaceutical formulations

The proposed methods were applied successfully for determination of MET in pharmaceutical formulations. Validation of the proposed methods A and B was performed statistically (Miller and Miller, 2005) by taking five replicate measurements and then comparing the obtained results with those corresponding data obtained by the official BP method (British Pharmacopeia, 2013). No significant difference was found by applying t-test and F-test at 99% confidence level with four degrees of freedom (Table 7). The results showed that the experimental t-test and F-test were less than the theoretical value (t=3.747, F=15.97)(Table 7) and indicating that there was no significant difference between the results obtained by the proposed methods and standard method.

**Table 7**
**Applications of methods A & B for the determination of MET and statistical comparison with the official method.**

| Pharmaceutical preparations of MET | % Found [1] ±SD[2] | | |
|---|---|---|---|
| | Proposed methods | | Official method |
| | Method A, t[3] & F[4] values | Method B, t[3] & F[4] values | |
| **Nidazole 200 mg (suspension), HIKMA Pharma. Jordan** | 97.2± 0.53<br>t=1.92<br>F=1.00 | 97.0±0.66<br>t=1.80<br>F=4.79 | 96.4±1.14 |
| **Metronidazole Awa 500 mg (tablet), Awamedica, Erbil, Iraq** | 101.6±0.82<br>t=1.98<br>F=٠.59 | 97.6±0.77<br>t=1.91<br>F=0.98 | 97.0±0.46 |
| **Flanizol 500 mg (tablet), United Pharma. Jordan** | 98.4±0.66<br>t=1.91<br>F=٠.85 | 95.4±0.97<br>t=1.98<br>F=1.48 | 99.2±0.16 |

[1] Average of five determinations; [2] SD=Standard deviation; [3] Tabulated t-value for four degrees of freedom; and p=0.01 is 3.747; [4] Tabulated F-value for four degrees of freedom; and p=0.01 is 15.97.

### 3.5. Comparison of the methods

Several analytical variables were taken into account to make a comparison between the developed methods and other previously reported spectrophotometric methods (Table 8).It indicates that the developed methods are sensitive due to the high values of molar absorptivity enough stability of the colored products.

**Table 8**
**Comparison between the proposed methods and previously reported methods for determination of MET**.

| Analytical parameters | Proposed methods | | Previouslyreported method * | Previously reported method ** |
|---|---|---|---|---|
| | Method A | Method B | | |
| Color | Purple | Blue | Pinkish-red | Purple |
| Medium | Aqueous (2M NaOH) | Aqueous (2M $Na_2CO_3$) | Aqueous (3% sulphamic acid) | 1, 4-dioxan/Acetonitrile (6:4) mixture |
| Type of reaction | Coupling reaction | Coupling reaction | Diazo-coupling reaction | charge-transfer complexation |
| Reagent | NBD-Cl | FCR | N-(1-naphthyl) ethylenediamine | chloranilic acid |
| Temperature (°C) | 25 | 25 | At room temperature | At room temperature |
| $\lambda_{max}$ , nm | 546 | 725 | 503 | 520 |
| Beer's law range (µg/ml ) | 0.3-30 | 0.5-40 | 0.8-20 | 5-40 |
| Determination coefficient, $R^2$ | 0.9982 | 0.9967 | 0.9999 | 0.9990 |
| ε (l/mol.cm) | $0.712 \times 10^4$ | $0.685 \times 10^4$ | $4.673 \times 10^3$ | $1.312 \times 10^3$ |
| LOD (µg/ ml) | 0.16 | 0.05 | …….. | …….. |
| RSD% | 0.225 | 0.31 | ±0.47 to ±1.08 | …….. |
| Stability of the color | 1 h | 3 h | 75 minutes | ……. |
| Applications | Suspension and tablets | Suspension and tablets | Suspension ,tablets and injection | Dosage forms |

* Ibrahim and Bashir, 2012.
** Adegoke, 2011.

## 4. CONCLUSIONS

The important features of the developed methods compared to other previously used techniques for the determination of metronidazole are sensitivity, easy accessibility to the instrument and cost –effectiveness. The reagents used in the proposed methods are safe, cheap and readily available. The statistical analysis showed that the data from the proposed methods are in a good agreement with those reported in literature. The high values of molar absorptivity, low values of Sandell's sensitivity and LOD indicate the high sensitivity of the proposed methods. The methods are free from critical optimum conditions such as heating, extraction step, pH control and using of toxic organic solvents. The colored products obtained from the reaction were enough stable to be analyzed by the analyst. Moreover, the developed methods are not affected by interferences such as excipients or additives. The presented data in this paper for determination of metronidazole in its pure and dosage forms demonstrate that the proposed methods have an acceptable linearity, high accuracy and precisio

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# Detection of Biofilm Producer Methicillin Resistant *Staphylococcus hominis* Isolated From Different Clinical Sources

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**A R T I C L E I N F O**

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**ABSTRACT**

*Staphylococcus hominis* is an opportunistic gram positive bacteria and is a member of coagulase-negative staphylococci (CoNS) . During the study, 213 clinical specimens were collected from patients admitted to different hospitals in Erbil city, Iraq. Only 18(8.45%) isolates of *S. hominis* were isolated including 7 isolates (17.07%) from blood, 4 isolates (8.70%) from urine, 3 isolates (7.14%) from ear, 2 isolates (6.67%) from wound, and one isolate from each nasal swab (4.55%) and oral cavity (3.13%), all *S. hominis* identified based on morphology, cultural, biochemical tests, and further confirmed by Vitek 2 compact system. To determine the most accurate assay for measuring methicillin resistance *S. hominis*, compared the detection of *mecA* by PCR with detection by National Committee for Clinical Laboratory Standards methods using oxacillin and Cefoxitin disk diffusion method. The results of oxacillin showed 13 (72.22%) isolates resistant to methicillin and 5 (27.77%) isolates were sensitive to it. While, the results of cefoxitin demonstrated that 16 (88.89%) isolates were resistant to methicillin and only 2 isolates (11.11%) were sensitive to it. However, the same results of the Cefoxitin disk diffusion method was obtained by PCR and by using *mecA* gene which 16 isolates (88.89%) were carried mecA gene with product size 499bp. The results of microtiter plate method revealed that 16 (88.89%) isolates of S. hominis were biofilm producer and only 2 isolates (11.11%) were non-biofilm producer. Moreover,of 16 biofilm producer isolates, 14 isolates (77.77%) were categorized as strong biofilm producers and 2 (11.11%) isolates were identified as moderate biofilm producers.

# 1. INTRODUCTION

*Staphylococcus hominis* is the third important species of coagulase-negative staphylococci (CoNS) and is a part of the commensal bacterial microflora of healthy people, in addition to that it recognized as a major cause of nosocomial infections and frequently isolated from specimens of hospitalized patients( Szczuka *et al*., 2014) . *S. hominis* found asymptomatically on the skin, arms, legs, and surfaces of the axilla and is an opportunistic pathogen found in blood and capable of causing a different diseases including bacteremia, septicemia, and endocarditis especially in immunocompromised patients (d'Azevedo *et al*., 2008; Al Wohoush *et al*., 2010; Bouchami *et al*., 2011). Infections caused by *S. hominis* are often highly resistant to antibiotics which make the treatment difficult. Furthermore, a growing concern is the emergence of methicillin resistant *S. hominis* , which is the important pathogen among methicillin resistant CoNS especially in clinical isolates and the methicillin resistance *S. hominis* is associated with the *mecA* gene (Zhang *et al*., 2013; Mendoza-Olazaran *et al*., 2013). Detection of *mecA* gene by Polymerase chain reaction is considered as the gold standard for methicillin resistance as this gene is highly conserved among Staphylococcal species (Khan *et al*., 2012). Indeed, many infections caused by *S.hominis* appeared to be associated with biofilms and the nosocomial infections by CoNS are primarily associated with the use of medical devices, likely because of biofilm formation (Fredheim *et al*. 2009; Mendoza-Olazaran *et al*., 2015). A biofilm can be defined as a sessile community, which are sets of microorganisms in which cells affix to each other on a surface that is a polymeric mixture generally composed of proteins, extracellular DNA and polysaccharides which facilitates the adherence of these microorganisms to the surfaces and protect them from host immune system and antimicrobial therapy (Jabra-Rizk *et al*., 2006; Novick and Geisinger, 2008). Nevertheless, little information is available regarding the ability of methicillin resistant *S. hominis* to form a biofilm . Because of that the main objective of the present study was isolation and identification of *S. hominis* from hospitalized patients in Erbil city- Iraq, also to characterize their susceptibility profile to methicillin resistant antibiotics and comparing with *mecA*, also to investigate the ability of methicillin resistant *S. hominis* to form biofilm.

## 2. MATERIALS AND METHODS

### 2.1 Collection, isolation and identification

During September 2015 to May 2016; 213clinical specimens were collected from different patients in Teaching, Rizgary, West Emergency hospitals, and Health Center Laboratory in Erbil city, Iraq. The included specimens were as follow: 41 specimens from blood, 42 from ear, 22 from nose infection, 32 from oral cavity, 46 from urinary tract infections, and 30 from wound infections . All specimens were taken by disposable cotton swabs or sterile containers. The specimens were plated on mannitol salt agar (MSA) media (Oxoid, England), and incubated overnight at 37$^{o}$C for 24 hours. All isolates were identified based on cultural, morphological, and different biochemical tests, Single, well isolated colonies with the typical appearance of *S. hominis* were sub cultured and confirmed by Vitek 2 compact system . On the other hand, the ability of isolated bacteria to produce some virulence enzymes were investigated depending on (Harley and Prescott. 2002; Cheesbrough, 2006; Atlas, 2010). The enzymes were catalase, oxidase, coagulase, gelatinase, lipase, DNase, protease, lecithinase, urease , beta lactamase and hemolysin .

**2.2. 1. Phenotypic detection of methicillin resistant *S. hominis***

The disk diffusion method with antibiotic oxacillin 1µg and cefoxitin 30 µg (BBL, England) was performed in Mueller Hinton agar as described by (CLSI, 2015).

**2.2.2. Detection of *mecA* gene by PCR**

The extraction of genomic DNA from the isolated bacteria was performed by using Presto$^{TM}$ Minig DNA bacterial kit following the manufacturer's recommendations. Lysostaphin was added for effective extraction of DNA from the *S. hominis*. The extracted DNA was stored at -20°C until PCR was performed. PCR was performed using the primer *mecA* primer were: 5'AGCTGATTCAGGTTACGGACAAGGT 3'and5'GCAACCATCGTTACGGATTGCTT CA 3'with expected size 499bp. The PCR reactions were prepared in 20µl volume, consisting primer 1.3µl of each forward and reverse, and 2.5µ of DNA template were added to AccuPower PCR tub. The amplifications were conducted using thermal Cycler (Eppendorf, Germany) programmed with the initial denaturation at 94°C for 5 min with 30 cycles, denaturation at 94°C for 60s, annealing at 63°C for 60s and extension at 72°C for 60s, and final extension at 72°C for 5 min. The PCR products were separated by gel electrophoresis

in 1.5% agarose gel (Igeltjorn, 2009 ;Mishra *et al*., 2010).

### 2.3.Detection of the bacterial ability to produce biofilm

All isolated bacteria were tested for biofilm formation by using the Microtitre plate method as described by (Mathur *et al*., 2006) .The isolated bacteria were inoculated in brain heart infusion broth and incubated for 24 hours at 37 °C. After incubation each culture was diluted 1:100 with sterile fresh medium. Then, 200 µL of the samples were added to each well of a 96-well microplate , in addition broth was used as blank. The microplate were incubated at 37 °C for 24 hours. Content of each well was gently removed. The wells were washed three times with phosphate buffer saline (pH 7.2) to take off unbound bacterial cells subsequently the plates were exposed to air-dry and 200 µL of 0.1% w/v crystal violet solution was added to each well and incubated at room temperature for 30 minutes . The plates were washed off with distilled water and kept for air-dry. The bound bacteria were quantified by addition of 200 µL of absolute ethanol to each well and the absorbance of dissolved dye was measured at a wavelength of 570 nm by using 96-flat wells of ELISA. The isolates were classified according to biofilm production (Christensen *et al.,* 1985; Mathur *et al*., 2006).

## 3. RESULTS AND DISCUSSION

### 3.1. Bacterial isolation and identification

Two hundred and thirteen specimens were collected from patients admitted to hospitals located in Erbil city, Iraq. Only 18(8.45%) isolates of *S. hominis* were isolated from clinical specimens including 7 isolates (17.07%) from blood, 4 isolates (8.70%) from urine, 3 isolates (7.14%) from ear, 2 isolates (6.67%) from wound, and one isolate from each nasal swab (4.55%) and oral cavity (3.13%) as illustrated in table (1), and the above results similar to results obtained by (Chaves *et al.,* 2005). All *S. hominis* identified based on morphology, cultural, and biochemical properties ( Kloos and Schleifer, 1986; Bannerman and Peacock, 2007). The isolates were yellow Gram positive cocci colonies that appeared round, smooth, raised, glistening, non- motile, and grow in 5-10% NaCl. Additionally, all isolates gave positive results for (catalase, lecithinase, and protease). In addition to that, they gave negative result for (novobiocin (5µg/disc), oxidase, coagulase, Dnase, lipase, ß-lactamase, urease, gelatinase, rhamnose, and dextrose). Moreover, they had the ability to ferment (fructose, lactose, maltose, sucrose, and produces acid from trehalose ). Whereas, they

differ in their ability to produce hemolysin which 6 isolates (33.33%) were β- hemolysin and 12 isolates (66.67%) were γ- hemolysin producers (Figure 1)and these results were similar to results reported by (Abdulla and Barzani, 2016; Barzani *et al*., 2017). All obtained *S. hominis* isolates were further confirmed by Vitek 2 system.

**Table 1. The number and percentage of *Staphylococcus hominis* isolates isolated from different clinical sources.**

| Specimens sources | Specimens number | Number of *S. hominis* | Percentage (%) of *S. hominis* |
|---|---|---|---|
| Blood | 41 | 7 | 17.07 |
| Ear | 42 | 3 | 7.14 |
| Nasal swab | 22 | 1 | 4.55 |
| Oral cavity | 32 | 1 | 3.13 |
| Urine | 46 | 4 | 8.70 |
| Wound | 30 | 2 | 6.67 |
| Total | 213 | 18 | 8.45 |

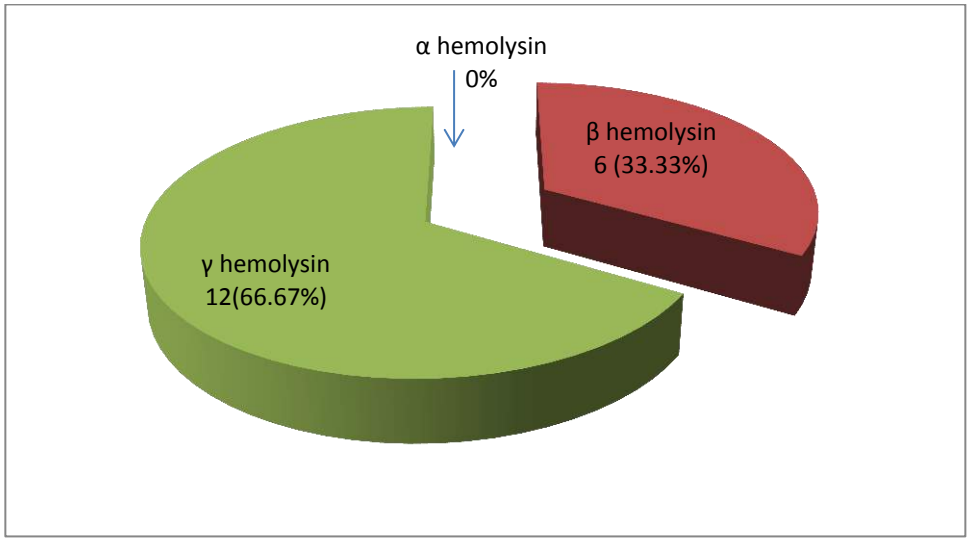

**Figure 1. Hemolytic activity of isolated *S. hominis***

**Detection of methicillin resistant *S. hominis***

To investigate the distribution of methicillin resistance *S. hominis* in the our community and among the patients in the Erbil city hospitals, the methicillin sensitivity test was applied to all 18 isolates, the test was performed by using disk diffusion method with antibiotic oxacillin and cefoxitin, additionally the *mecA* gene by using PCR was used to confirm the detection of methicillin resistant *S. hominis*. The results of oxacillin disk diffusion revealed that from all 18 isolates, 13 isolates (72.22%) showed resistant to the methicillin while 5 isolates (27.77%) were sensitive to the methicillin. On the other hand, the results of cefoxitin disk diffusion demonstrated that 16 isolates (88.89%) were resistant to methicillin and only 2 isolates (11.11%) were sensitive to methicillin (Table 2). However, the same results of the Cefoxitin disk diffusion method was obtained by PCR and by using *mecA* gene which 16 isolates (88.89%) were carried *mec*A gene with product size 499bp (Figure 3), and these results similar to results recorded by (Palazzo *et al*., 2008; Mendoza-Olazarán *et al*., 2015). Additionally, all above methicillin resistant isolates of *S. hominis* which detected phenotypically were harbor *mecA* gene, it means the Cefoxitin disk diffusion method was more accurate method for detection of methicillin resistant isolates in addition to PCR methods and *mecA* gene. Although, the rate of methicillin resistance *S. hominis* isolates were differ according to clinical specimens sources and also similar results obtained in both Cefoxitin disk diffusion method and by PCR (*mecA* gene). Generally methicillin resistant Staphylococci have become a serious problem in many country of the world. In spite of, the incidence of methicillin resistant strains varies from country to another, and from hospital to another, and may be also due to specimens size and source of isolation, it has been steadily increasing resistant to methicillin and oxacillin worldwide in the last decade (Calderon-Jaimes *et al*., 2002; Fung-Tomc *et al*., 2002). The most satisfactory explanation to this phenomenon is that even before methicillin resistance was reported for *S. aureus*, it was recognized in coagulase-negative Staphylococci (Chambers, 1988) and many data support the hypothesis that *mecA* originated in a coagulase-negative Staphylococcus species and resistance to methicillin is due to the acquisition of *mecA*, that encodes PBP2a -a transpeptidase with a low affinity for beta-lactam antibiotics

(Enright *et al*., 2002; Brased and Weigelt, 2007).

**Table 2. Detection of methicillin resistant *S. hominis* isolates by different methods.**

| Specimens sources | Number of *S. hominis* | Oxacillin disk diffusion (No. & %) | Cefoxitin disk diffusion (No. & %) | PCR (*mec*A gene) (No. & %) |
|---|---|---|---|---|
| Blood | 7 | 5 (71.43%) | 7 (100%) | 7 (100%) |
| Ear | 3 | 2 (66.67%) | 2 (66.67%) | 2 (66.67%) |
| Nasal swab | 1 | 1 (100%) | 1 (100%) | 1 (100%) |
| Oral cavity | 1 | 1 (100%) | 1 (100%) | 1 (100%) |
| Urine | 4 | 3 (75%) | 3 (75%) | 3 (75%) |
| Wound | 2 | 1 (50%) | 2 (100%) | 2 (100%) |
| Total | 18 | 13 (72.22%) | 16 (88.89%) | 16 (88.89%) |

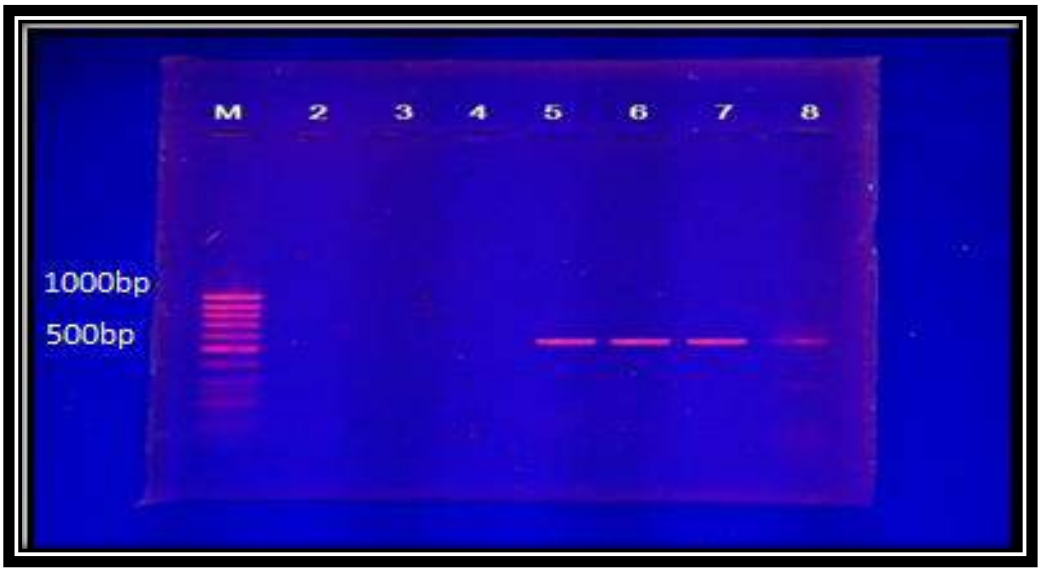


**Figure 3. Polymerase chain reaction products on gel electrophoresis (1.5%) for *mecA* gene. M: DNA ladder (1000bp). Lanes 5,6,7, 8 : Amplified PCR product of *mecA* gene (499 bp) for *S. hominis* isolates. Lanes 3, 4: *S. hominis* isolates negative for *mecA* gene .Lane 2: negative control.**

**Biofilm formation in *S. hominis* isolates**

The results of microtitre plate method revealed that 16 (88.89%) isolates of *S. hominis* were biofilm producer and only 2 isolates (11.11%) were non-biofilm producers, and these results similar to those obtained by (Garza-González *et al.,* 2011; Soroush *et al*., 2017). Of the 16 biofilm producer isolates, 14 isolates (77.77%) were categorized as strong biofilm producers and 2 (11.11%) isolates were identified as moderate biofilm producers (Figure 4) as defined by (Christensen *et al.,* 1985; Mathur *et al*., 2006) , and these results accordance with those reported by (Mendoza-Olazarán *et al*., 2015, Abdulla and Barzani, 2017) . On the other hand, the results showed that all 16 biofilm producer isolates were methicillin resistant and carried *mecA* gene, whereas the two non-biofilm producer were methicillin sensitive and not contained *mecA* gene. A biofilm is a community of bacteria living together in an organised structure as cellular clusters or microcolonies and it is enclosed in amatrix composed of an extracellular polymeric substance . However, the biofilm allows bacteria to adhere to inert materials and to experience increased antibiotic resistance (Davies, 2003; Hoiby *et al*., 2010). Moreover, the biofilm producer CoNS species are more resistant to antibiotics than when they exist as free-swimming planktonic cells.The formation of a stable biofilm on medical devices or on host clinical specimens is thought to be the major pathogenicity factor of *S. hominis* (Götz 2006; Rodhe *et al*. 2006). Additionally, many infections caused by staphylococci species found to be associated with biofilms. In addition to that, the knowledge about the ability of these bacterial isolates to form biofilm is relatively limited especially in Iraq.

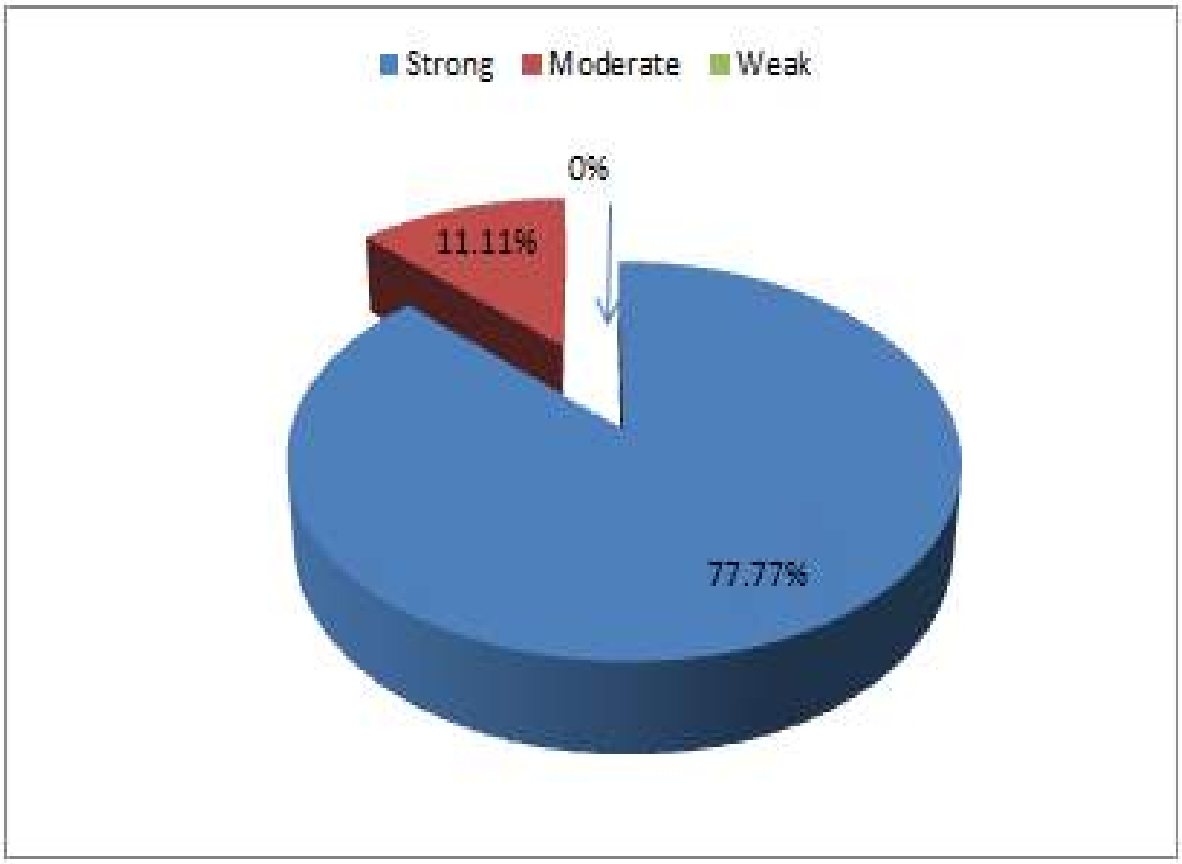


**Figure 4. Percentage of strong, moderate and weak biofilm production of isolated *Staphylococcus hominis* by using microtiter plate method.**

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# The Relationship between Temperature, *Tetranychus urticae* and Cucumber Hybrids

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**A B S T R A C T**

Three different temperature degrees (25, 30 and 35˚C) were used to study their effect on the biology of two spotted spider mite (*Tetranychus urticae*). The results demonstrated that the longest developmental time was 9 days at 25˚C and the shortest developmental time was 6.8 days at 35˚C with R.H.65±5%. The maximum period of adult longevity was 17 days at 25˚C, and minimum longevity was 13.8 days at 35˚C. The pre- oviposition period was 1.5, 1.3, and 0.9 days at 25, 30 and 35˚C, respectively. Oviposition period was 8, 7 and 6 days as above mentioned temperature. The highest number of eggs (31.67) was laid at 25 ˚C after three days and minimum eggs (6.67) were deposed after five days at 35˚C. The highest number of larvae which 43 larvae was observed at 35˚C after six days and the minimum number 1.17 was recorded at 25˚C after three days. As well as, the highest number of nymphs (27.67) was noticed after seven days at 30˚C, whereas the lowest number of nymphs (1 nymph) found after four days at 25˚C. The Unigene cucumber hybrid showed more resistant to *T. urticae* with lowest mean numbers (267.89, 54.67 and 247.11) of eggs, adults and lesions respectively. While, Saef hybrid was the most susceptible variety with (694.44, 315.89 and 1226.67) for the eggs, adults and lesions respectively.

## 1. INTRODUCTION

The cucumber (*Cucumis sativus* L.) is one of the most important cultivated crops that belong to the Cucurbitaceae family, have benefits for example contains alkaline forming minerals, an ascorbic acid antioxidants and vitamin B complex. (Maheshwari *et al*., 2014). *Tetranychus urticae* is one of the most serious agricultural pests in the world. This mite is polyphagous and attacks the broad range of crops. The importance of this mite pest is not only due to direct damage to the plants (i.e. defoliation, leaf burning, and an excessive outbreaks plant death) but also indirect damage to the plants which decrease both photosynthesis and transpiration. The rapid developmental rate and high reproductive of *T. urticae* allows them to cause damage very quickly when growth conditions aresuitable, resulting in a rapid decline of host plant quality (Skorupska, 2004).

The high temperature and non-humid climates are suitable for the development and outbreak of the two-spotted spider mite (Leite *et al*., 2003). The population growth parameters of *T. urticae* such as developmental rate, survival, reproduction and longevity are

varying according temperature, host phenological stage and relative humidity (Liu and Tsai 1998, and El-Halawany 2001).

Host plant preference and performance are positively correlated, but not always in some cases no correlation or a negative relationship was found between preference and performance (Mayhew, 1997). Wilson and Huffaker, (1976) mentioned that the use of resistant plants could affect pest population density, herbivore damage, and the efficiency of natural enemies and in turns reduce pesticide applications in agricultural ecosystems.

Female spider mites forced to feed on non-preferred host plants generally have low fecundity (Greco *et al*., 2006). Spider mites disperse mostly in a passive way through wind they are likely to end up near new hosts, hence their selection of host plants should be thought of in terms of acceptance, rather than host finding (Sabelis, 1985). On the other hand, as they are constantly exposed to herbivore damage, plants are known for their ability to defend themselves against pathogens and herbivores (Karban and Baldwin, 1997).

## 2. MATERIALIS AND METHODS

### 2.1. Preparing stock culture of *T. urticae*

*T. urticae* specimens were collected from cucumber (Cucumis sativa L.) leaves at fields located around Erbil province. They were maintained in the laboratory at 26±2˚C. The spider mites were reared on fresh leaves. After several generations, the mites from the stock colony were used for the tests (Riahi *et al*., 2013).collected mites were maintained on the detached sprouts and leaves of cucumber. Infested plants were kept in wood-framed rearing cages (120x60x60 cm).

### 2.2. The Effect of Temperature on Biology of *T. urticae.*

The effect of temperature on the biology of T. urticae was studied using leaf disk, according the method proposed by El-Halawany (2001), with some modifications, as one leaflet from the first fully expanding leaf at the top per plant of the cucumber, was taken and then it was washed with running water to remove any possible residuals or mites, which may be found on these leaves. Leaf discs of about 3 cm in diameter were made and surrounded by Arabic gum, which acts as a barrier to prevent mite individuals from escaping. These discs were placed on pieces of moisten cotton wool in petri dishes (10-cm diameter ) with two couple (male and female) was placed on each disc, on the lower surface of the leaf, for each replicate (six replicates for each temperatures). These petri dishes were kept at three different temperatures (25, 30, and 35 ±2˚C and 70 ± 5% R.H.), for 24 hours to allow mating. Thereafter, males were removed, while female served as a source for eggs, which in turn produce known-age larvae. The moisture was kept constant by adding few drops of water to the cotton wool. About 60 hatching larvae were trans and kept singly to a leaf of each plant and left to continue their life span. Observation was made twice daily, early in the morning, and in the evening, under a dissecting microscope (40X). For each temperature, data were recorded for seven days; only data in oviposition period was recorded for five days (El Halawany, 2001and Romeih *et al.,* 2013).

### 2.3. Effect of host plant and their variety (hybrid) on the mite population dynamic.

From Erbil Research Center of Ministry of Agriculture, field was prepared for planting cucumber in greenhouse. Three different hybrids, (Saef hybrid, Unigene hybrid and Non- Hamus hybrid) of cucumbers were planted in three rows in order to determine which hybrid is resistant or susceptible to the mites infestation. Ten days after planting cucumbers, Topsin at concentrate 1g/5m and Rival at concentrate 5ml/5m$^2$ (Fungicides) were added to the soil according to the

manufacture instructors to control soil fungus; the process was repeated each three weeks. Additionally, in the first stage growth of plants, nutrient deficiency was observed in some leaves like P, Fe, and Mg…. etc. NPK was used once a week to prevent the nutrient deficiency. HumiMax it's a fertilizer was added to the soil to increase growth of flowers. To prevent fungal disease Goldazane fungicide was used according to manufacture instructors (5ml/5m$^2$). In the last stage plants growth they infected with 20 mites artificially in order to determine the resistant and susceptibility of plants (Razmjou *et. al*., 2009). Cucumber resistance to mite infestation in greenhouse (plastic house) were tested. Seeds were planted in perlites that were filled with. Patmos after five days; the seedlings were transplanted to the plastic house. Three hybrids of cucumber were planted which were Saef hybrid, Unigene hybrid and Non Hamus 14 hybrid. Each greenhouse of cucumber hybrid was tested with 15 replicates including 5 replicates as negative control (healthy plant) then seedlings were kept in plastic house at 28±2˚C. Samples were taken from each hybrid after one week infecting by the mite; three leaves were included for each hybrid. The samples were conveyed to the lab to numerate the number of eggs, immature and mature stages and the number of spots (lesions) per cubic centimeter (Mohammadi *et al*., 2015). The data was recorded every two days throughout the study period

## 3. RESULTS AND DISCUSSION

Data in table (1) indicated that, the shortest average developmental time of T. urticae 6.8 days at 35˚C with R.H.65±5% and the longest developmental time was 9 days at 25˚C. Moreover, the mean generation times 7 and 9 days at 35˚C and 25˚C subsequently were recorded. The average pre-oviposition periods was 1.5, 1.3, and 0.9 days at 25, 30 and 35˚C, respectively. Average, oviposition period was 8, 7 and 6 days respectively as above mentioned temperature degrees. Results in table (1) clearly demonstrated that the maximum average period longevity was 17 days recorded at 25˚C. While the minimum time value was 13.8 days at 35˚C. Different results were obtained by Riahi et al., (2013) who recorded insignificant impact of temperature on the male longevity in contrast; adult female longevities of T. urticae were significantly different among the different temperatures examined the longest at 25 ˚C (12.91 days) and the shortest at 30 ˚C (3.56 days). These differences can probably be attributed to the following two reasons: (a) the geographical origin and adaptation of the *T. urticae* population (b) different laboratory conditions such as photoperiod and humidity. Data present in table (2) showed that the average number of eggs, larvae and nymphs was decreased as the temperature increased as it was 17.5, 11.92 and 10.96 eggs at 25, 30 and 35˚C respectively, and 14.71, 12.17 and 9.13 larvae at 25, 30 and 35 ˚C respectively, and 10.96, 6.79 and 8.5 nymphs at 25, 30 and 35˚C respectively. While in the adult the average number of adult was increased as the temperature increased as it was 1.46, 2.38 and 4.63 at 25, 30 and 35˚C respectively. Results above showed that there are no significant differences between number of eggs, larvae, nymphs and adults at the different temperature (25, 30 and 35˚C), but the highest mean were observed at 25˚C in all immature stages, while in adult showed reversed result that the highest mean number were observed at 35˚C. the results also could be agree with Cossins and Bowler,(1987) that the developmental rate, expressed as the reciprocal of time taken to change from one stage to another, is nothing at the low temperature threshold, increases with temperature and levels off at the optimum, and then decreases rapidly as the high threshold is

approached. El-Halawany, (2001) recorded the longest longevity 26.45 and 20.3days at 15 and 20˚C, of *T. urticae*, and duration of different developmental stages was 7.23 days at 30˚C when fed on Cadota fig variety.

Fig. (1) revealed that the maximum eggs were laid after three days among tested temperatures. Meanwhile the highest number of eggs (31.67) at 25 ˚C were laid by *T. urticae* after three days, and the minimum were 10.67 after one day at same temperature, followed by 30˚C which recorded highest and lowest number of eggs (22.67 and 6.67) after (3 and 5) days respectively.

The larvae not observed among determined temperatures in the first two days, whereas the maximum larvae (4.33, 23, 22.67, 43 and 28 respectively) recorded during last five days at 35˚C. Meanwhile minimum numbers of larvae (1.17, 16.33, 20, 37 and 20 respectively) were observed by 25˚C at same days, as well as the 30˚C showed variation in a number of larvae with 3.33, 20.33, 20.67, 38 and 23 respectively (Fig.2).

In the first three days there was no nymph observed among tested temperatures, while the number of nymphs was increased by all tested temperatures. Thus, lowest numbers of nymphs were recorded 1.00, 1.33, and 1.67 at 25, 30 and 35˚C respectively during the 4th day whereas maximum number of nymphs were occurred in the last day of the experiment 26, 27.67 and 25.67 at 25, 30 and 35˚C respectively (Fig.3). Dicke, (2000) and James and price, (2002) indicated that the population growth parameters of *T. urticae* such as developmental rate, survival, reproduction and longevity may vary in response to changes in temperature, host plant species, host plant nutrition, cultivar kind, phonological stage, exposure to pesticides and relative humidity. Gulati, (2004) revealed that *T. urticae* population showed positive correlation with high temperature and negative correlation with low temperature. Sunita, (1996) reported positive correlation between mite population and minimum temperature.

Table (3) revealed the relationship between *T. urticae* and three hybrids of cucumber plant. The results showed that the highest population of *T. urticae* eggs, adults and spot lesions caused by sucking mites were observed in Saef hybrid with 694.44 eggs, 315.89 adult and 1226.67 spot lesions on lower leaf surface with average of 306 $cm^2$. While the lowest mean number of eggs, adults and lesions were recorded on Unigene hybrid of cucumber with 267.89 eggs, 54.67 adults and 247.11 lesions, respectively on lower leaf surface with average of 253 $cm^2$. According the statistical analysis there were no significant differences between Saef hybrid and Unigene hybrid in number of adults, eggs, and lesions.

According to the results Unigene hybrid showed greatest resistant to *T. urticae* according to number of eggs, adults and spot lesions, meanwhile *T. urticae* was well adapted to Saef hybrid and caused high number of spot lesions (1226.67) of cucumber leaves by sucking out the contents of leaf sap. Similar results were also obtained by Ghallab *et al*., (2011) who revealed that Nemsse cultivar harbored the highest infestation of spider mites, with mean numbers of 77.8 individuals, followed insignificantly by Sweet crunch cultivar (52.5individuals), while Xena cultivar recorded the lowest infestation (18.9 individuals / 40 leaves). The results also are in accordance with Mondel and Ara, (2006); Kumaran *et al*., (2007) they demonstrated that *T. urticae* is responsible for causing the loss of foliage of the crop resulting in reduction of the economic yield of fruits ranging from 20-45 % depending upon cropping season and agro-climatic conditions. *T. urticae* is well adapted to various environmental conditions, causing

loss of quality and yield or death of plants by sucking out the contents of leaf cells.

Host plants of spider mites differ in the degree of food quality, which either depend on the level of primary plant metabolites, or on the quantity and nature of secondary metabolites (Rosenthal and Berenbaum, 1991). Many secondary metabolites found in plants have a responsibility in defense against herbivores, pests and pathogens. These compounds can perform as toxicants, deterrents, digestibility reducers or act as precursors to physical defense systems (Bennett and Wallsgrove, 1994; Balkema- Boomstra *et al*., 2003).

Wilson and Huffaker, (1976) indicated that the nutritional quality, host plant species, cultivar, physiological, ecological and chemical traits of the host plant may influence the life history parameters of spider mites and therefore the degree of plant resistance.

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Table (1): The temperature effects on the life table parameters of *T. urticae*

| Parameter | Temperature (˚C) | | |
|---|---|---|---|
| | 25 | 30 | 35 |
| Developmental time (days) | 9 | 7.6 | 6.8 |
| Pre-Ovipostion period (days) | 1.5 | 1.3 | 0.9 |
| Ovipostion period (days) | 8 | 7 | 6 |
| Generation time (days) | 9.7 | 7.8 | 7 |

| | | | |
|---|---|---|---|
| Longevity (days) | 17 | 15.4 | 13.8 |

Table (2): Effect of Temperature on *T. urticae* stages on cucumber hybrid

| Mite stage | ˚C | Mean ± SD | F | Sig |
|---|---|---|---|---|
| Egg | 25 | 17.5 ± 13.49 | 2.96 | 0.06 |
| | 30 | 11.92 ± 7.6 | | |
| | 35 | 10.96 ± 8.01 | | |
| larva | 25 | 14.71 ± 17.26 | 1.13 | 0.33 |
| | 30 | 12.17 ± 12.14 | | |
| | 35 | 9.13 ± 7.24 | | |
| nymph | 25 | 10.96 ± 17.92 | 0.67 | 0.52 |
| | 30 | 6.79 ± 7.5 | | |
| | 35 | 8.5 ± 9.69 | | |
| Adult | 25 | 1.46 ± 3.97 | 1.49 | 0.23 |
| | 30 | 2.38 ± 5.42 | | |
| | 35 | 4.63 ± 9.12 | | |

Table (3): The susceptibility of three cucumber hybrids to infested by *T. urticae*

| Cucumber variety | | Mean ± SD | F | Sig. |
|---|---|---|---|---|
| Eggs | Saef hybrid | 694.44 ± 1010.38 | 0.93 | 0.41 |
| | Unigene hybrid | 267.89± 490.93 | | |
| | Non Hamus 14 hybrid | 371.67±417.78 | | |
| Adults | Saef hybrid | 315.89±455.70 | 1.37 | 0.27 |
| | Unigene hybrid | 54.67±91.16 | | |
| | Non Hamus 14 hybrid | 252.33±386.50 | | |
| Lesions | Saef hybrid | 1226.67±1509.77 | 1.15 | 0.33 |

| | | | | |
|---|---|---|---|---|
| | Unigene hybrid | 247.11±348.25 | | |
| | Non Hamus 14 hybrid | 1138.33 ±2115.67 | | |

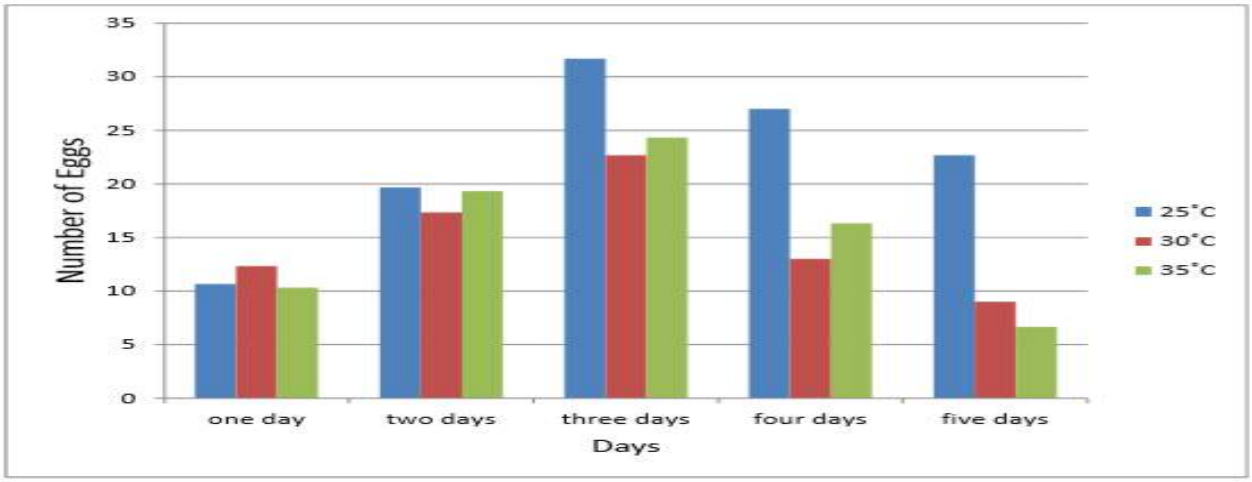


Fig.1: Effect of temperatures on the egg laying by *T. urticae* on cucumber

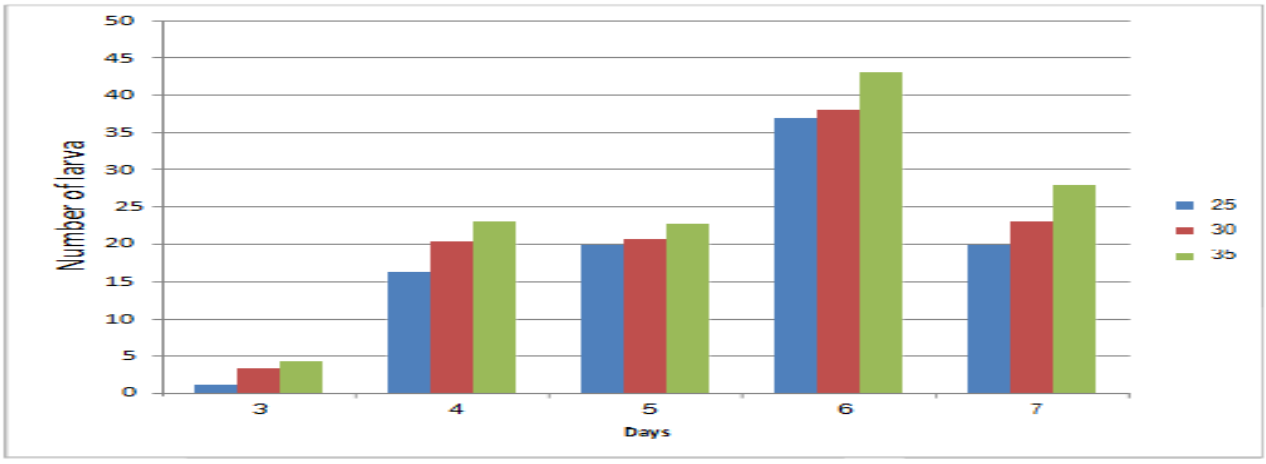


Fig.2: Effect of temperatures on the larvae appearance of *T. urticae* on cucumber

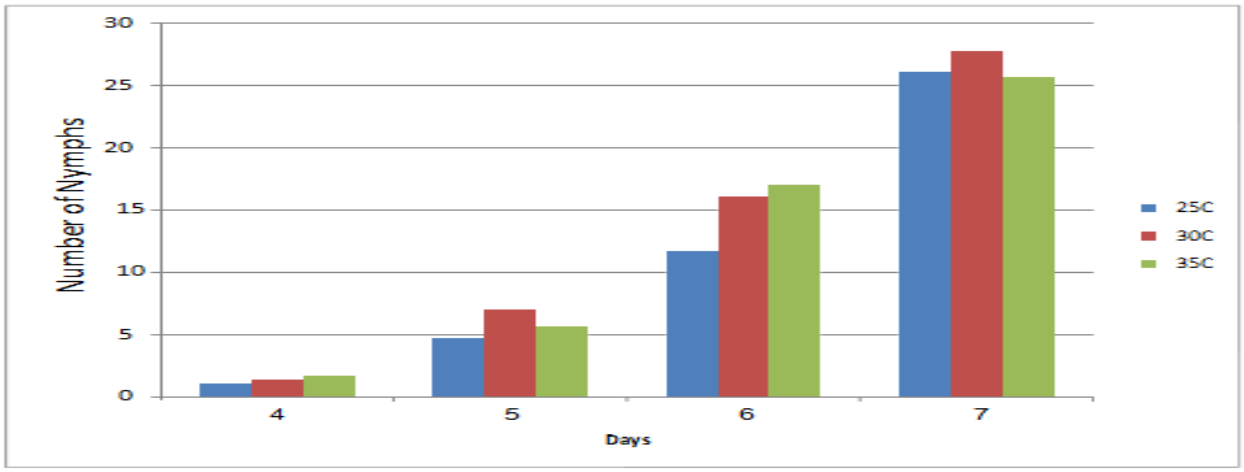


Fig.3: Effect of temperatures on the Nymphs appearance of *T. urticae* on cucumber

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# Benthic community study and seasonal variation of zooplankton biomass in Dukan Lake Kurdistan region-Iraq

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**A R T I C L E I N F O**

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**ABSTRACT**

A study of benthic invertebrates and seasonal variation of zooplankton biomass has been carried out at three selected sites on Dukan Lake. Monthly samples of water, benthic and zooplankton invertebrates were collected during period from July 2016 to the February 2017. Some physical and chemical properties of water were studied and the results showed that the air temperature ranged from 1 to 36.5°C, water temperature ranged from 4 to 32.5°C, hydrogen ion concentration (pH) of studied lake were ranged from 7.01 to 8.6. electrical conductivity ranged from 163.39 to 801.19 µs.cm$^{-1}$, turbidity level of lake during studied period were ranged from 1 to 8NTU, dissolved oxygen from 3 to 6.1 mg.l$^{-1}$, and $BOD_5$ were ranged from 1 to 26 mg.$^{-1}$. Concerning to benthic invertebrates the results showed that a total of 18 species were recorded belonged to Nematoda, Annelida, Arthropoda and Mollusca. Regarding to planktonic communities, total zooplankton number was ranged from 10 to 8726 ind.l$^{-1}$. While, the total count of phytoplankton was ranged from 99573 to 754001cell.l$^{-1}$. Positive correlation between total count of phytoplankton and total count of zooplankton was reported with r=0.455, r =0.478 and r =0.381 in the site1, site 2 and site 3 respectively.

## INTRODUCTION

Invertebrates are a major component of the aquatic ecosystem such as ponds, pools, streams, rivers and lakes. They are good biological indicators due to their long lived in aquatic ecosystem and including species of varying degree of tolerance for different source of pollution (Klemm et. al., 2001).

Many studies on invertebrates community were conducted on different lakes in Iraq among them; (Salih et al., 1990) made a study on effects of some factors on the distribution of Chironomid larvae in Mosul dam. The effects of Samarra Impoundment on zooplankton distribution was studied by (Sabri et al., 1993), they observed that the zooplankton community decreased during the drainage season. While, (Sabri et al., 1997a) investigated the distribution of benthic and zooplankton invertebrates community of some dams and Reservoirs water in center of Iraq. On the other hand, the effects of Himreen Impoundment on the benthic and planktonic invertebrates in Diyala river was carried out by (Saadalla, 1998).

Dukan lake is one of the important and largest lake in Iraqi Kurdistan region, and there were few research projects have been

carried out on it, in that manner, (Bilbas, 2014) made an ecosystem health assessment of Dukan lake. While, a study of the shore zooplankton community in Dukan Lake was carried out by (Dhahir, 2016). She identified 37 species belonged to three main groups; Cladocera, Copepoda and Rotifera. The present project aimed study of the benthic community and seasonal variation of zooplankton invertebrates in Dukon Lake.

## MATERIALS AND METHODS

Dukan Lake is located on the Lesser Zab River, about 65km northwestern of Sulaymaniyah city. Samples for physical, chemical and biological variables in three sites from small part of Dukan Lake were collected during period extended from July 2016 to the February 2017(Fig. 1). Water samples were collected for analysis using pre-washed polyethylene bottle by water sample twice before filling. Physico-chemical measurements were conducted including: Water temperature, pH, Electrical conductivity and Turbidity by using pH meter (AllA France pH-meter-1-15), EC meter and Turbidity meter (HACH, 2100A). While, Dissolved Oxygen using Azide modification method, as well as, BOD test according to (APHA, 2012).

Benthic invertebrates samples were collected in selected sites by using Surber sampler. While, the zooplankton were collected by passing 55L of lake water through a planktonic net with 55µm pore size. The collected samples were fixed with 5% formalin and later preserved in ethanol alcohol 70% (A.P.H.A., 2012).

Identification of invertebrates species and counting of zooplankton was undertaken in the laboratory using a compound microscope and the following references were used: (Edmondson, 1959; Smith, 2001; Ruppert et al., 2003; Thorp and Covich, 2010 and Hammadi et al., 2012).

Enumeration of phytoplankton was conducted based on a modification of the membrane filtration technique of (McNabb, 1966) and (Hinton and Maulood, 1979).

## RESULTS

In this study, a number of phisco-chemical parameters were studied (table 1), the results showed that the air temperature in the studied sites ranged between 1 to 36.5°C with significant monthly variation ($p<0.05$) and non-significant differences ($p>0.05$) between studied sites, while the water temperature showed regional and monthly variations with significant differences ($p<0.05$) and it ranged between 4 to 32.5°C.

The results of hydrogen ion concentration showed significant differences ($p<0.05$) between studied sites and date of sampling. The pH values of the studied lake were ranged from 7.06 to 8.6.

Electrical conductivity level of the studied lake at the selected sites were ranged between 163.39 to 801.19µs.cm$^{-1}$, and the statistical analysis showed a significant differences ($p<0.05$) among sampling date only.


Fig. (1): Map of Iraq, inset location of sampling sites on Dukon Lake (Google Earth)

The turbidity level of the present study was ranged between 1 to 8 NTU and statistically the significant differences ($p<0.05$) were observed between studied sites and date of sampling.

Dissolved oxygen concentrations revealed that they were a significant differences ($p<0.05$) between studied sites and date of sampling, it was ranged between 3 to 6.1 $mg.l^{-1}$.

$BOD_5$ level fluctuated between all sites and ranged from 0.7 to 26 $mg.l^{-1}$ and the statistical analysis showed that the regional and monthly variation with significant differences ($p<0.05$).

Collection of benthic invertebrates were made at the studied lake for eight months, and a total of 18 species were recorded (table 2) including 3 species of Nematoda, followed by Annelida with 4 species (3 Oligocheata and 1Leech), then by Arthropoda 5 species (3 insects, 1 crab and 1 shrimp), and then by Mollusca with 6 species (5 gastropoda and 1 bivalvia).

Concerning to the total count of zooplankton, the results showed it was ranged from 10 to 8726 $ind.l^{-1}$. While, the total count of phytoplankton was ranged from 99573 to 754001$cell.l^{-1}$. The results statistical analysis revealed that there are a positive correlation between total count of phytoplankton and total count of zooplankton with $r = 0.455$, $r = 0.478$ and $r = 0.381$ in the site1, site 2 and site 3 respectively (Fig. 2,3 and 4).

**Table 1: minimum and maximum value of studied parameters during study period**

| Physico-chemical Parameters | Site 1 | Site 2 | Site 3 |
|---|---|---|---|
| Air temperature | 1-36 ˚C | 1-36.5 ˚C | 1-34 ˚C |
| Water temperature | 4-31˚C | 5-32.5 ˚C | 4-32 ˚C |
| Hydrogen ion concentration (pH) | 7.1-8.6 | 7.2-8.5 | 7.06-8.5 |
| Electrical conductivity (EC) | 163.39 -710.1µs.cm$^{-1}$ | 215-720µs.cm$^{-1}$ | 189.16-801.2µs.cm$^{-1}$ |
| Turbidity | 2.5-8 NTU | 1.5-6.6 NTU | 1-8 NTU |
| Dissolved oxygen | 3.9-6 mg.l$^{-1}$ | 4.1-6.1 mg .l$^{-1}$ | 3-6 mg .l$^{-1}$ |
| Biochemical oxygen demand ($BOD_{5)}$ | 4-26 mg .l$^{-1}$ | 1-2.5 mg.l$^{-1}$ | 0.7-2 mg .l$^{-1}$ |

**Table 2: list of benthic invertebrates recorded during studied period in Dukan lake**

| **Invertebrates** | | | **Site1** | **Site 2** | **Site 3** |
|---|---|---|---|---|---|
| **Nematoda** | **Adenophorea** | *Mylonchulus* sp. | + | | |
| | | *Plectus* sp. | + | | |
| | | *Rhabdolaimus* sp. | + | | |
| **Annelida** | **Oligochaeta** | *Chaetogaster diaphanus* | + | | |
| | | *Chaetogaster* sp. | + | | |
| | | *Aelosoma* sp. | + | | + |
| | **Hirudinea** | *Dina* sp. | | | + |
| **Arthropoda** | **Insecta** | *Ablabesmyia* sp. | + | + | + |
| | | *Caenis moesta* | + | + | + |
| | | *Tendipes* sp. | | | + |
| | **Malacostraca** | *Potamon magnum* | + | | |
| | | *Gammarus fasciatus* | + | + | |
| **Mollusca** | **Gastropoda** | *Lymnaea auricularia* | + | + | |
| | | *Physa gyrina* | + | + | |
| | | *Physa* sp. | + | + | + |
| | | *Promenetus exacuous* | + | + | |
| | | *Lymnaea auricularia* | + | + | |
| | **Bivalvia** | *Unio pictorum* | | + | |

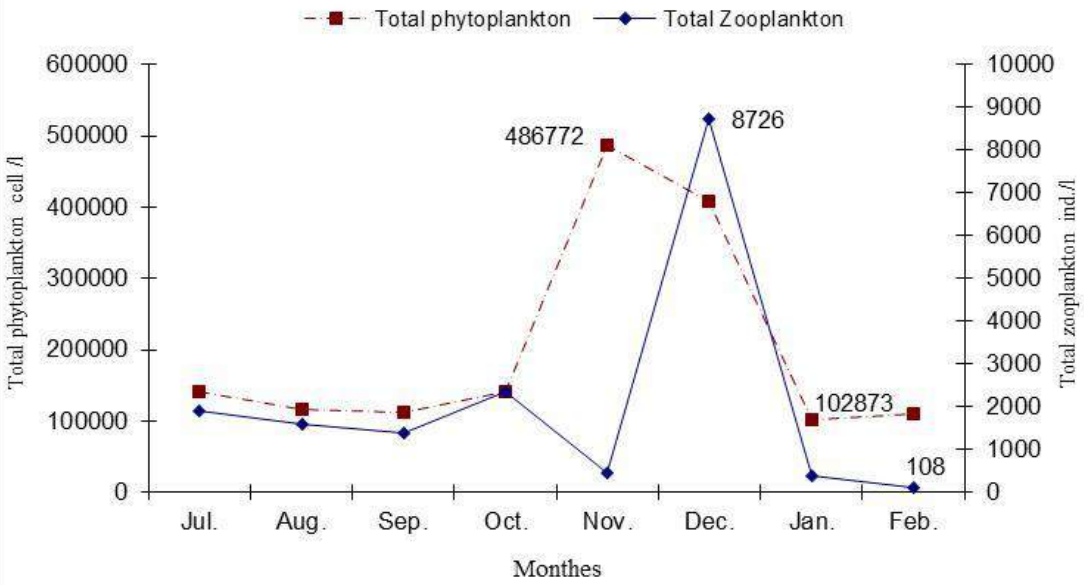


Fig. (2)Relationship between total zooplankton and total phytoplankton in Site 1 during study period

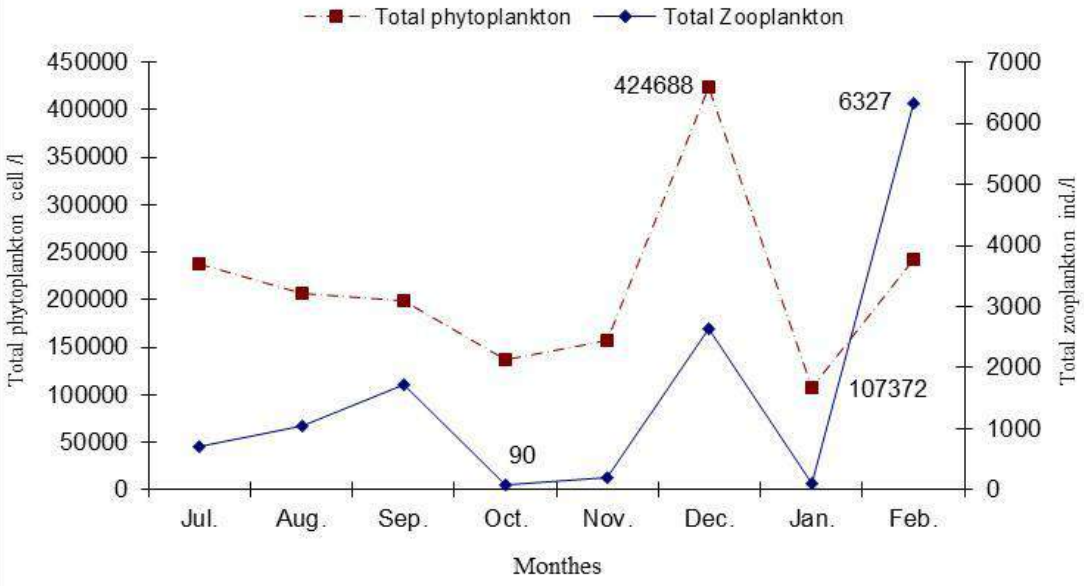


Fig. (3)Relationship between total zooplankton and total phytoplankton in Site 2 during study period

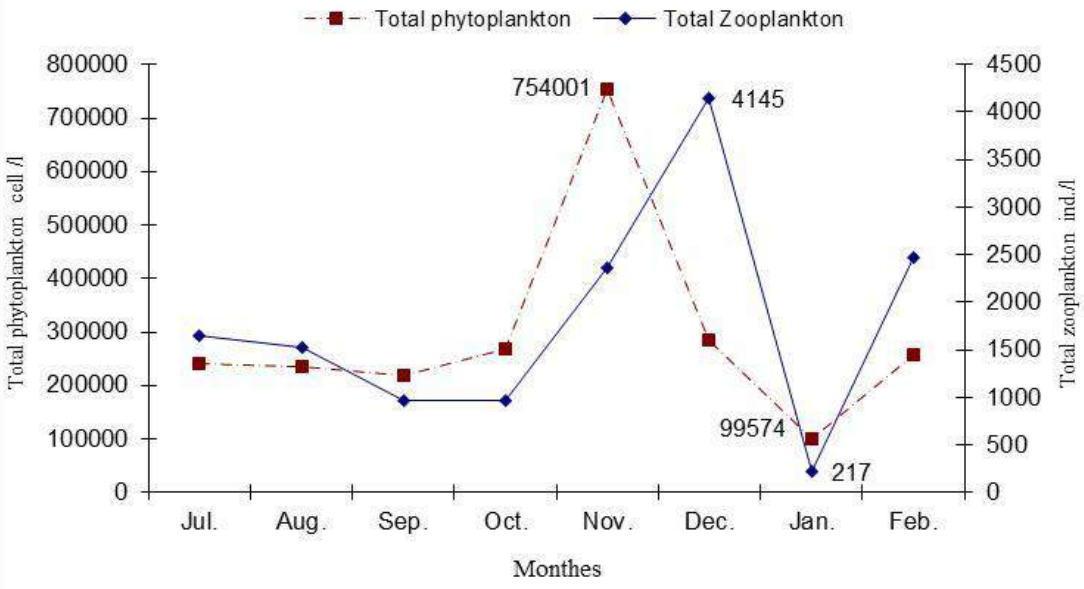


Fig. (4)Relationship between total zooplankton and total phytoplankton in Site 3 during study period

## DISCUSSION

In this study, the results of limnological parameter showed that the higher air and water temperature were 36.5°C and 32.5°C recorded at site 2 during July 2016. While, the lower air was 1°C recorded in all studied sites and the lower water temperature was 4°C was recorded in sites 1 and 3 during January 2016. The results of air temperature indicate that the studied area is characterized by cold winter and autumn, moderate spring, and warm summer and this come in accordance with (Guest, 1966). Also the variation of water temperature may be due to change in air temperature in addition to the other factors as solar radiation and turbidity (Dance and Hyness, 1980). Similar results were reported by (Bilbas, 2014) and (Dhahir, 2016).

Hydrogen ion concentration of studied lake were more than 7 and reached up to 8 many times during studied period and this is a normal condition for Iraqi inland water, which reflecting the geological formations of the area. In the present study the maximum pH value was 8.6 recorded in site 1 during August and the minimum value was 7.06 recorded in site 3 during November 2016. This result was agreed with the results reported by (Bilbas, 2014) and (Dhahir, 2016).

In the present study, electrical conductivity level was lower in summer and higher in winter season. The EC level of studied lake at the studied sites was ranged from 163.39 to 801.19µs.cm$^{-1}$. Lower level of EC was recorded in site 1 during July, while the higher level of EC was recorded in site 3 during January 2016. The fluctuation of EC

may be linked to the presence of chloride and dissolved ions that are the main constituents in water and directly affect the Ec value. The present results are close to the results reported by (Al-Ghafily and Al-Tamimi, 2009) in Habbaniya lake, and higher than that reported by (Toma, 2011) and (Bilbas, 2014) in the Dukan lake. On the other hand, the minimum value of turbidity (1 NTU) was recorded at site 3 during September 2016, while the maximum value (8 NTU) was recorded at site 1 during August 2016. The high level of turbidity in site 1 and 3 may be attributed to many reasons among them the activities of fisherman, tourists and discharge of pollutants from residential surrounding area.

Dissolved oxygen concentration of studied lake was ranged from 3 $mg.l^{-1}$ to 6.1 $mg.l^{-1}$ observed in site 3 during October and site 2 during December 2016 respectively. However, $BOD_5$ values were ranged between 0.7 to 26 $mg.l^{-1}$, the lower value of $BOD_5$ was recorded at site 3 during July, whereas the higher value was recorded at site 1 during August 2016. The results of present study come in accordance to the findings given by (Toma, 2013) and (Bilbas, 2014) in Dukan Lake.

Concerning to the benthic invertebrates study, 18 species were recorded belonged to 4 phylum and 7 classes with dominancy of insects in all studied sites during studied period and this results come in accordance with that reported by (Saadalla, 1998) in Himreen impoundment. However, Nematoda, Oligochaeta, Gastropoda and crab were observed mostly in site 1, while leech, shrimp and Bivalvia were recorded only in site 3 during the sampling period.

On the other hand, the results of total count of zooplankton and phytoplankton showed a clear peaks of growth in November and December and they revealed that there are a direct grazing relationships with r =0.455, r =0.478 and r =0.381 in the site1, site 2 and site 3 respectively.

The seasonal variations of zooplankton population in studied lake suggest that the most favourable period for growth is from October to the December, and this may be due to increase of phytoplankton population. The similar phenomenon was reported by (Ali, 2010).

Finally, from the results appear that the more study should be conducted on seasonal variation of zooplankton and phytoplankton in Dukan Lake especially in the other remaining months.

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# CFD Modeling of Simultaneous Flow Over Broad Crested Weir and Through Pipe Culvert using Different Turbulence Models

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**A B S T R A C T**

Culverts and broad crested weirs are hydraulic structures that could be used for measuring the flow rate in open channels. In this research, a simultaneous flow over broad crested weir and through pipe culvert was simulated using the numerical software ANSYS CFX. Three turbulence models were used for the modeling of flow turbulent to determine the best turbulence model for combined flow simulation. To achieve this purposes, results of simulations have been compared with data gathered from lab experiments from literature. The computational results showed a close agreement with obtained experimental data, but that of the (RNG) k – ε model provides more accurate results compared with other two turbulence models used in this study. Therefore, it can be concluded that this turbulence model (RNG) k – ε model can be used for simulation of simultaneous flow over broad crested weir and below through culverts.

## 1. INTRODUCTION

In the case of culvert overtopping with water, where the culvert cross sectional area is insufficient to drain the incoming flood, the ordinary solution is either to replace the old culvert with a bigger capacity one or to add new vents to the original one. An alternative solution is to use a hydraulic structure (broad crested weir with circular opening). In this case a part of the flow will go through the culvert vents (circular openings) and the rest will overtop it. (Mahmoud S. M. 2002) and (Negm A. M. 2002) conducted an experimental investigation on simultaneous flow through box culvert and over contracted broad crested weir. The flow at the culvert outlet is considered as submerged flow, a discharge prediction models have been developed by means of multiple linear regression techniques. (Othman K. Mohammed 2010) simulated experimentally the combined flow through pipe culvert and over broad crested weirs of different side slopes, he developed empirical relations between the discharge coefficient and geometrical parameters of the combined weir culvert model. Due to high cost in construction of the physical models (Kositgittiwong, 2012). . In addition, because of the difficulties in solving the high order partial differential equations of many fluid flow (Aziz, 2016). Nowadays, most researchers turn to the use of numerical methods. (Sarker and Rhodes 2004) compared the free surface profile over a laboratory rectangular broad crested weir with numerical CFD model using commercial software FLUENT applying both slandered k – ε model and (RNG) k – ε model, they reported that the

uncertainties in predicting the water levels above and D/S the crest were higher compared to the wave-like profile observed in the laboratory experiments. (Hargreaves D. M. 2007) conducted a serious of CFD simulations using version 6.2 of FLUENT, for predicting free surface profiles over broad crested weir, they used the experimental data of (Hager W. and Schwalt M. 1994) to verify the validity of the computational code in prediction the position of free surface profile, velocity and pressure distributions for different flow rates. (Afshar, H. and Hoseini, H. 2013) used CFD together with laboratory model in order to simulate the flow over rectangular broad-crested weir. Simulations were performed using three turbulence models of the RNG k–ε, standard k–ε and the large eddy simulation (LES) to find the water level profile and streamlines. Their results indicated that RNG model has lowest error compared with the other models. (S. Hoseini, S. Jahromi and M. Vahid 2013) used ANSYS FLUENT V.14 together with laboratory model for determining the discharge coefficient of the rectangular broad-crested side weir located on the trapezoidal channel, they found that both results of CFD and physical model showed that Cd coefficient decreases with increasing values of Fr and Cd coefficient increases with increasing values of Re. (Hoseini S. H. 2014) simulated the free surface flow over the triangular broad-crested weir using FLOW 3D. The simulation results were found in reasonable agreement with experimental observations. (Jalil, Shaker and Qasim, Jihan 2016) used FLOW-3D and HEC-RAS software's to predict the free surface profile of Flow over Single-Step Broad- Crested Weir, they found that HEC-RAS has limited ability to produce curved profiles past vertical faces, while FLOW-3D produced more accurate results. (Al-Hashimi A. S. 2017) used Fluent Software to compare four different turbulence models accuracy in computing free surface flow over broad crested weir and stepped weir with rounded corner. Results are compared with the experimental data and showed that the predictions provided by the standard k–ε model are closer to the experimental data, whereas those obtained from the standard k–ω model deviate the most. As found from literature survey that the characteristics of flow over broad crested weir along with the development of CFD codes have attracted the attention of many investigators. In this study, the flow characteristics through Combined Pipe Culvert and Broad Crested Weir were investigated using ANSYS-CFX 14. The results of the numerical model were compared with the experimental data of (Othman K. Mohammed 2010).

## 2. THEORETICAL ANALYSIS

Computational Fluid Dynamics (CFD) involves the solution of the equations of fluid flow (in a special form) over a region of interest, with specified (known) conditions on the boundary of that region. The set of the governing equations of fluid flow which are solved by ANSYS-CFX are the Reynold average Navier-Stokes equations. The governing equations of continuity and momentum for incompressible flow can be written as:

$$\frac{\partial}{\partial x_i}(\rho u_i) = 0 \qquad \dots\dots\dots (1)$$

$$\frac{\partial}{\partial t}(\rho\, u_i) + \frac{\partial}{\partial x_i}\left(\rho\overline{u_\iota u_j}\right) = -\frac{\partial P}{\partial x_i} + \frac{\partial}{\partial x_i}\left[\mu\left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i}\right)\right] + \rho g_i + \vec{F} \qquad \dots\dots\dots (2)$$

Where:

ρ = fluid density, $\overline{u_\iota u_j}$= average velocity in x and y directions, x and y = space dimensions, t = time, P = the pressure, μ =μo + μt , μo is dynamic viscosity and μt is turbulence

viscosity, gi = acceleration due to gravity and $\vec{F}$ = the body force.
The suffices i and j indicate that the stress component acts in the j-direction on a surface normal to the i-direction. (Versteeg H. K. and Malalasekera W. 2007)

ANSYS-CFX code uses finite volume method to convert governing equations to algebraic equation in order to be solved numerically. The Naiver - Stokes equations with time average velocity called Reynolds averaged Naiver – Stokes (RANS) equations. since the Navier-Stokes equations are non-linear, it is difficult to solve them analytically especially for turbulent flow. Because the size of the computational cells should be smaller than the length scale of the smallest turbulent this is impossible which cannot be achieved in many cases (Versteeg H. K. and Malalasekera W. 2007).

Turbulent models have been classified based on the application of their design and number of differential equations to create relation between turbulence stresses and averaged rates or their gradients. Among these models, two-equations model for modeling turbulence with RANS equations have been used, one-layer model such as k – ε and (RNG) k – ε and two-layer model such as shear stress transport (SST).

### 2.1. Standard k – ε model:

This model expresses the turbulent viscosity in terms of turbulent kinetic energy (k) and its dissipation rate (ε). The following two transport partial differential equations are solved for the values of k and ε (Launder and Spalding 1974)**:**

$$\frac{\partial}{\partial t}(\rho k)+\frac{\partial k}{\partial x_i}(\rho k u_i) = \frac{\partial}{\partial x_j}\left[\left(\mu+\frac{\mu_t}{\sigma_k}\right)\frac{\partial k}{\partial x_i}\right]+G_k-\rho\varepsilon \quad \ldots\ldots\ldots (3)$$

$$\frac{\partial}{\partial t}(\rho\varepsilon)+\frac{\partial \varepsilon}{\partial x_i}(\rho\varepsilon u_i) = \frac{\partial}{\partial x_j}\left[\left(\mu+\frac{\mu_t}{\sigma_\varepsilon}\right)\frac{\partial \varepsilon}{\partial x_i}\right]+C_{1\varepsilon}\frac{\varepsilon}{k}G_k-C_{2\varepsilon}\rho\frac{\varepsilon^2}{k} \quad \ldots\ldots\ldots (4)$$

The eddy viscosity μt is written as follows

$$\mu_t=\rho C_\mu\frac{k^2}{\varepsilon} \quad \ldots\ldots\ldots (5)$$

where $G_k=\mu_t S^2$

$S=\sqrt{2S_{ij}S_{ij}}$ and $S_{ij}=\frac{1}{2}\left(\frac{\partial u_i}{\partial x_j}+\frac{\partial u_j}{\partial x_i}\right)$

Model constants: C1ε = 1.44, C2ε = 1.92, Cμ =0.09, σk =1.0, and σε =1.3.

### 2.2. Renormalization Group (RNG) k – ε model (Choudhury D. 1993):

The (RNG) k-ε turbulence model is derived from the instantaneous Navier-Stokes equations, from using a mathematical technique called, “renormalization group" (RNG) methods. The analytical derivation results in a RNG model with constants different from those in the standard k-ε model and additional terms and functions in the transport equations for k and ε**.**

$$\frac{\partial}{\partial t}(\rho k)+\frac{\partial k}{\partial x_i}(\rho k u_i) = \frac{\partial}{\partial x_j}\left[\left(a_k\mu_{eff}\right)\frac{\partial k}{\partial x_i}\right]+G_k-\rho\varepsilon \quad \ldots\ldots\ldots (6)$$

$$\frac{\partial}{\partial t}(\rho\varepsilon) + \frac{\partial\varepsilon}{\partial x_i}(\rho\varepsilon u_i) = \frac{\partial}{\partial x_j}\left[\left(a_k\mu_{\varepsilon ff}\right)\frac{\partial\varepsilon}{\partial x_i}\right] + C_{1\varepsilon}\frac{\varepsilon}{k}G_k - C_{2\varepsilon}^{*}\rho\frac{\varepsilon^2}{k} \quad \dots\dots\dots(7)$$

$$G_{2\varepsilon}^{*} = G_{1\varepsilon} + \frac{C_\mu\rho\eta^3\left(1-\frac{\eta}{\eta_o}\right)}{1+\beta\eta^3} \quad \dots\dots\dots(8)$$

in which $\eta = \frac{S\,k}{\varepsilon}$

In above Equations, C1ε, C2ε, and Cμ are constants and equal to 1.42, 1.68, and 0.0845, respectively. ak and aε equal to 1.393, ηo equal to 4.38, μεff equal to 1 and β equal to 0.012**.**

### 2.3. Shear Stress Transport (SST) model (Menter FR, 1994):

Menter (1994) developed the SST turbulence model to blend effectively the robust and accurate formulation of the k- ω model in the near-wall region with the free stream independence of the k- ω model in the far field. It is an eddy-viscosity model which includes two main novelties:

- It is combination of a k-ω model (in the inner boundary layer) and k-ε model (in the outer region of and outside of the boundary layer);
- A limitation of the shear stress in adverse pressure gradient regions is introduced.

The transport equations and effective viscosity are modelled in SST k-ω model, by the following equations**:**

$$\frac{\partial}{\partial t}(\rho k) + \frac{\partial}{\partial x_i}(\rho k u_i) = \frac{\partial}{\partial x_j}\left[\left(\mu+\frac{\mu_t}{\sigma_k}\right)\frac{\partial k}{\partial x_j}\right] + G_k - Y_k \quad \dots\dots\dots(9)$$

$$\frac{\partial}{\partial t}(\rho\omega) + \frac{\partial}{\partial x_i}(\rho\omega u_i) = \frac{\partial}{\partial x_j}\left[\left(\mu+\frac{\mu_t}{\sigma_\omega}\right)\frac{\partial\omega}{\partial x_j}\right] + G_\omega - Y_\omega + D_\omega \quad \dots\dots\dots(10)$$

$$\mu_t = \frac{\rho k}{\omega}\frac{1}{\max\left[\frac{1}{\alpha^*}, \frac{\sqrt{2\Omega_{ij}\Omega_{ij}}F_2}{\alpha_1\omega}\right]}$$

in which $\Omega_{ij} = \frac{1}{2}\left(\frac{\partial u_i}{\partial x_j} - \frac{\partial u_j}{\partial x_i}\right)$

α* damps the turbulent viscosity causing a low Reynolds number correction

$G_k$, $G_\omega$ is the generation of $k$ and $\omega$

and $D_\omega = 2(1-F_1)\ \frac{\rho\sigma_{\omega 2}}{\omega}\frac{\partial k}{\partial x_j}\frac{\partial\omega}{\partial x_j}$

$$\sigma_k = \frac{1}{F_1/\sigma_{k1} + (1-F_1)/\sigma_{k2}}$$

$$\sigma_\omega = \frac{1}{F_1/\sigma_{\omega 1} + (1-F_1)/\sigma_{\omega 2}}$$

F1, F2 are the blending functions

$Y_k$, $Y_\omega$ represent the dissipation of $k$ and $\omega$ due to turbulence

## 3. EXPERIMENTAL DATA

The experimental data (Table 1) used for the comparisons were taken from laboratory tests conducted by (Othman K. Mohammed 2010). The geometry and dimensions of the combined broad crested weir and pipe culvert model are stated in Fig. (1). The experiments were conducted in a horizontal research flume with a width of 0.5 m, a height of 0.5 m and a total length of 12 m. the laboratory model was made of concrete box shape like of dimensions (50 x 50 x 13.1 cm), containing a plastic pipe 10.6 cm diameter. The notations in this paper are kept identical to those defined by (Othman K. Mohammed 2010).

Table 1, Experimental data.

| Run | H/P | Q l/sec | Cd |
|---|---|---|---|
| 1 | 0.180 | 10.780 | 0.526 |
| 2 | 0.240 | 12.360 | 0.521 |
| 3 | 0.310 | 15.140 | 0.542 |
| 4 | 0.400 | 17.950 | 0.527 |
| 5 | 0.480 | 21.620 | 0.531 |
| 6 | 0.510 | 22.720 | 0.535 |
| 7 | 0.560 | 24.900 | 0.53 |
| 8 | 0.590 | 28.270 | 0.569 |
| 9 | 0.760 | 37.010 | 0.566 |
| 10 | 0.930 | 47.350 | 0.575 |

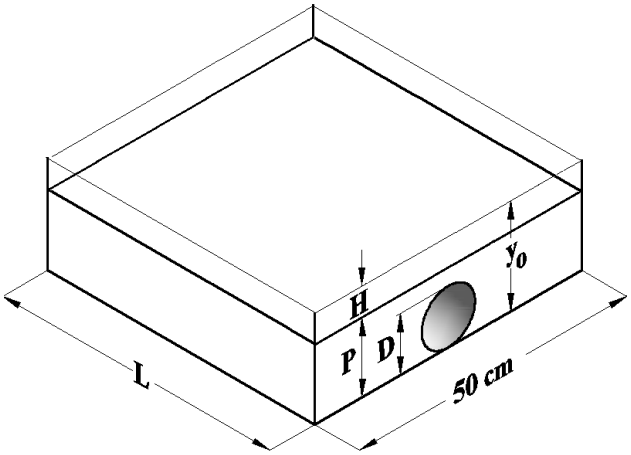


Fig. (1) Geometry of the tested Model D = 10.6 cm, P = 13. 1cm, L = 50 cm

## 4. NUMERICAL MODELLING

The numerical model was constructed at the same dimensions as the physical model. This allows direct comparison of the predicted results with physical model results.

### 4.1. Mesh Design

Meshing is an important step to solve the hydraulic systems in numerical modelling. According to earlier studies, the smaller the mesh size the greater is the accuracy and the more is the computational time (Aziz, Y. W. 2016). ANSYS ICEM was used for mesh generation as it contains many methods for mesh generation. In this study the Multi-zone method was used with the maximum and minimum mesh size of 0.05 m and 0.000196 m respectively such as mesh sizes used by (Aziz, Y. W. 2016), and the hexahedron mesh type was provided as shown in Fig. (2).

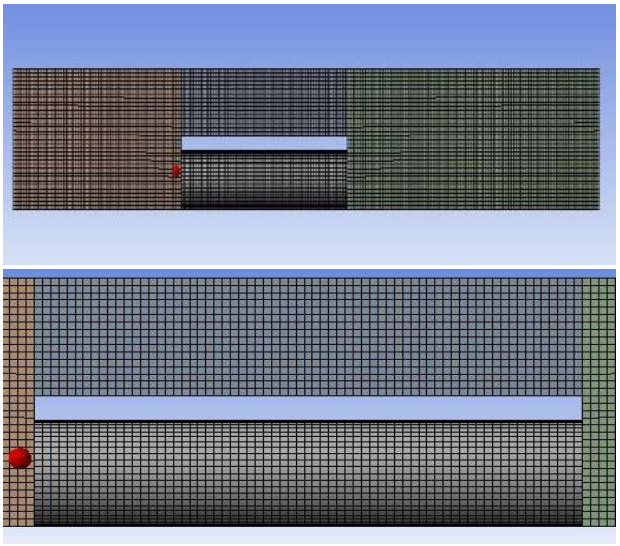

Fig. (2) Meshing and its Distribution

### 4.2. Boundary conditions

ANSYS-CFX contains several boundary conditions including inlet, outlet, opening and wall. Fig. (3). Inlet boundary condition imposed at inlet section with the average velocity, water and air volume fraction. Static pressure used at the outlet and opening boundary condition was specified for the top of the fluid domain. On the walls, the no slip wall boundary condition was applied; that is the fluid velocity next to the wall immediately is equal to zero. Walls were assumed to be smooth, since the channel sides were made from glass.

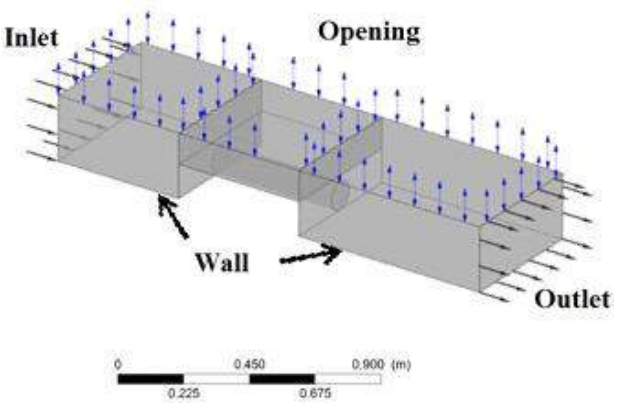


Fig. (3) Boundary conditions

## 5. RESULTS AND DISCUSSION

To have access to an appropriate turbulence model for the simulation, the numerical model is examined with different models of turbulence such as standard k – ε model, RNG k – ε model and SST model under the same conditions (boundary condition, material, mesh and so on). Then the results of these turbulence models are compared with those provided by the experimental data

The experimental and numerical results of discharge through combined broad crested weir and pipe culvert were plotted as shown in Fig. (4). The results for all turbulence models with the experimental data are very close to each other, but some of them are in closer agreement to the experimental data as presented in table 1.

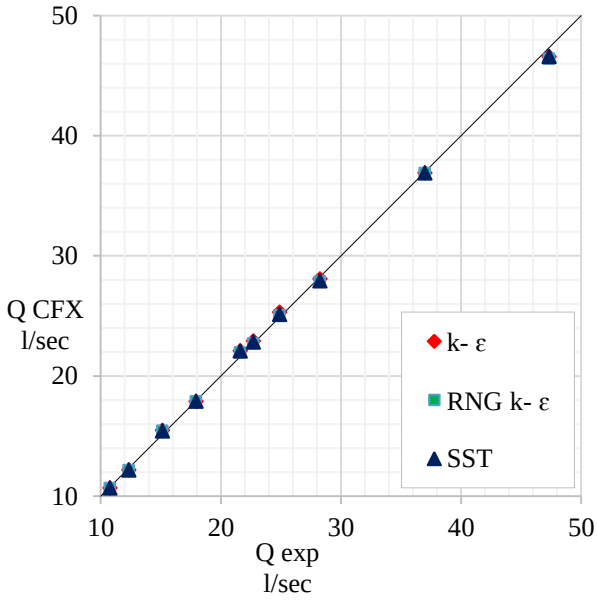


Fig. (4) Comparison of $Q_{CFD}$ with $Q_{Exp}$ for Combined Weir and Culvert

Slight deviations are observed between the predicted by numerical model and the measured values from Fig. (4)

Fig. (5) shows the 3D and longitudinal section at the center line of the simulated velocity distribution through the culvert and over the weir predicted by the k- ε turbulence model for discharge flow rate of 28.27 l/s. Since from the experiment there is no any measurement of velocity, so the comparion with the CFD modelling can not be done.

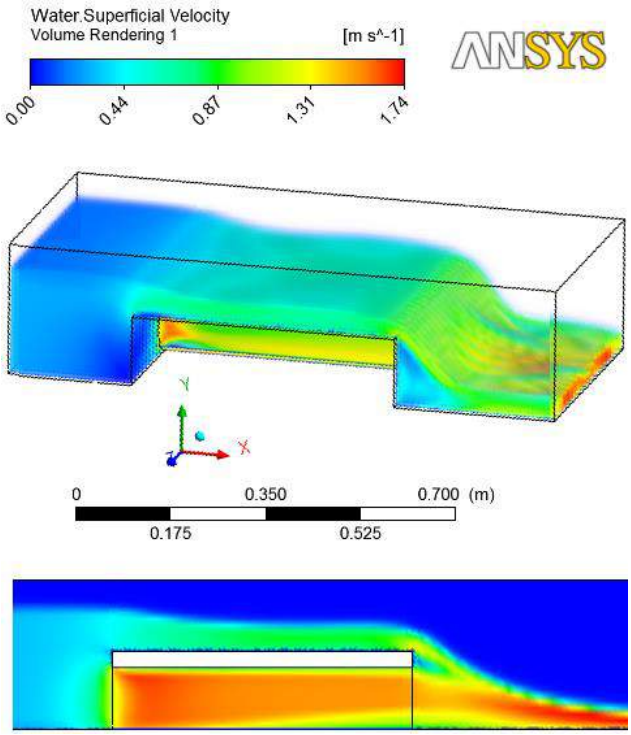


Fig. (5) Velocity distribution through combined structure for discharge 28.27 (l/sec) using k-ε turbulence model.

Table (2) shows that discharge coefficient results obtained from SST turbulence are mostly closer than the other models to the experimental data. Further, it can be observed that the RNG k- ε turbulence model performs better than k- ε model. In addition, k- ε has lesser agreement with the experimental data as it has higher error percentage.

Table (2) Discharge coefficient and relative error of the numerical simulations

| Exp. | CFX | | | | | |
|---|---|---|---|---|---|---|
| | Standard k- ε | | RNG k- ε | | SST | |
| Cd | Cd | Error % | Cd | Error % | Cd | Error % |
| 0.526 | 0.521 | 1.018 | 0.519 | 1.267 | 0.521 | 0.939 |
| 0.521 | 0.514 | 1.433 | 0.512 | 1.742 | 0.513 | 1.541 |
| 0.542 | 0.554 | 2.163 | 0.552 | 1.849 | 0.552 | 1.934 |
| 0.527 | 0.525 | 0.391 | 0.525 | 0.468 | 0.525 | 0.288 |
| 0.531 | 0.542 | 2.079 | 0.539 | 1.468 | 0.542 | 2.064 |
| 0.535 | 0.539 | 0.840 | 0.535 | 0.023 | 0.538 | 0.564 |
| 0.53 | 0.539 | 1.658 | 0.533 | 0.554 | 0.535 | 0.888 |
| 0.569 | 0.565 | 0.675 | 0.560 | 1.536 | 0.562 | 1.280 |
| 0.566 | 0.565 | 0.202 | 0.565 | 0.263 | 0.565 | 0.188 |
| 0.575 | 0.566 | 1.625 | 0.563 | 2.064 | 0.566 | 1.582 |

Discharge coefficients Resulted from Applying turbulent models together with experimental values plotted against relative upstream water depth (H/P) are shown in Fig. (6). In this figure H and P stand for the head of water above the weir and weir height respectively. It can be seen that, for all cases Cd increased with (H/P) increasing this is due to increase the ratio of (Flow cross sectional Area/Contracted parameter).

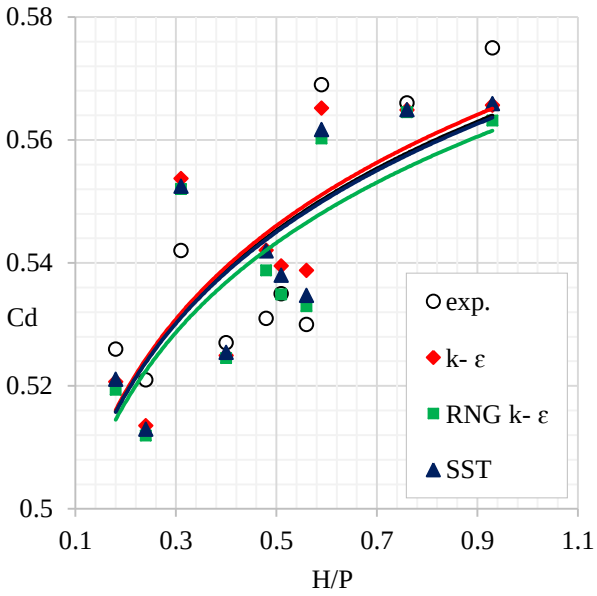


Fig. (6) Head-discharge coefficient of the numerical simulations and Experimental data

In order to determine the accuracy of the simulation results, the Relative Error percent (E %) of the experimental and the numerical discharge results are calculated using the equation:

$$E\,\% = 100\frac{1}{n}\sum_{i=1}^{n}\left|\frac{Cd_{CFX} - Cd_{Exp}}{Cd_{Exp}}\right|$$

Table (3) Relative Errors of the average discharge coefficient

| Exp. | CFX | | | | | |
|---|---|---|---|---|---|---|
| | Standard k- ε | | RNG k- ε | | SST | |
| Cd | Cd | Error % | Cd | Error % | Cd | Error % |
| 0.542 | 0.543 | 1.208 | 0.540 | 1.123 | 0.542 | 1.127 |

Errors for discharge coefficient Resulting from applying different turbulent models are shown in Fig. (7).

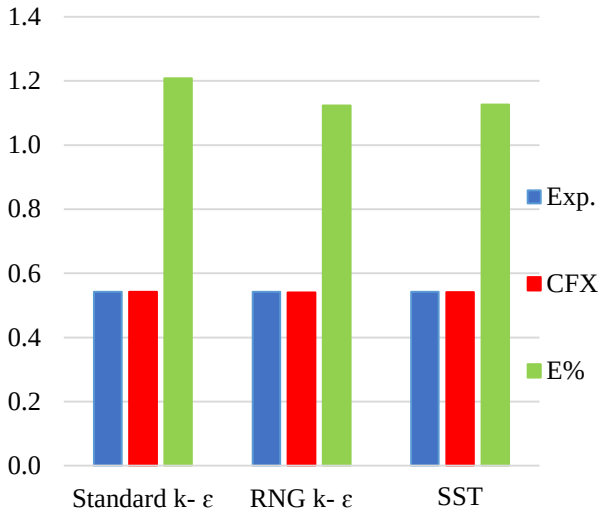


Fig. (7) Discharge coefficient and their Relative Errors from Applying Different Turbulent Models

It can be seen that (Fig 6 and Fig 7) the results of k- ε turbulence model for discharge coefficient are greater than those obtained by experiments, but those of both (RNG) k – ε and SST are lower than experimental results. From table (3) it is clear that higher percentage error obtained by using k – ε turbulence model, while using RNG k – ε has lower average percentage error with the experimental data. These differences can be clearly seen in charts shown in Fig. (7).

## 6. CONCLUSIONS

In the present study, flow over broad crested weir combined with the circular culvert is simulated using ANSYS – CFX. The sensitivity of the results obtained from CFD modeling of different turbulence models is examined, to determine the turbulence model that gives accurate predict of flow through the combined structure. The summery of the results of this study can be defined as follows:

1) SST model perform much better has the maximum accuracy in comparison with other turbulence models for most discharges values, but for average relative percentage error, (RNG) k- ε has a bit greater accuracy than SST.
2) The results of k- ε have less accuracy compared with other turbulence models.
3) The discharge coefficient in all methods slightly increased with upstream relative head (H/P) which was (from 0.526 to 0.575) such variation may be considered as constant, same conclusion indicated by (Hager W. and Schwalt M. 1994) for broad crested weir flow.
4) It is also concluded that, such type of software is useful to study the number of fluid flow problems without going for expensive and time consuming experiments.

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# Effect of Hybrid Teaching Methodology and Student Group Policy on Object Oriented Problem Solving

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**A B S T R A C T**

Teaching programming at university for beginner's level represents one of the most difficult tasks for faculty members. It has many challenges such as wide diversity of student's background knowledge, not able to understand programming concept, identifying programming language syntax usage etc. Through the literature, many methods and tools have been covered to make programming concepts easier to understand for students. In this paper, hybrid approach is proposed to efficiently increase the understanding capacity of the students. The hybrid method is based on problem-based and puzzle-based learning (PBL & PZBL) methods. In order to test the proposed approach, students enrolled in object oriented programming courses in software engineering department were considered as test case. In addition, the paper addresses the benefits of using non-fixed student group policy with the proposed hybrid method to get a better understanding of student learning outcome. The proposed method scored a better result when compared to standalone methods. The enhancement was reflected both in student questionnaire and final course work results.

## 1. INTRODUCTION

Programming languages represents one of the most important topics in computer science. It is a core subject where students will gain the necessary tools for creating software programs. In software engineering, programming language represents the core tool used during development phase of software development cycle. Therefore, it is very crucial for developers to have a very good knowledge in programming language, especially, in object oriented programming, as it is the main programming paradigm used in developing windows, web and mobile based applications (Eckerdal, 2006).

Learning object oriented programming (OOP) is one of the core requirements for every modern developer. It is one of the main subjects in computer and software related departments where learners or students will gain the required skills for understanding the concepts of OOP. However, teaching this subject is one of the main challenges for academic staff, as most of the students will find it difficult to understand (Pereira, 2010). Therefore, many researches in the field of teaching OOP emerged to establish the best tool or methodology to simplify the subject, as well as, to motivate the student and get them

involved in the learning process (Teague, 2007).

In this paper, a hybrid algorithm based on two well-known strategies which are Problem-based and Puzzle-based learning methodologies. The proposed strategy tries to merge the strength of both strategies so that a maximum learning outcome is achieved. The remaining of this paper is organized as follows, in section 2, a comprehensive literature is presented that covers the tools and methods used in teaching programming as general and OOP as special. In section 3, the proposed hybrid method based on Problem-based Learning (PBL) and Puzzle-based Learning(PZBL) is introduced. Section 4, covers the result and discussion of applying the proposed strategy. Finally, the final driven conclusion is covered in section 5.

## 2. RELATED WORKS

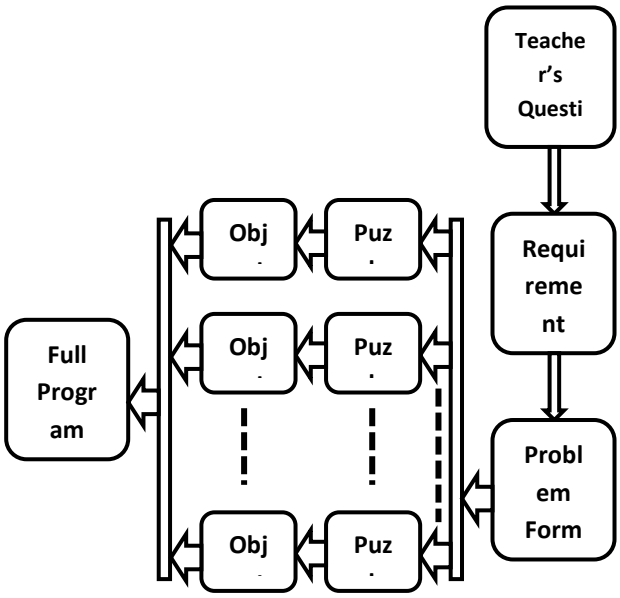
Teaching methodology in programming was initiated at the early 80's. The subject importance was gaining momentum because of the use of computers in various aspect of life (Nuutila, 2008). The focus was on procedural and low-level programming at college level to help students gain maximum knowledge on the subject (Montero, 2010). However, during 90's, when OOP based languages, such as Java, C++ and ADA, started to spread rapidly, researchers started to find new methods and tools to teach programming (Pilkington, 2010; Looi, 2014; Lykke, 2014).

There are numerous papers and researches on teaching methodology in programming. The main focus of these papers is either to introduce the students with new teaching paradigms, or designing a special tool for simplifying the understanding of the subject (Vogel-Heuser, 2012; Teague, 2007). OOP languages such as Java and C++, has been used to develop various applications and software for many years. The methodologies used with OOP paradigm varied from problem based, buzzle based, game based and hybrid based (Ala-Mutka, 2005; Bellstrom, 2009; Fee, 2010; Montero, 2010; Dietrich, 2014). The problem based methodology was basically used to motivate medical school students to find a solution for any problem they face during their medical education (Beaumont, 2003; Dijkstra, 1989). Each problem must pass through seven steps to find a propoer solution. These steps are 1) terms and concept clarification; 2) problem identification; 3) brainstorming; 4) explanatory model sketching; 5) learning issue formulation; 6) self-learning and 7) information synthesising and testing (Falkner, 2010). These concepts can be easily integrated in programming problems, as the students will try to solve any problem by writing a program (Kay, 2000). These researches have been motivated by the increased importance of programming languages in computer science and software engineering (Nuutila, 2007; Falkner, 2010, Livovsky, 2014). In PBL for Programming, the problem is also seen as a learning core for student to understand how problem is formed, analyzed and solved (Nuutila, 2007; Malik, 2017). The process involves a learning scenario (Yoneyama, 2008), or concerning a large scale problem (Livovsky, 2014; Montero, 2010) or related to learning outcomes of the course (Nuutila, 2007; Livovsky, 2014; Kramer, 2016; Thota, 2010; Sooriamurthi, 2015). The algorithm also was integrated fo some challenging problems (Livovsky, 2014; Kramer, 2016, Malik, 2017), or complex problem (Peng, 2010), and ill-structured. The features of the studied solutions may relate to special type of problems that requires an open-mindedness (Mathews, 2013; Santos, 2013), or realworld simulation (Kramer, 2016; Peng, 2010), activation of prior knowledge (Thota, 2010; Santos, 2013), integration of theory and practice (Thota, 2010), increase of problem

complexity and sufficient solution for a duration of time (Karmer, 2016), followed by validation (Malik, 2017). However, the study found several problematic characteristics in PBL. Due to time and erratic changing of the curriculum constraint in Computing courses, problems that are well-defined, well-structured, and uncomplicated are much more preferable (Yoneyama, 2008).

Puzzle based learning (PZBL) is a creative way of learning in which students deals with any programming problem as a puzzle (Pereira, 2010). The idea is to initiate the thinking of students about a problem in a way that it will motivated them to think about it in different way (Redmond, 2001). There was a various researches in the field of puzzle based problem for various problems, however, there were few that dealt with programming in computer science and engineering courses (Pereira, 2010), or by using it for OOP language learning (Merrick, 2010), or to evaluate its empact on introductory courses in computer science (Redmond, 2001). It was adapted with student learning history in programming to efficiently enhance the outcome of student programming learning process (Tuikys, 2016). It was also used to help students understand the design concept of Design Patterns for software engineering students (Rusu, 2011).

**Figure 1: Proposed system for Hybrid learning method for OOP programming**




In this research, a hybrid learning method is proposed to solve various programming problems in OOP. The method uses both methods to consider some of the problems studied in second year software engineering. The following sections will thoroughly explain the methodologies used in this research.

## 3. PROPOSED METHOD

The following Fig. 1, explains the steps used in this research. The steps show how the question forms a problem and then the problem

is divided into puzzles and finally is merged to form one program.

In the first step, the question is initiated by the teacher for the students to find a programming solution. Then, the students will try to find a problem-based form of the question such that the requirements are clear and the final targets are specified. These specified requirements are a little hard to understand from programming view and need to be simplified. In most cases, the problem in hand can be complex where multiple independent requirements are formed. Therefore, each problem is divided into smaller sub problems which can be easily understood, and clearer set of requirements are obtained for each one.

The puzzle-based learning is used to transform these requirements from a problem-based to a puzzle per sub-problem. These puzzles are easier to understand by the students and will from a brain challenge for them to find a proper solution (O'Grady, 2012). Hence, an object (class) is will represent each puzzle. These objects are programmed and tested independently and therefore, can be assigned to different groups to solve each one of them. Finally, all these puzzles (objects) are merged together to form one big program which then is tested for integration and verified against meeting the initial requirements.

## 4. RESULTS AND DISCUSSION

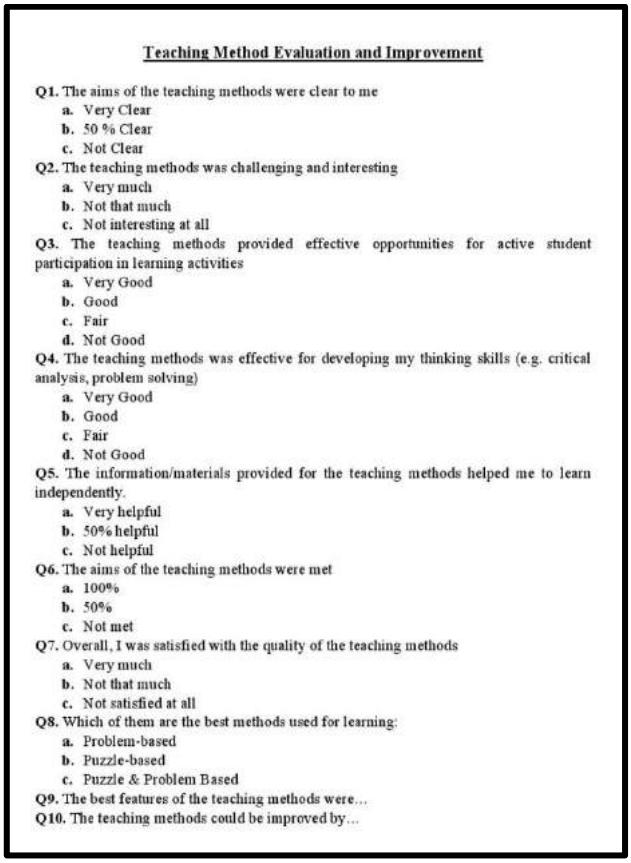
### 4.1 Experiment setup

The proposed system was integrated in software engineering second and third year courses of Object Oriented Programming and Mobile Application using Android during the academic years between 2013-2015. Both courses use Java as a programming language which is an OOP programming based language. The students were setup in groups that can form a sub groups within it. During the subject curriculum, the students were presented with various type of software systems which varies in difficulty, starting with simple problems and finishing with complex systems. In the first study year the problem-based learning was integrated in the studying system without the puzzle-based learning. While in the second year only puzzle-based year was integrated. Finally, the proposed method was integrated in the third year.

At the end of the courses a questionnaire was distributed among the students to find the impact of the learning methods on the students and the feedback was used for further assessment. Table 1 lists the questionnaire used and the question types asked during the process. It is worth noting, that the student groups were selected randomly and fixed during the experiment to guarantee the correctness of the results.

### 4.2 Results and discussion

The result of the questionnaire is illustrated in Table 1 for the above questions with the results clearly demonstrated that the proposed method has a good impact on student learning outcome.



**Teaching Method Evaluation and Improvement**

**Q1.** The aims of the teaching methods were clear to me
- **a.** Very Clear
- **b.** 50 % Clear
- **c.** Not Clear

**Q2.** The teaching methods was challenging and interesting
- **a.** Very much
- **b.** Not that much
- **c.** Not interesting at all

**Q3.** The teaching methods provided effective opportunities for active student participation in learning activities
- **a.** Very Good
- **b.** Good
- **c.** Fair
- **d.** Not Good

**Q4.** The teaching methods was effective for developing my thinking skills (e.g. critical analysis, problem solving)
- **a.** Very Good
- **b.** Good
- **c.** Fair
- **d.** Not Good

**Q5.** The information/materials provided for the teaching methods helped me to learn independently.
- **a.** Very helpful
- **b.** 50% helpful
- **c.** Not helpful

**Q6.** The aims of the teaching methods were met
- **a.** 100%
- **b.** 50%
- **c.** Not met

**Q7.** Overall, I was satisfied with the quality of the teaching methods
- **a.** Very much
- **b.** Not that much
- **c.** Not satisfied at all

**Q8.** Which of them are the best methods used for learning:
- **a.** Problem-based
- **b.** Puzzle-based
- **c.** Puzzle & Problem Based

**Q9.** The best features of the teaching methods were…

**Q10.** The teaching methods could be improved by…

**Figure 2: The questionnaire used for student feedback about the proposed hybrid method compared to the problem-based and puzzle-based learning**

Out of 32 students, 19 students preferred the proposed method over the problem-based (6 students) and puzzle-based (5 students). In addition, the method feedback showed a better impact in the sections of learning understanding, develop thinking skills, clearness and satisfaction.

In addition to the above questionnaire, the impact of the proposed method where observed in the field of student improvement during exams. The students were divided into 7 groups with each group consists of 4-5 students and these students can be sub-grouped into 2-3 subgroups.

**Table1: Questions and Result of the Questionnaire about the Proposed Method**

| Question | a | b | c | d |
|---|---|---|---|---|
| The aims of the teaching methods were clear to me? | 16 | 15 | 1 | |
| The teaching methods were challenging and interesting | 16 | 13 | 3 | |
| The teaching methods provided effective opportunities for active student participation in learning activities | 5 | 22 | 4 | 1 |
| The teaching methods were effective for developing my thinking skills (e.g. critical analysis, problem solving) | 7 | 18 | 6 | 1 |
| The information/materials provided for the teaching methods helped me to learn independently | 20 | 12 | 0 | |
| . The aims of the teaching methods were met | 4 | 27 | 1 | |
| Overall, I was satisfied with the quality of the teaching methods | 11 | 19 | 2 | |
| Which of them are the best methods used for learning? | 6 | 5 | 19 | 2 no vote |

The practical testes where introduced to the groups as a part of their activities during lab sessions, such that, each week, the student attempt to solve the same problem using problem-based, puzzle-based and hybrid method. Then, the time and mark was registered (out of 10) for each method to find the improvement in student performance. The following Tables 2 and 3 show the seven test results of problem-based and proposed hybrid method for seven group of students. The tests various from simple to complex problem.

**Table 2: Problem-Base Learning Test Results**

| Group | Test1 | Test 2 | Test 3 | Test 4 | Test 5 | Test 6 | Test 7 |
|---|---|---|---|---|---|---|---|
| 1 | 8.3 | 8.6 | 7.1 | 9.3 | 10.0 | 10.0 | 10.0 |
| 2 | 6.0 | 7.4 | 8.1 | 10.0 | 10.0 | 9.7 | 2.8 |
| 3 | 7.7 | 7.7 | 3.9 | 8.3 | 10.0 | 7.2 | 10.0 |
| 4 | 8.0 | 8.6 | 7.4 | 10.0 | 8.9 | 7.2 | 6.4 |
| 5 | 9.7 | 8.9 | 9.0 | 9.7 | 10.0 | 7.8 | 10.0 |
| 6 | 10.0 | 10.0 | 7.1 | 10.0 | 10.0 | 9.7 | 6.4 |
| 7 | 10.0 | 8.0 | 7.4 | 10.0 | 7.2 | 9.7 | 9.5 |
| **Average Mark** | 8.53 | 8.46 | 7.14 | 9.61 | 9.44 | 8.76 | 7.87 |

Table 3: Hybrid Learning Test Results

| Group | Test1 | Test 2 | Test 3 | Test 4 | Test 5 | Test 6 | Test 7 |
|---|---|---|---|---|---|---|---|
| 1 | 9.3 | 8.6 | 9.4 | 10 | 10.0 | 10.0 | 9.7 |
| 2 | 10.0 | 9.4 | 9.0 | 10 | 10.0 | 10.0 | 10.0 |
| 3 | 9.7 | 8.9 | 9.7 | 10 | 10.0 | 10.0 | 10.0 |
| 4 | 10.0 | 9.7 | 9.4 | 10 | 10.0 | 10.0 | 10.0 |
| 5 | 10.0 | 9.7 | 9.0 | 10 | 8.9 | 10.0 | 10.0 |
| 6 | 8.3 | 9.7 | 9.7 | 10 | 7.8 | 10.0 | 10.0 |
| 7 | 7.3 | 10.0 | 9.4 | 10 | 8.3 | 9.7 | 4.6 |
| **Average Mark** | **9.23** | **9.43** | **9.37** | **10.00** | **9.29** | **9.96** | **9.19** |

From the above two tables, it is obvious that the average mark and group marks shows a great improving in marks except for one of the tests. The improvement reflects the easier understanding of the problem when it is divided into multiple puzzles to form objects. This is also reflected in total time required to solve a problem using the proposed method

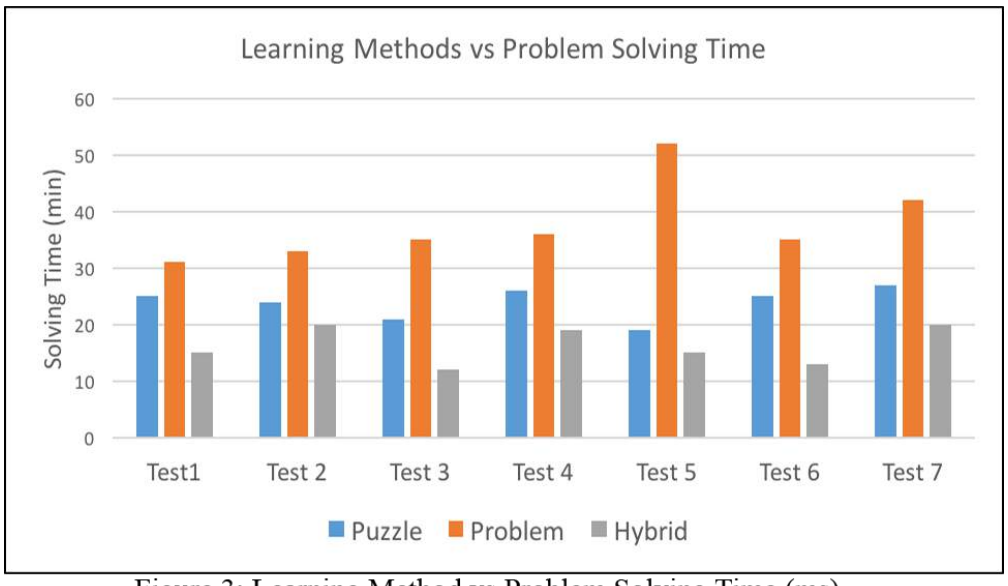



Figure 3: Learning Method vs Problem Solving Time (ms).

compared to problem-based learning method as illustrated in table 4 and Fig. 3.

Table 4: Comparing Solving Time (min) for each Learning Method

| Group | Test1 | Test 2 | Test 3 | Test 4 | Test 5 | Test 6 | Test 7 |
|---|---|---|---|---|---|---|---|
| Puzzle | 25 | 24 | 21 | 26 | 19 | 25 | 27 |
| Problem | 31 | 33 | 35 | 36 | 52 | 35 | 42 |
| Hybrid | 15 | 20 | 12 | 19 | 15 | 13 | 20 |

The main reason beyond the long time in problem-based learning compared to the other two methods, is that, the problem based method gives the problem as an overall requirement which the student needs to understand and break to pieces. This process is Thus, simplifying the problem in this form makes it understandable and easier to understand the purpose of OOP programming. Finally, it is worth mentioning that not all problems will prove solvable and understandable using the proposed method. In some cases, when the problem is much simpler, the student can solve the problem using one object, and thus the method loses its efficiency.

## 5. CONCLUSION

Teaching programming to university students represent one of the great challenges facing lecturers in computer related department especially OOP. This paper presents a hybrid method using both problem-based learning and puzzle-based learning methods. The method works in forming a basic problem with requirements from the question in hand. Then, this problem is broken into a set of puzzles and these puzzles will form an individual object. This object will be easier to program and test compared to the basic method. The method was tested on a group of student studying OOP in software engineering and the obtained results showed the improvement in student learning level compared to stand alone problem and puzzle based methods.

the most important and difficult part of programming. Hence, the student will take much time to analyze the problem. Meanwhile, in puzzle-based learning, the student will deal with a set of requirements as a puzzle, which make it much easier to understand than problem-based but it will take a little longer for student to form the problem statement. Finally, the hybrid method will make it simpler to the student to obtain the problem, divided into pieces of puzzles and creating an object for each piece of them. Therefore, the total time will be much shorter than the other two methods.

In general, it has been shown through this paper that, the proposed hybrid method proved to be more efficient than both problem-based and puzzle based used by many researches to teach programming language especially OOP. The method strength comes from the strategy used in forming the main problem into a set of puzzles and making each puzzle works as an object.

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# Knocking out the *pptA* gene in *Aspergillus fumigatus* could result in new protein profile and promising targets for antifungals

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**A B S T R A C T**

The filamentous fungus *Aspergillus fumigatus* is the most considerable common opportunistic pathogen. This is due to the prevalence of its occurrence in patients with immune debilities. It can cause diseases with impact extends to life-threatening seriousness. This fungus recently showed interesting means of adaptation, biofilm formation, and morphogenesis within the host biome. Its ability to invade and systematically disperse in the patient's body is of great concern. The complications of antifungal therapy and the limited target available to treat such infections, urge intensive and thorough research to uncover new more effective targets. The present study revealed a novel possibility of making the *pptA* an effective address in the fungus for antifungal treatment. Knocking-out the *pptA* gene resulted in halting pathogenecity as it correlated with essential genes of pathogenecity *medA* which showed considerable down regulation. Significant increase of protein profile has been discovered in Δ*pptA* strains as *UBC* gene highly activated and protein assay revealed high rate of production. In conclusion, the present study emphasize *pptA* as a possible strong antifungal target and declares high protein output from Δ*pptA* which may be a promised strain for industrial and beneficial protein production.

## 1. INTRODUCTION

Annually, there are reports of deaths of around 2 million people in the world due to fungal infections (Brown *et al.*, 2012). Such ratio of death is even beyond the mortality rate of malaria (Vos *et al.*, 2012). The most encountered and considered infections by a filamentous fungus are related to the *Aspergillus fumigates* (*A. fumigatus*) (Hospenthal, *et al*, 1998). It can cause life threatening invasion in patients. Vast majority of infections with *Aspergillus fumigates* is ended up with death (Gupta *et al*, 2012). This is specifically in patients with immune system debility. Immune system may be debilitated due to many reasons, such as Acquired immunodeficiency Syndrome (AIDS), leukemia, transplantation, and chemotherapy receiving patients (Brown *et al.*, 2012). The fungus has the ability to produce high rate of conidia. These spore structures are then spread through air into the environment (Filler and Sheppard, 2006). Human without noticing may inhale more than

hundred of these spores a day while breathing, specifically in areas contaminated with such conidia. These small tiny structures can easily penetrate the air passages into the alveoli of the lung (Chandrasekar *et al*, 2000). However, in normal circumstances as the body defense system working, it can wipe out these spores through the innate and acquired immune army. Nevertheless, for patients who suffer immune system debility or suppression the presence of those spores are problematic, as failure to eradicate those conidia may eventually lead to germination and growth of the *Aspergillus fumigatus* (Ben-Ami *et al*, 2010). This can cause tissue invasion and aspergillosis may arise. Aspergillosis is one of the most infective life threatening diseases in those patients. The germination and growth of the fungus inside the human host are mostly dependent on the proteins and metabolic activities of the fungus. These pathways of pathogenecity and invasiveness are based on the active gene expression of the fungus to survive and nourish in such a complex new environment (Dagenais and Keller, 2009). The onset of the infection and success of the pathogen are of many types. Patients with previous history of asthma or fibrosis can show allergic response known as Allergic Brnonchopulmonary Aspergillosis (ABPA). Also fungal sensitization may occur in asthma patients. Coughing, sneezing, running nose, and hay fever like symptoms may appear. The success of germination may cause long persistence of the fungi in the chest known as Chronic Pulmonary Aspergillosis (CPA). Patients with underlying lung diseases such as tuberculosis are more prone to such infection and complications. Aspergillus can grow in pre-existing cavities of the lung and form a ball like structure of hyphae known as (Fungus Ball) (Denning *et al*, 2009).The fungus ball in addition to the mycelia it may contain necrotic tissues, mucus and immune responded cells. Patients may suffer from fatigue, muscle pain, long term coughing and difficulty of breath. The most dangerous and life-threatening type of infection is invasive aspergillosis (IA). Invasive aspergillosis is of a great medical concern as it can cause high mortality rate in patients with critically ill conditions in addition to the immunocompromised individuals (Denning *et al*, 2003). None specific symptoms may appear such as fever, pain of the chest and production of mucus sputum. Treatment of fungal infections and especially aspergillosis, so far, has not succeeded in limitation of the high mortality rate (Kousha *et al*, 2011). The invasive systematic infections by *Aspergillus fumigatus* and other fungi require systematic interference with highly affective antifungal (Walsh *et al*, 2004). This is in order to deliver the drug to the targeted area in deep tissue infections. Intravenous and oral administrations of systematic antifungal are used widely nowadays in order to eradicate the infection. However, the emergence of antifungal resistance and the lack of variety of targets declined the success possibilities of cure. In addition, the fate of the antifungal and their biochemical activity in the tissues also has been of medical mycology concern (Denning and Bromley, 2015). Consequently, researches are ongoing toward the discovery of new antifungal targets with more potential treatment bioactivity. Billions of dollars annually been spent for the available antifungals in the market. These are includes echinocandins, azoles, polyenes and pyrimidine analogues, but however the death rate are still high. These classes have fenced ability to attack fungal cell rather than chitin and sterols (Denning and Hope, 2010). Thus, finding a new target for antifungals with high

specificity, vital essentiality and accessible feature is a goal of the present study. The target, however, should be crucial for biological activity of the pathogen. The Phosphopantetheinyl transferases (PPTase) is shown to be a fundamental metabolite in many bacteria as well as plant fungal pathogens (Walsh *et al*, 1997). With little investigation of this imperative target in human pathogens, the current study shed lights on the metabolites and protein profiles of *pptA* gene and its possibility to be an antifungal target. Phosphopantetheinyl transferases (PPTase) is a necessary enzyme for the adjustment of many other proteins after translation such as polyketide synthases, nonribosomal peptide syntheases, and fatty acid synthases.

## 2. MATERIALS AND METHODS:

### 2.1. Growth of fungus

The ATCC3626 strain of *Aspergillus fumigatus* used to be grwon under standard condition on Potato Dextrose Agar PDA (Temperature: 24°C to 26°C Atmosphere: Typical aerobic).

### 2.2. DNA Extraction:

Slightly tuned and optimized Cetyl Trimethyl Ammonium Bromide (CTAB) method for DNA extraction was used as described by Fraczek *et al* 2013. Where spores were collected using surface wash out with PBS/Tween20 and centrifuged at 14000 rpm for about 2 minutes. The sediment was then added for a suspension of the extraction buffer of CTAB. The sediment then added to a 2mL screw capped tubes that previously filled up to 300µL with sterile glass beads. Respective three cycles of vortexing were then applied intervened by 10 minutes incubation of the tubes at 60˚C. The tubes then left for rest at room temperature 5 minutes and centrifuged later at 1400 rpm for 5 minutes. Amount of 700µL of the supernatant transferred to sterile 2mL tubes and 4µL of RNase (Qiagen) to be added. The tubes were incubated at 37˚C for about 10 minutes. Using 25:1 chloroform:isoamyl alcohol to eliminate any protein content, amount of 700µL and mix by vortexing for 10 seconds only. Centrifugation of the tubes then performed with 1400 rpm for 2 minutes. An amount of 600µL of the supernatant then added to a set of new sterile 1.5mL tubes. Isopropanol was added to the tubes and mixed manually by inverting the tubes up and down. Another round of centrifugation for 5 minutes at 14000 rpm was performed and the supernatant discarded. Ethanol with 70% concentration were added (500µL) and vortexed for only 5 seconds in order to wash the sediment from any debris and proteins. The mixture centrifuged again at 9000 rpm for 2 minutes. The ethanol supernatant removed and the pellet let to dry at room temperature. DNA were harvested by adding 100µL of molecular grade dH2O.

### 2.3. Primers:

The primers were designed, optimized and efficiency test performed. Table 2.1 shows the list of primers used in the present study.

**Table 2.1. List of primers used in this study (Johns, 2015)**

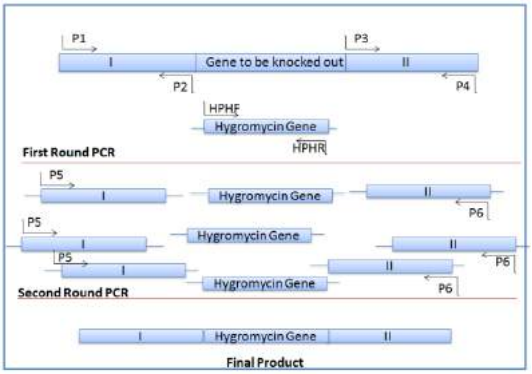
| *Gene* | Sequences |
|---|---|
| *pptA_P1* | CCGGTCTCTTTCTCTGCATC |
| *pptA_P2* | TAGTTCTGTTACCGAGCCGGTC AAACGAGGGAGGAGTCAG |
| *pptA_P3* | GCTCTGAACGATATGCTCCCC CATGCAATATTCCACAGGA |
| *pptA_P4* | CGGCGTACAGTTCGACATTA |
| *pptA_P5* | CGTCCACCTGGATACCTTGT |

| | |
|---|---|
| *pptA_P6* | TCTTCATTGGCAACCATCAG |
| *pptAF* | ACCACCTCAGGGACAGACAC |
| *pptAR* | CTCCTTGAGAGCCCAGTACG |
| *HPHF* | CCGGCTCGGTAACAGAACTAA CGGCGTAACCAAAAGTCAC |
| *HPHR* | GGGAGCATATCGTTCAGAGCT CTTGACGACCGTTGATCTG |
| *medA*F | CGTTACCCAACTTAATCGCCTT GTATTCATTACCCGACCCTTCC |
| *medA*R | CGAAACGACGTAGATGAAAGA |
| *UBC*F | ACCAGCAGAGGCTGATCTTT |
| *UBC*R | ACCTCTGAGGCGAAGGACTA |

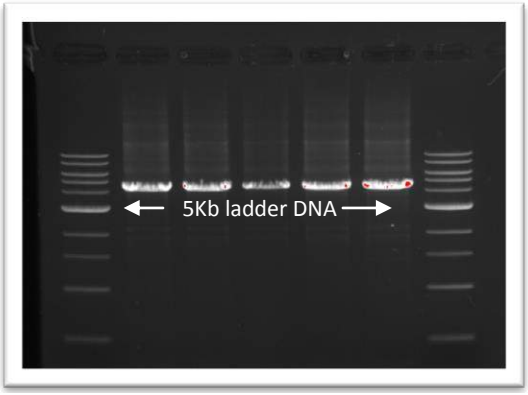
*medA* and *UBC* are designed specifically for this study.

### 2.4 Generation of Knockouts in *A. fumigatus*:

The methodology for generating a knockout strain of *A. fumigatus* through PCR fusion along with transformation of the fungus has been well addressed by Berl Oakley's group (Szewczyk, 2006).




A




B

**Figure 1: A: The fusion PCR technique for knocking out the gene of interest (adapted from Szewczyk *et al.*, 2006). Both flank ends along with a selected marker were amplified in the first round of PCR reaction. The second round included nested primers P5 and P6 to increase the precision of product amplification.**
**B: Generation of knowckout sequence after 2 cycles of fusion pcr resulted in 4kb DNA sequence.**

### 2.5. Growth rate determination:

The growth rate of both radial on agar and in broth were determined using Vogel`s Minimal Media (VMM). However in order to harmonize the growth rate of both knockout and parental strains, 0.5 mM FeSO4 or 10 mM lysine or both 0.5 mM FeSO4 and 10 mM lysine has been added (Fraczek *et al* 2013).

### 2.6. The Bradford (Coomassie) protein production assay:

The amount of $2x10^6$ harvested spores of knockout strain and parental strain of *A. fumigates* of were added to 250 ml broth VMM in flasks. The negative control was established as media only. The samples were each of 5 replicates. Shaker incubator was used at 200 rpm. From the supernatant of the growth medium, one millilitre were taken and sterilized by filtration using Whatman #2 filter paper. The Bradford assay (Coomassie) protein detection kit were used (thermo scientific). The protein

estimation were performed as per the manufacturer protocol. The replicates of samples were added to microplates and their absorbance were determined using scanning spectrophotometer (Bio TEK Synergy). A T-Test used to indicate significance of protein production.

### 2.7. Real- Time PCR:

Gene expression for the knockout strain along with parental strains was compared by using the development modification gene *medA* gene along with the ubiquitin C *UBC* in both. The gene expression rates were determined using the real-time PCR (BIO-RAD). The data were analysed using REST2000 program.

### 2.8. Statistical Analysis:

The T-test paired group analysis were used to determine difference between parental (WT) and knockout (KO) strain of *A. fumigatus*. Prism graphpad were used to present the data and calculate correlations. REST2000 program were used to analyse data of the real-time PCR.

### Results and Discussion:

The present study aimed to generate a knock-out strain of *A. fumigatus* that lacks the pptA gene. This is as a novel step toward finding new targets for the antifungal used against this filamentous invasive fungus. The results showed interesting approaches when parental wild type compared to the knockout strain. The growth rate and the protein profile showed promising results toward further investigation into this essential gene and seeking vital antifungal target. Generation of the knockout strain successfully performed (Figure 2) and the strain showed effective growth on media supplemented with nutrients such as $FeSO_4$ and Lysin (Figure 3).

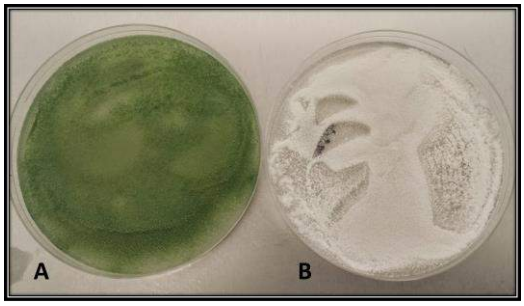



**Figure 2: The two tested strains of parental (A) and the Δ*pptA* (B).**

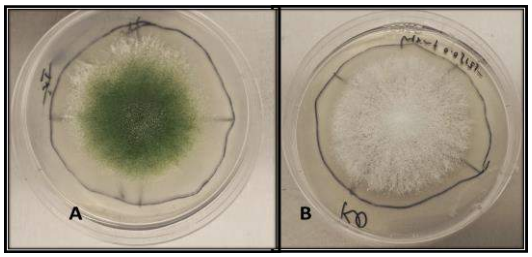



**Figure 3: Growth rate determined through radial growth diameter determination of both parental strain (A) and the knockout strain (B). The knockout strain was supplemented with 1.5 mM FeSO4 and 10 mM lysine.**

Growth on broth medium were also determined and tuned in order to get the optimal equal growth of both strains. However providing 1.5 mM FeSO4 and 10 mM lysine were also necessary for the growth of the knockout in broth medium to come in parallel with the wild type strain (Figure 4)

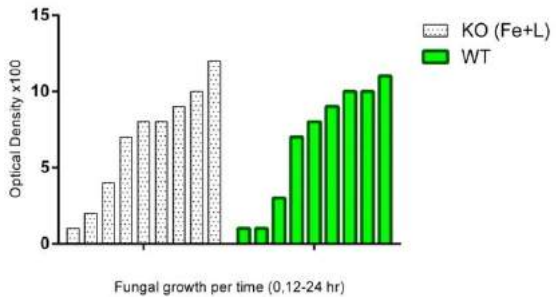



**Figure 4: Growth rate determined through optical density determination of both parental strain (WT) and the knockout strain (KO). The knockout strain was**

**supplemented with 1.5 mM FeSO4 and 10 mM lysine.**

This indicated that the deletion of the *pptA* gene urges the fungi for the need of lysine and ferrous. Thus essentiality of the *pptA* for functions related to the cellular virulence as ferrous is strongly needed to activate set of genes that have crucial role in adaptation, ergosterol biosynthesis, and drug resistance (Haas, 2012). Lysine was shown to be a possible antifungal target. Depletion of lysine resulted in activated lysine uptake by the fungus (Schöbel *et al*, 2010). The result of this study shows possibility of targeting *pptA* products to starve the fungal cell from both iron and lysine.

The protein production assay for both strains showed interesting profile for the knockout. Compensation of the depleted and deleted enzymes results in the high production of various proteins (Figure 5). The results of the present study showed significant increase of protein production profile of the knockout strain, specifically when the media supplemented with 1% of Carboxymethyle cellulose (CMC) and 1% Glycerol.

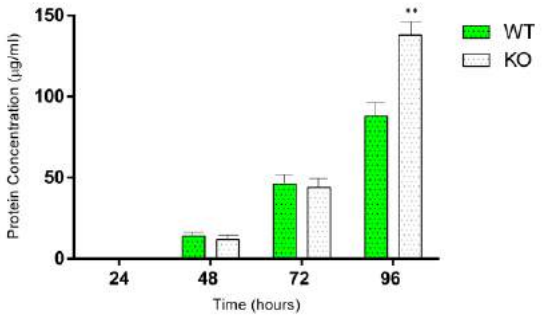



**Figure 5: Bradford (Coomassie) protein assay. The total protein outputs of the knockout strain have been increased significantly by time.**

Increase of the protein production is a promising approach toward further study of the *pptA* mutant. Seeking essential proteins and their reliability on each other is a considerable concern. The protein production tested for the knockout strain through a CMC contained medium. It turned out that the knockout strain have the ability to assimilate cellulose effectively and significantly more than the wild type (Figure 6).

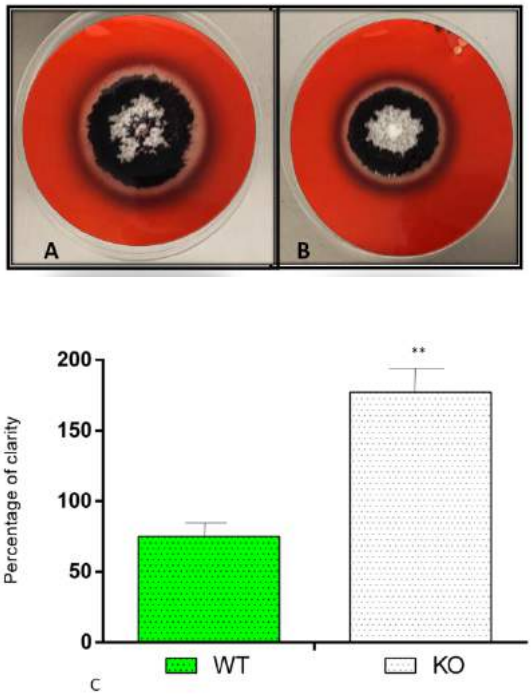



**Figure 6: Growth on CMC contained medium showed significant radial progress of the knockout strain (A) in compare to the parental strain (B). The statistical analysis showed significant differences of the percentage of clearance of cellulose (C).**

From the protein production assay and the growth characteristics, the study showed that the knockout strain can present high protein yield. This is in order to compensate the secondary metabolites that will be missing when the gene is disrupted, indicating the essentiality and crucial role of the *pptA* gene in the fungal growth and progression. The new protein profile can also provide closer insight for the ability of the organism to produce some new industrial proteins that can be

harvested. Cellulose assimilation test indicated high rate of cellulase enzyme providing a promising approach toward the capability of the fungi to be directed for industrial production of cellulase enzyme.

Results of gene expression and transcription profile for selected genes of pathogenecity and ubiquitin protein activity have revealed an interesting up-regulation of the genes when *pptA* has been knocked out in compare with the parental strains. Table 2 shows the real time PCR interpretation result. The *medA* gene was down-regulated by 3 folds while *UBC* is actively expressed with 10 folds expression increase.

The medA gene is shown to have a great role in pathogenecity and adaptation of A. fumigatus. It has roles in morphogenesis and hyphal growth. Recently the gene is indicated a high efficiency in fungus ball formation and biofilm triggering (Busby *et al*., 1996, Yu *et al*., 2006). In a comprehensive study of Twumasi-Boateng and others (2009), *medA* shown to be one of the most effective genes contributed to virulence.

**Table 2: The real time PCR results of gene expression for transcription profile of selected genes.**

| Gene | Type | Reaction Efficiency | Express | P | Result |
|---|---|---|---|---|---|
| ***medA*** | TRG | 0.99 | 3.077 | 0.014 | Down |
| ***UBC*** | TRG | 0.99 | 10.76 | 0.012 | UP |
| | | *medA* is UP-regulated in sample group (in comparison to control group) by a mean factor of 3.077 (S.E. range is 1.27 – 1.32). | | | |
| **Interpretation** | | *UBC* is UP-regulated in sample group (in comparison to control group) by a mean factor of 10.766 (S.E. range is 1.73 - 1.88). | | | |

Thus, the down-regulation of *medA* in Δ*pptA* indicates correlation of the gene with the pathogenecity, which eventually present it as a promising novel antifungal target. On the other hand, *UBC* up-regulation may reveal the response of the cell toward high protein production profile as indicated in the present study when *pptA* is knocked out.

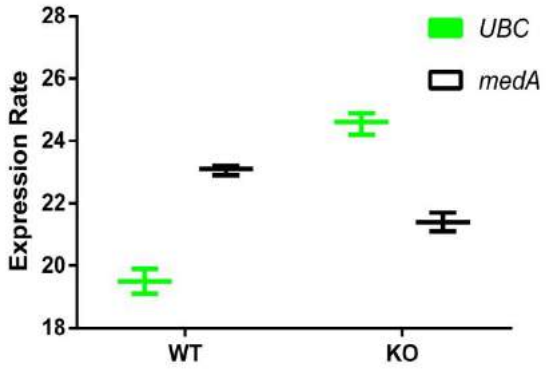



**Figure 7: The transcriptomic profile of the selected genes expression levels of both wild type (WT) and knockout strains (KO) in a mixed biofilm.**

The *UBC* is shown to play a role in pathogenecity and virulence of *A. fumigatus* as an important step of successful adhesion and initiation of disease related to endothelial response of the host (Richie *et al*., 2011). Also in other studies has been used as housekeeping gene for gene expression studies (Ren *et al.,* 2010). ). In fact protein elimination and cellular protein level regulation are robust functions of the *UBC* (Shankar *et al.,* 2005). The current findings of this study showed interesting response of *UBC* gene expression when protein levels is increasing. This correlating the high rate of protein with gene expression changes in the knockout strain of the pathogenic *A. fumigatus*. Nevertheless, such a high input of proteins may pave the way toward finding new antifungal targets as well as beneficial proteins and

metabolites that can be industrially produced.

## 3. Conclusion:

In conclusion, the present study showed that the gene *pptA* is strongly related to pathogenecity of *A. fumigatus*. This is in addition to its vital role in essential cellular mechanisms of growth and protein production control. The deletion of this gene could reveal a new protein profile of which further study is crucial to identify the possibility of industrial and production harvest of those proteins.

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# Onion (Allium Cepa) and Garlic (Allium Sativa L,) Oil effects on Blood Glucose Levels and Body Weight of Local Quails in Erbil Province

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**A B S T R A C T**

The current study was conducted on 38 week old of local quails. The quails were divided into four groups, randomly allocated to four dietary treatments. The diet of the first group was supplemented with 2 ml/kg of onion oils, diet of the second group supplemented with 2 ml/kg of garlic oils, the third group was supplemented with a mix of (1 ml/kg of onion oils and 1 ml/kg garlic oils), and the fourth group designed as a control with no oil supplementations. Results show that the blood glucose level, and body weight was significantly reduced by onion oil supplementation (2 ml/kg) compared to control group Whereas quails with Garlic oils (2 ml/kg) and a mixture of onion and garlic oil (1 ml/Kg of garlic and 1 ml/kg of onion oils) supplementation exhibit no significant differences in experimented parameters compared to control group. Overall, our data presented that onion can serve as good diet additive for lowering blood sugar in diabetics and decreasing body weights, It is suggested that the oils of onion protect against risks like heart failure. The varied results about hypoglycemic role of garlic oils Demanding scientists to perform further works on the garlic extract.

## 1. INTRODUCTION

Diabetes mellitus known as the highest metabolic disorder globally. Animals are varied based upon their utilization of hepatic glucose and until now, the singular animal species with largest utilization of hepatic glucose is Quail (Golden et al., 1982). The levels of blood glucose decreased by age in the birds. Researchers reported that bird ages affect biochemical parameters, while hematological profiles remained sustained (Ali & Hmar, 2012). Despite all the drug discoveries made by scientists to manage hyperglycemia and diabetes mellitus, there are still many traditional theories are based upon by many nations to cure many health disorders using plant oils. Although, efforts has not been enough toward the efficiency improvement of the used medicinal plants (Baldé et al., 2006). Lots of Traditional therapy as a cure for diabetes mellitus has been used without knowing action onset most of these herbs (El-Soud & Khalil, 2010). Unlike previous works which they use Diabetic mellitus induction like

streptozotocin (STZ), Our study compared healthy quails feeding on different supplemented plant oils and observing the level of differences in their Blood glucose and body weight.

Allium genus chiefly garlic and onion confirmed by many studies to have a critical role in countless continuous diseases. As they are known to be full of organosulfur compounds and quercetin (Zeng et al., 2017). Onion (Allium cepa) belongs to Liliaceae family which is loaded in with copper magnesium and potassium, also contains small amounts of vitamins, fat, and sugar (Gabor et al., 2012). Purposes of using onions are variously known as curative plant serving as an antibacterial, antifungal agent, an antibiotic, antiseptic and anti-infectious, and also inform of vegetable and spice as a food. In addition it was proven to have an antioxidant with some anticancer properties (Ramos et al., 2006). According to (Yoshinari, Shiojima, & Igarashi, 2012) onion derivation efficacy looks to be dosage dependent. Also proven that onion derivation with its sulfur content is able to abolish lipid aggregation or differentiation in adipocyte. A study by (Goodarzi et al., 2013) showed that onion derivation can have a beneficial effect on meat-type broiler chickens by improving its growth performance. It has been reported that onion contains *S*-methyl-l-cysteine sulfoxide which showed by (Kumari & Augusti, 2002) to have hypoglycemic effect in alloxan diabetic rats.

Garlic (Allium sativum, Liliaceae) is a compound that loaded with bio actives, serving as a curative plant for various medical disorders. Garlic contains an amino acid loaded with sulfur called S-allyl cysteine (SAC) which have a critical role as an antioxidant (Saravanan & Ponmurugan, 2010). Garlic derivation showed to have a beneficial effect in conversion ratio and cumulative feed of broilers, by rising villus height of small intestine and process activation of absorption (Fadlalla et al., 2010).

In 2016, it has been demonstrated that overweighting and obesity scales tripled dramatically since 1975, in adults overweighting reached more than 1.9 billion with more than 650 million obesity cases. The prevalence of obesity has been significantly risen due to rapid urbanization and individuals total income improvement. The obese percentages in 2016 were nearly 13% of the world's adult population (11% of men and 15% of women). In general, men are much less likely to be obese than women. The prevalence of obesity is also related to better total incomes, mainly in urban areas. Obesity is the most abundant condition that's age independent. Heading toward further complications like type 2 diabetes mellitus, heart disease, and stroke (Nasri & Shirzad, 2013). The use of synthetic drugs to control obesity are varied but the usage of these drugs regarding their safety and efficacy are not promising (Taghikhani et al., 2012).

Many medications have been used for the controlling obesity and overweight like sibutradmine and Orlistat. These drugs side effect and their efficacy limitation in the treatment of obesity become a major concern, regarding their high costs (Bahmani et al., 2016). Plant extractions, remedial sleep, acupuncture, and homeopathy become the substitutes and better options to control overweight and obesity disorders. They were already present for long times (Pittler & Ernst, 2005). The action onset of medicinal plants as anti-obesity still remained unknown. Although many theories like rising energy expenditure, lowering lipid absorption, rising differentiation of pre-adipocyte and proliferation have been suggested for those plants ( Yun, 2010). It has been indicated that onion which is known to

have *S*-methyl-l-cysteine sulfoxide is able to lower lipid levels in the serum and tissue of rats feeding on high cholesterol diet by rising lipids catabolism, subsequent excretion and decreasing endogenous lipogenesis (Kumari & Augusti, 2007).

The objective of this study was to investigate the anti-hyperglycemic and anti-obesity effect of garlic and onion oils as a supplementation additives to quails diet.

## 2. MATERIALS AND METHODS

The experimental work of this study was carried out at the GRDA RASHA field, Agriculture College, from 7th May 2017 to 7 th July 2017.

### 2.1 Animal Model

60 local quail hens (40 weeks of age) of similar weight about (210g±5) were randomly assigned to four treatments. All quails were maintained under the standard laboratory condition for a temperature controlled room 35-38°Cat afternoon, 28-30 C° at night and morning and were allowed free access to food and water. The quail hens were reared in special cages 65cm×60cm×50cm length, width and height respectively, designed for quails.

### 2.2 Plant Oils

Quails randomly allocated to 4 dietary treatments (Commercial plant oils purchased from markets). Each treatment comprised 3 replicates of 5 quails (at 1 male to 4 female ratios) Therefore, 4 groups containing 15 quails each were arranged. Diet is given in mash form and water supplied continuously. The diet of the first group was supplemented with 2 ml/kg of onion oil, Diet of the 2nd group supplemented with 2 ml/kg of garlic oil, the third group supplemented with a mix of (1 ml/kg of onion oils and 1 ml/kg garlic oils), and fourth group designed as a control without garlic or onion oils supplementations.

### 2.3 Diabetic Induction

All quail groups were healthy and no Diabetic mellitus inductions were injected to any of treated groups. Because the aim was to find the effect of these selected plant oils on healthy quails under normal conditions.

### 2.4 Blood glucose determination

The concentration of blood glucose was estimated by Glucometer (Viva check, Iso 15197: 2013 gm/dl, UK) at zero time (T0), fourth week (T1) and Eighth week (T2). Mortality has been recorded during last 3 weeks of the study. (Ibrahim, J. *et al.,* 2015)

### 2.5 Body weight determination

The electrical balance was used to record body weights of all quails at zero time (T0), fourth week (T1) and Eighth week (T2) of the experimental period. The Blood glucose data and body weight of the quails were subjected to statistical analysis using SPSS programs.

## 3. RESULTS AND DISCUSSION

### 3.1 Hypoglycemic Effect

The data of the current study show (table1) that the mean levels of blood glucose of control group of quails during the period of the study are T0=219.7±259, T1=236.2±45.6 and T2=227.4±17.17. They are significantly decreased during the period of the study in quails feeding with onion oil in diet (T0 217.28±8.32, T1 204.7±15.1 and T2 198.1±25.65).

**Table 1:** Shows the mean levels of blood glucose mg/dl of four treatment quail groups (Control, onion, garlic, and Mixture) at 3 different time periods (T0, T1 and T2).

| Treatments | MEAN ± ST.DEV. | | | |
|---|---|---|---|---|
| | T0 time | T1 time | T2 time | Significance |
| Control | 219.7±259 | 236.2±45.6 | 227.4±17.17 | P > 0.05 |
| Onion | 217.28±8.32 | 204.7±15.1 | 198.1±25.65 | P < 0.05 |
| Garlic | 195.4±37.83 | 199.83±2742 | 198.28±9.42 | P > 0.05 |
| Mix | 218.8±33.17 | 228±22.78 | 216.85±28.81 | P < 0.05 |

Whereas there are no significant differences in the mean levels of blood glucose during the period of the study in quails feeding with garlic oil in diet (T0=195.4±37.83, T1=199.83±2742 and T2=198.28±9.42) and mixture of garlic and onion oil (T0=218.8±33.17, T1=228±22.78 and T2=216.85±28.81).

Our study demonstrates that Quail groups feeding on supplemented onion oil exhibit significant dropping in blood glucose levels (Table 1) which may be attributed to antioxidant properties. This is in agreement with previous studies that showed similar effects of onion oil on the level of blood glucose (Kook et al., 2009, El-Demerdash et al., 2005, Augusti & Benaim, 1975). Lee et al., 2013 reportedd the hypoglycemic activity of ripe onion juice at two dose levels (5 and 15 mL/kg b.w.) in the rates.

The similar data found by (Jalal et al., 2007) suggesting that the mode of action of Allium cepa (onion) as anti-diabetic may be caused by the antioxidant properties of its essential oil components thereby preventing hyperglycemia. In addition it has been reported that the ingestion onion (Allium cepa) (100 g) caused a considerable reduction in fasting blood glucose levels which could be used as a dietary supplement in management of type 1 and/or type 2 diabetes mellitus (Taj Eldin et al., 2010).

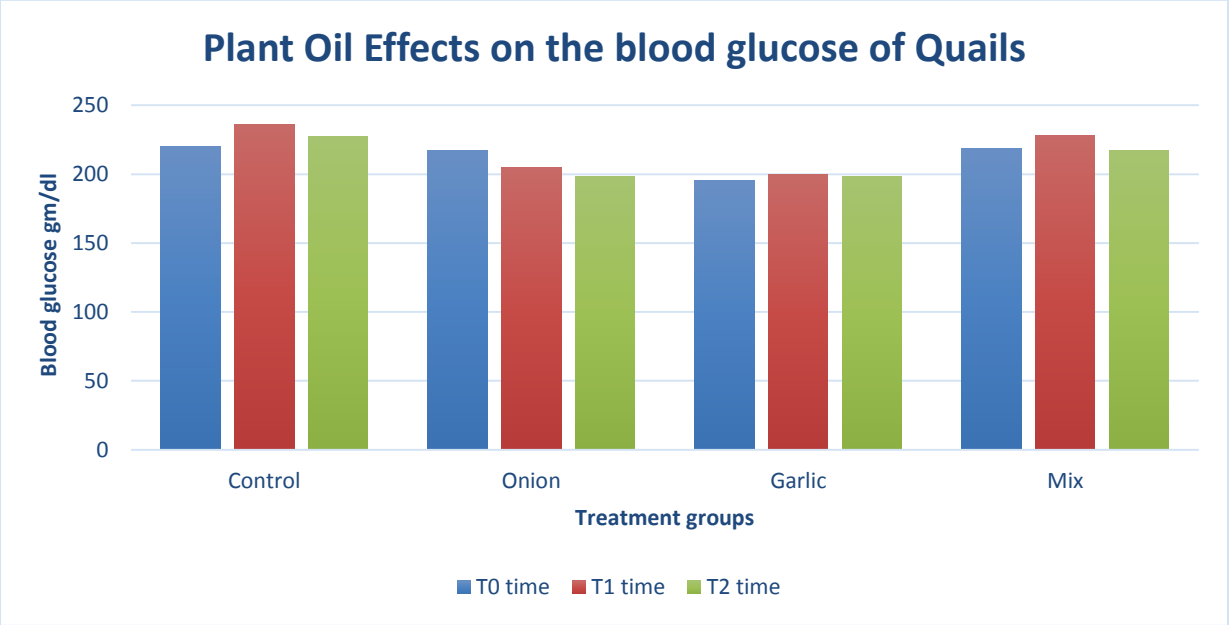


**Figure 1:** Shows the mean levels of blood glucose pooled (males and females) quails feeding on different supplemented oils (Garlic, Onion, and Mixture of both oils). T0: Time zero, T1: After four weeks, T2: After eight weeks.

This study shows that there is no effect of Garlic oil on the mean levels of blood glucose of quails (figure 1). This finding can be supported by (Kook et al., 2009). In accordance with our study, another work done by (Ashraf et al., 2011) concluded that garlic only not enough but a Combination of garlic with typical anti-diabetic remedy has shown to improve glycemic control. Also (Baluchnejadmojarad & Roghani, 2003) found no hypoglycemic effect of an aqueous extract of garlic in streptozotocin-induced diabetic rats. Treatment of fructose-induced insulin resistance rats with aqueous garlic extract (500 mg/kg BW/day, i.p.) for a period of four weeks did not have any effect on the intraperitoneal glucose tolerance (Jalal et al., 2007). The garlic powder did not show significant effect on body weight of quails. (Yalçin et al., 2007). It has been reported that diet supplemented with garlic oils (1ml/100 g) didn't significantly change the rat body weight (Masjediet al., 2013b).

While the salutary role of garlic in type 1 diabetes are established by Several studies documenting the efficacy of garlic in reducing blood glucose in various animal models of type 1 diabetes mellitus (Banerjee et al., 2002, Ohaeri, 2001, Kumar & Reddy, 1999, Padiya et al., 2011).

Sustained blood glucose of quails supplemented on mixture (1% garlic and 1% onion) oils presented by us, may be due to its low percentage of plant oil

supplementations presented in the quail's diet. Until now there is no study showing that mixture oils of Sativium sp. could have hypoglycemic activities.

### 3.2 Body weight Effect

Our results show that at zero time the body weight of all three experimental groups were not significantly different and they were staid under 270 g (table 2). The body weight of quails at T1 (at 4 weeks) were slightly raised in all the studied groups but not significantly different. There was a significant decrease in the body weight of quails under supplemental onion oil diet, from 281.66±42.93 g at fourth week to 223.33±32.65 g at the Eighth week of the study. The body weight of quails feeding on diets with garlic and mixture oils showed no significant differences between T1 and T2 periods of the study.

**Table 2:** Shows body weights in g for four treatment quail groups (Control, onion, garlic, and Mixture) at 3 different time periods (T0, T1 and T2).

| | MEAN ±ST.DEV. | | | |
|---|---|---|---|---|
| Treatments | T0 time | T1time | T2 time | Significance |
| Control | 251.5±34.46 | 264.54±21.04 | 230±15.63 | P > 0.05 |
| Onion | 265.35±44.18 | 281.66±42.93 | 223.33±32.65 | P < 0.05 |
| Garlic | 261.78±48.82 | 292.5±40.15 | 250.55±27.73 | P > 0.05 |
| Mix | 257.08±31.14 | 292.72±40.86 | 270±35.74 | P > 0.05 |

These data show that onion oils reduced significantly body weight of quails ($P < 0.05$), this is in agreement with Kook et al., 2009. Body and adipose tissue weights, fasting blood glucose level were also improved in 5% of onion extract-fed group. Onion extract and its sulfur-containing compounds, suggesting that these compounds may play a vital role in suppressing obesity. The present study showed that the anti-obesity effect of onion in the rodent that may be beneficial for human health (Yoshinari et al., 2012). Goodarzi & Nanekarani, 2014 demonstrated that possibly onion could improve growth performance of chicks due to the content of organosulfur compounds. Similar results of Aji et al., 2011 and Goodarzi et al., 2013 reported the positive influence of broilers fed diets containing fresh onion on body weight.

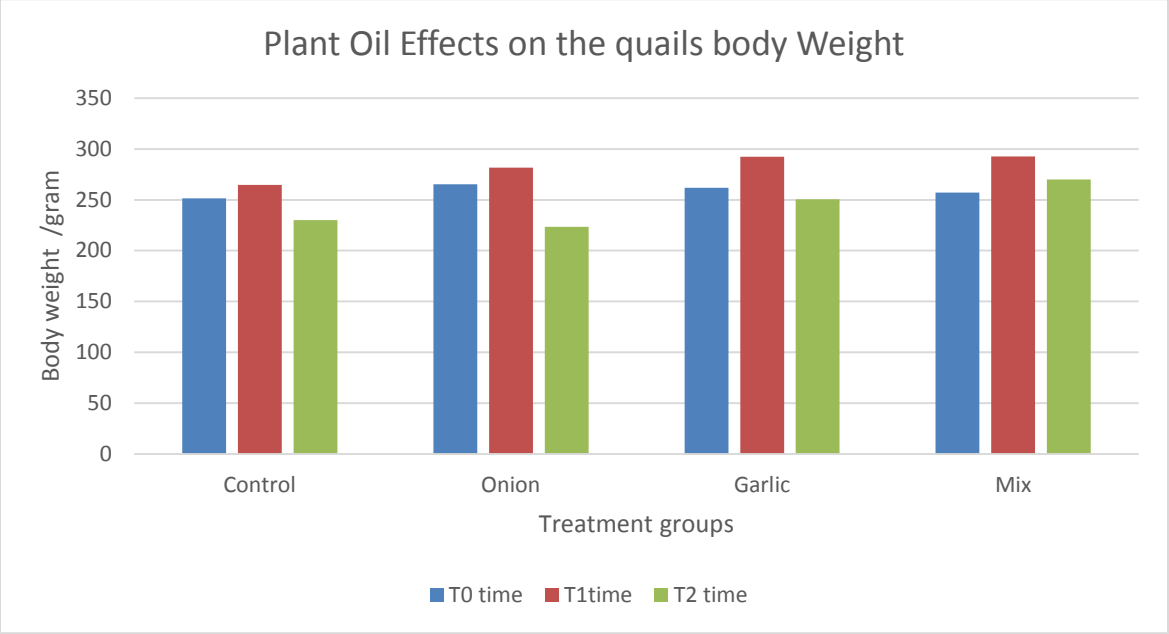


**Figure 2:** Shows that the body weight not effected when quails feed on supplemented plant oils (2 % onion oils, 2% garlic oils and 2% mixture of both oils ) during two-month study project.

The present study shows that garlic oil does not influence the body weight of quails throughout the studied period (figure 2). This is in agreement with Kook et al., 2009. Whereas it is in disagreement with Karangiya et al., 2016, who Showed that diet containing 1% garlic significantly increased body weight of broiler chicks. Our finding present that mixture oils of garlic and onion have no significant influences on the body weights. Similar result has been found by Karangiya et al., 2016, concluded that the body weight gain (g/bird) was not significantly ($p<0.05$) changed by diet treated with garlic and ginger mixture supplements.

## 4- CONCLUSION

The onion extract could have beneficial effects in reducing the levels of blood sugar suggesting that dietary onion supplementation may help diabetics to reduce over Dependence on drugs. our data presented that onion can serve as a good diet additive for body weight loss, which can be used by the physicians to help overweight individuals before facing series disorders like hypertension and cardiovascular diseases. Further studies must be done to analysis onion oils contents responsible for these beneficial effects. There are some contradictory results about the hypoglycemic role of garlic oils, therefore scientists need to perform more investigations in order to determine the exact dosage of garlic oils additives to the

diets to be effective in reducing blood sugar levels.

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# Effect of Seed Exposure to Direct Electrical Current on Germination and Seedlings Growth of three Cowpeas *(Vigna unguiculata* L.) Cultivars

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**A B S T R A C T**

The experiment was carried out during three weeks with San Martin, Gabs ton and West hillier cultivars of cowpea*(Vigna unguiculata* L.), seeds were exposed to 12V direct current (DC) electrical field in different exposure periods (0, 10, 20 and 40 minutes) before seed sowing. Generally, the best results were obtained from 20 min. exposure to DC electrical field of all studied cultivars, however, highest root characteristics except root dry weight obtained from control treatment. The highest germination percentage was in Gabs ton cultivar when exposed to DC electrical field for 20 min., and for the shoot length, shoot elongation speed/plant were obtained from two treatments (gaps ton cultivar without seed exposure to DC electrical field and with 20 min. exposure period). However the best root dry weight obtained from the seedlings of San martin cultivar when exposed to DC electrical field for 10min. The results shows that exposure of *(Vigna unguiculata* L.) seeds to (DC) electric (12V) improved germination percentage and seedlings vegetative growth.

## 1. INTRODUCTION

Cowpea is a member of the Dicotyledon plants, order Fabales, family Fabaceae, subfamily Faboideae and genus Vigna, the species *Vigna unguiculata* contain the cultivated cowpeas, this crop is important in many countries of South America, tropical Africa and Asia. Cowpeas grain and leaves are are rich and cheap sources of high-quality protein so they are edible products (Kabululu, 2008).

The movements of direct electrical current have effect on plant growth; the using of electrical devices in the agricultural biotechnology attracted the attention in the last few years. Many investigations have been done to evaluate the enhancement of direct electrical current on plant seed germination and growth, weeds can be exposed to electrical energy by use of electrostatic fields, microwaves, electric discharges or direct electric shocks using either alternating current or direct current and they have more advantages in weed control on compression to traditional methods (Diprose and Benson, 1984).

Tomato (*Lycopersicun esculentum L.*) seeds were treated with AC electric fields range from 4 to 12 kV/cm and AC magnetic flux densities ranging from 3 to 1000 Gauss for three time periods (started from 15 to 60 s) were accelerated seed germination rates about 2.8 times, however, AC electric fields more than 12 kV/cm for more than 60 s had an inhibitory effect (Moon and Chung, 2000). In another study the 30 KV/30 s electrical current treatment before sowing seeds were decreased growth of sticky spots of five cotton cultivars which belong to *Gossypium hirsutum* L. ( Mustafayev *et al*., 2001). Lynikiene (2006) was used continuous current discharge electric field on carrot, radish, beet, beetroot and barley seeds, was accelerated seed germination in compression to non-treated seeds. Dannehl, *et al.* (2009) was applied the intermittent (DC) electric (200 mA, 600 Ma and 1000 mA) on radish (*Raphanus sativus* L.) plant during growth period ( one hour per day) passed horizontally through the nutrient solution the plants, and was increased the current phenol content, anthocyanin and antioxidant activity. Gandhare and Patwardhan (2014) were found improvement germination, root shoot length and seed vigor of tomato plants by electrostatic field (2 kV/mm for 20 second interval). Ahmad *et al.* (2015) was used the electric fields and the electrical fields treated water which influenced the germination rate and stems height of young vegetables (Choy Sam' and bean sprout plants). A few studies have been reported the effects of different duration of electric field on plant seeds. Accordingly, the current study was conducted with the main objective to evaluating the influence of different seed exposing time to weak DC electrical field on seed germination and first stages of growth of the seedlings of three cowpea cultivars.

## 2. MATERIALS AND METHODS

The experiment was carried out during 5$^{th}$ to 26$^{th}$ September with three cultivars of cowpea (San martin, Gabs ton and West hillier) for each cultivar 200 uniformly seeds were soaked in 250mL tap water for 90 minutes and treated immediately with DC electrical field.

### 2.1 Application of DC electric fields

Soaked seeds were exposed to DC electrical field by inserting them into the parallel aluminum plates connected with the 12V DC as in figure 1. The seeds were inserted into the parallel plate type (Moon and Chung, 2000). The exposing times were 0, 10, 20 and 40 minutes before seed sowing (Gabdrakhmanova and Qussiny, 2011; Sedighi *et al*., 2013 and Gui *et al*., 2013 ).

Ten uniform seeds were selected per replicate (Chowdhury and Havern,2012; Sozharajan and Natarajan, 2014 ), polystyrene seedling trays were used with 77 cells, each cell about 59.31 cm$^3$ capacity, 2.5cm in width and 6.5cm in height filled with peat moss (Pokon Naturado BV, Veenendaal, Holland), pH of 5.2-6.2, NPK (14:16:18) and 50% organic matter. The climatologically data of temperature and humidity during the experiment period are shown in the table 1.

### 2.2 Experimental parameters

#### 2.2.1 Germination percentage (GP)

After two weeks from seed sowing the germination percentage (GP) was calculated following the International Seed Testing Association (ISTA) method according to Mousavizadeh *et al*. (2013) and Sozharajan and Natarajan (2014).

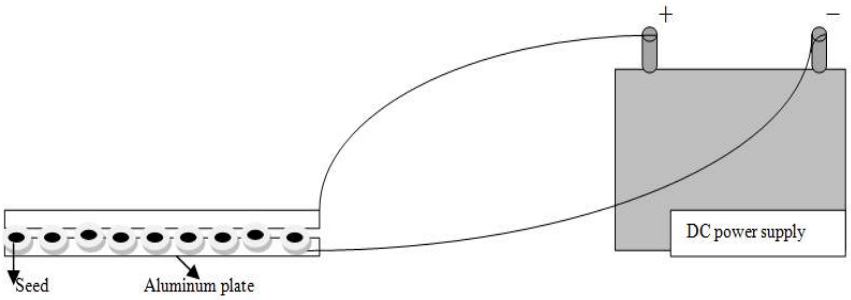


**Figure 1, Experimental set-up: Seed exposure to DC.**

**Table 1, Maximum and minimum air temperature and humidity throughout the experiment period.**

| September days | Air temperature C° | | Relative humidity% | |
|---|---|---|---|---|
| | maximum | minimum | maximum | minimum |
| **5** | 39.54 | 24.03 | 35.18 | 5.64 |
| **6** | 38.33 | 22.89 | 34.65 | 14.85 |
| **7** | 39.04 | 23.83 | 28.35 | 8.77 |
| **8** | 38.74 | 23.79 | 36.36 | 11.42 |
| **9** | 37.42 | 23.03 | 37.95 | 11.83 |
| **10** | 35.81 | 20.96 | 37.05 | 16.20 |
| **11** | 36.94 | 22.06 | 34.35 | 9.21 |
| **12** | 38.62 | 20.59 | 37.52 | 5.41 |
| **13** | 38.59 | 21.63 | 32.43 | 7.19 |
| **14** | 36.61 | 22.08 | 32.05 | 10.35 |
| **15** | 36.84 | 21.62 | 31.22 | 12.00 |
| **16** | 37.78 | 24.10 | 38.54 | 12.43 |
| **17** | 36.04 | 22.12 | 41.22 | 17.00 |
| **18** | 35.44 | 19.58 | 40.21 | 15.60 |
| **19** | 35.81 | 22.76 | 32.39 | 12.77 |
| **20** | 37.41 | 23.24 | 34.28 | 13.91 |
| **21** | 38.90 | 20.93 | 42.04 | 12.36 |
| **22** | 33.95 | 18.46 | 33.15 | 14.55 |
| **23** | 32.40 | 19.75 | 39.21 | 12.20 |
| **24** | 32.57 | 20.39 | 40.42 | 17.34 |
| **25** | 33.28 | 22.76 | 41.89 | 18.46 |
| **26** | 34.25 | 20.92 | 56.58 | 23.74 |

$$GP = \frac{\text{Number of normally germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

### 2.2.2 Morphological characteristics

The morphological characteristics of the three tested cultivars seedlings were calculated according to Qadir (2006) and Sozharajan and Natarajan (2014). All of seedlings were used for measuring the studied parameters at the end of the experiment,

-shoot length (cm) was measured from plant tip to the surface of the soil.

-Number of leaves/plant (visible leaves had been counted), longest leaf (from the base to the leaf to the top end). -Shoot elongation speed (stem length at the end of experiment / number of days required for elongation).

-Shoot fresh and dry weights (g).

-Shoot elongation speed (stem length at the end of experiment / number of days required for elongation).

-Shoot fresh and dry weights (g).

-Root length (cm) was measured from the contact point with the stem to the tip of longest root, -root elongation speed (means of root length at the end of experiment / number of days required for elongation).

-Root fresh and dry weights (g).

For dry weights of shoot and root systems were separately dried in an oven at 70$^{o}$C to constant weights then measured using sensitive balance (Mohammad-Amin, 2008).

### 2.3 Data analysis

Factorial complete randomized design was conducted (Al-Rawi and Khalaf-Alla 1980), totally, 12 treatments represented three cultivars of cowpea (San martin, Gabs ton and West hillier) and 4 exposing time to DC electrical field (0, 10, 20 and 40 minutes) each with three replicates, the replicate contained ten seedlings (thirty seedlings for one treatment). Analysis of variance (ANOVA) was used to analyse the data, and the significant differences of the means were compared using the Duncan's multiple range tests at 5% significant level using SAS program (SAS institute, 2005).

## 3. RESULTS AND DISCUSSION

### 3.1 Germination percentage:

Table 2 illustrated that Gab ston cultivar had a high response among the cultivars. However, 20 min. exposing time gave high significant results.

Results of multiple ranges Duncan's method indicated that exposing of San martin, Gabs ton and West hillier cultivars, time of exposure to different times(0, 10, 20 and 40 minutes) to DC electrical field before seed sowing were affected significantly on germination percentage. The highest germination percentage found in Gabs ton cultivar when exposed for 20 min. to DC electrical field, and further increase in the exposing time to DC electrical field caused a decrease in Germination percentage (table, 2).

### 3.2 Morphological characteristics

#### 3.2.1 Vegetative growth characteristics

Figure (2) shows that type of cultivars did not affect significantly on the shoot length, number of leaves/plant, longest leaf shoot elongation speed, shoot fresh and dry weights. While, exposing time of seeds to DC electrical fields caused significant effects on shoot fresh and dry weights of seedling vegetative growth, the best results were recorded from 20 min. exposure period (figure, 3).

**Table 2, Response of *(Vigna unguiculata* L.) cultivars and exposing time to DC electrical field represented by germination percentage.**

| Cultivars | Exposing time to DC electrical field (min.) 0 | 10 | 20 | 40 | Mean. cultivars |
|---|---|---|---|---|---|
| **San martin** | 40.00 d | 46.67 cd | 60.00 bcd | 46.67 cd | 49.09 c |
| **Gabs ton** | 90.00 a | 70.00 abc | 83.33 ab | 66.67 abc | 77.50 a |
| **West hillier** | 60.00 bcd | 63.33 bcd | 70.00 abc | 53.33 cd | 61.67 b |
| **Mean. exposing time** | 66.25 ab | 60.00 ab | 71.11 a | 55.56 b | |

*Values within each column followed with the same latter are not significantly different from each other according to Duncan's Multiple Range test at the 0.05 level.

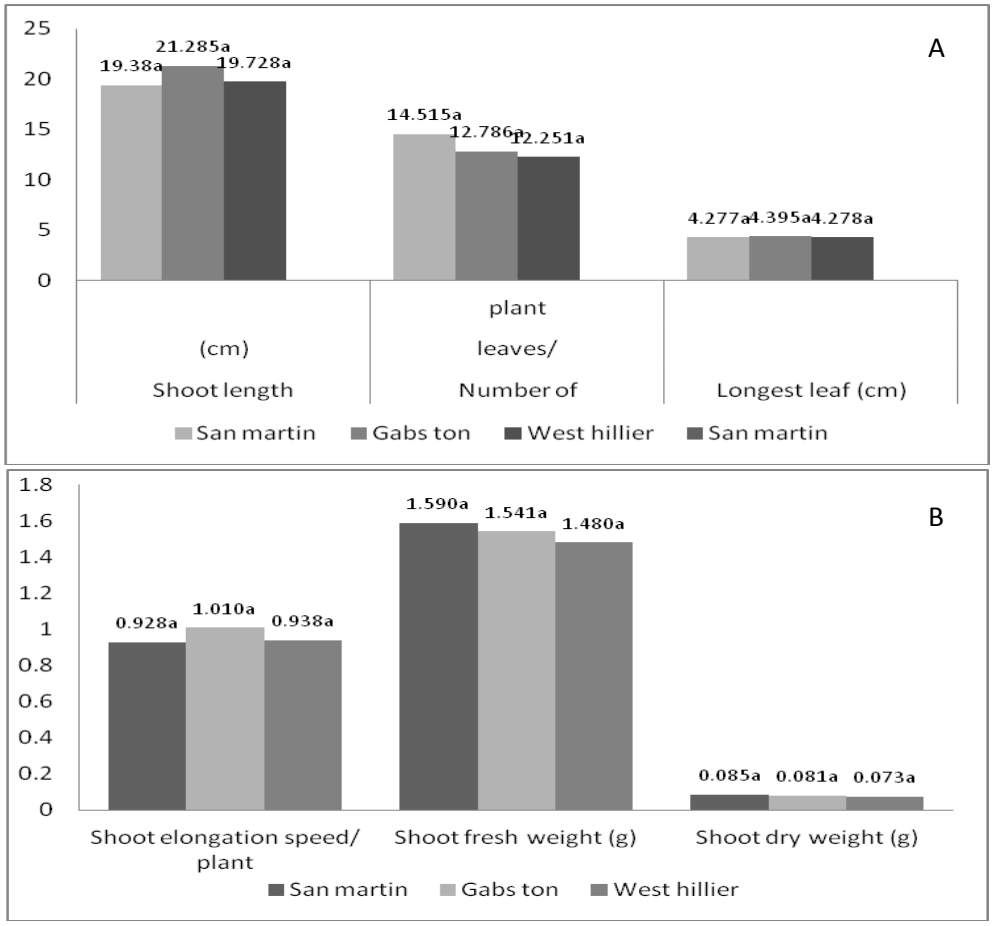


**Figure 2(A and B), Effects of *(Vigna unguiculata* L.) cultivars on vegetative growth characteristics according exposing time to DC electrical field.**

*(columns with the same letter are not significantly different from each other according to Duncan's Multiple Range test at 0.05 level).

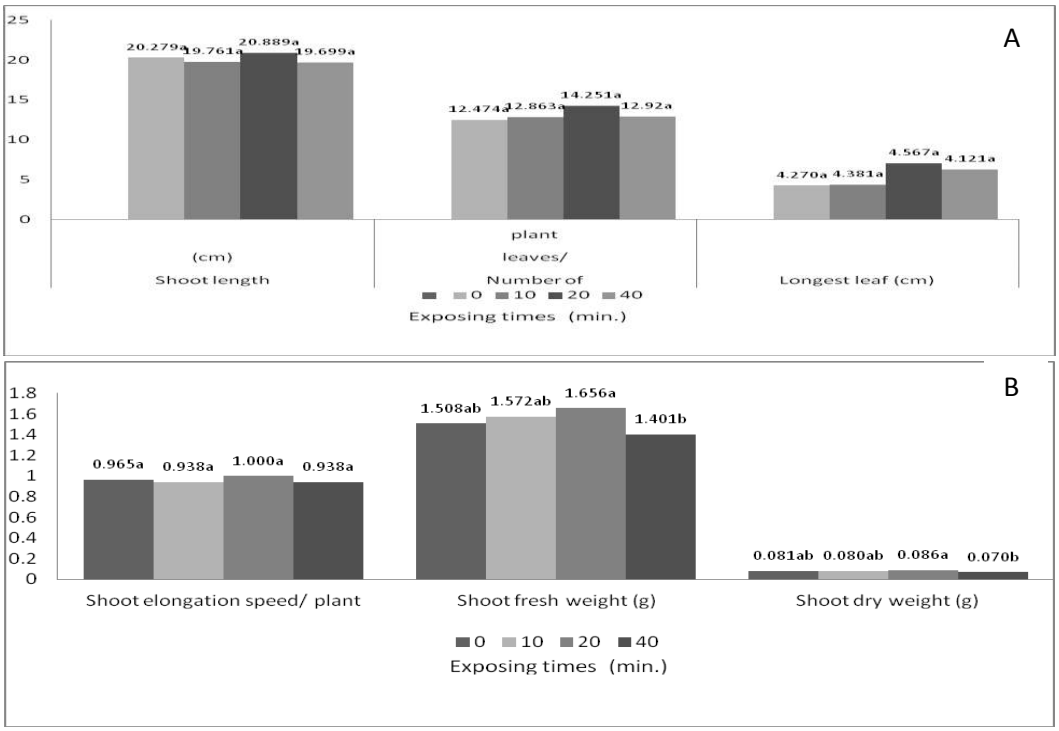


**Figure 3 (A and B), Effects of seed exposing time to DC electrical field on vegetative growth characteristics of *(Vigna unguiculata* L.).**

*(columns with the same letter are not significantly different from each other according to Duncan's Multiple Range test at 0.05 level).

The effects of cultivars and exposing time on vegetative growth parameters value are illustrated in table, 3. Significant effects were recorded except in number of leaves/plant and longest leaf. The highest values of shoot length, shoot elongation speed/plant were obtained from gaps ton cultivar with 20 min. exposing time when compared with the other treatments. The comparison between the treatments showed that the best values of fresh and dry weights were recorded from the San Martin cultivar when the seeds exposed to DC electrical field for 10min.

### 3.2.2 Root growth characteristics

There was no significant response among studied cultivars on root growth characteristics (figure, 4). Root length, root elongation speed and root dry weight decreased with increasing of exposing time to DC electrical field and the best results were obtained from the control. While, 10 min. exposing time to DC electrical field gave more significant value of root dry weight with no differences with control (figure 5).

Exposure of San martin, Gabs ton and West hillier seeds for different times to DC electrical field caused significant decline of root length, root elongation speed and root dry weight. It can be observed that the highest values of root length and root elongation speed were recorded from the control of West hillier cultivar. However, the highest root dry weight obtained from San martin cultivar and 10min exposing time to DC electrical field when compared with the other treatments with no significant differences with the control of the cultivars (table,4).

**Table 3, Response of *(Vigna unguiculata* L.) cultivars to seed exposing time to DC electrical field represented by vegetative growth characteristics**

| Cultivars | Exposing time (min.) | Shoot length (cm) | Number of leaves/ plant | Longest leaf (cm) | Shoot elongation speed/ plant | Shoot fresh weight (g) | Shoot dry weight (g) |
|---|---|---|---|---|---|---|---|
| **San martin** | **0** | 16.060 b | 13.415 a | 3.640 a | 0.765 b | 1.360 ab | 0.075 abc |
| | **10** | 20.663 ab | 14.577 a | 4.800 a | 0.987 ab | 1.740 a | 0.093 a |
| | **20** | 20.327 ab | 14.867 a | 4.533 a | 0.983 ab | 1.656 ab | 0.090 ab |
| | **40** | 19.363 ab | 14.833 a | 4.133 a | 0.923 ab | 1.526 ab | 0.080 abc |
| **Gabs ton** | **0** | 21.997 a | 12.593 a | 4.380 a | 1.047 a | 1.530 ab | 0.087 ab |
| | **10** | 20.797 ab | 10.097 a | 4.453 a | 0.980 ab | 1.616 ab | 0.08 abc |
| | **20** | 22.630 a | 14.877 a | 4.667 a | 1.077 a | 1.700 ab | 0.087 ab |
| | **40** | 19.717 ab | 13.577 a | 4.080 a | 0.940 ab | 1.316 b | 0.070 abc |
| **West hillier** | **0** | 21.373 a | 11.727 a | 4.580 a | 1.017 a | 1.586 ab | 0.080 abc |
| | **10** | 17.823 ab | 13.917 a | 3.890 a | 0.847 ab | 1.360 ab | 0.067 bc |
| | **20** | 19.700 ab | 13.010 a | 4.500 a | 0.937 ab | 1.613 ab | 0.083 abc |
| | **40** | 20.017 ab | 10.350 a | 4.143 a | 0.950 ab | 1.360 ab | 0.060 c |

*Values within each column followed with the same latter are not significantly different from each other according to Duncan's Multiple Range test at the 0.05 level.

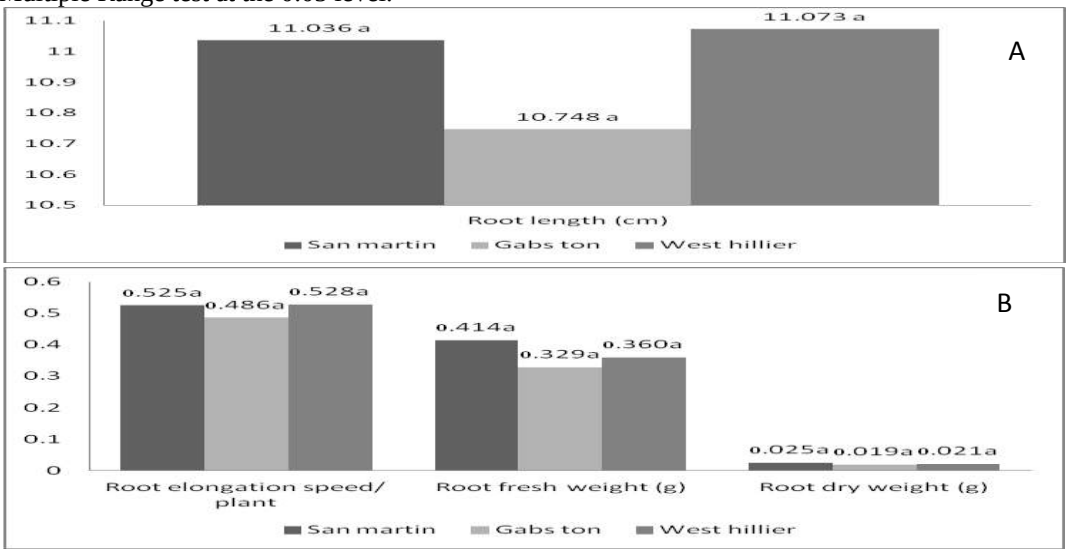


**Figure 4 (A and B), Effect of *(Vigna unguiculata* L.) cultivars on root growth characteristics according exposing time to DC electrical field.**

*(columns with the same letter are not significantly different from each other according to Duncan's Multiple Range test at 0.05 level).

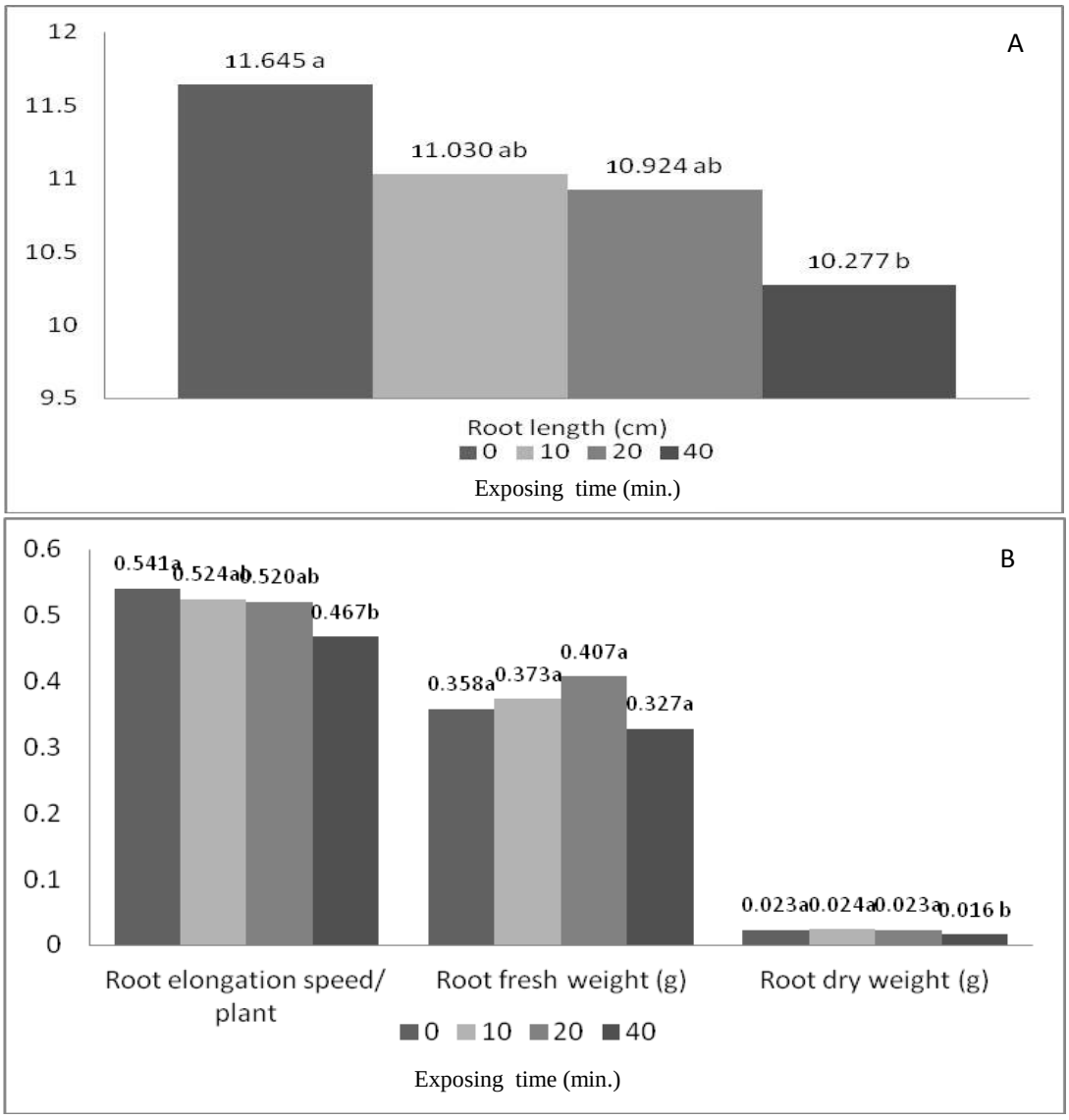


**Figure 5 (A and B), Effects of seed exposure periods to DC electrical field on root growth characteristics of *(Vigna unguiculata* L.).**
*(columns with the same letter are not significantly different from each other according to Duncan's Multiple Range test at 0.05 level).

## 4. DISCUSSION

The difference between the germination values as related to the studied factors (three cultivars of cowpea; San martin, Gabs ton and West hillier seeds exposed to 12V DC at different times (0, 10, 20 and 40 min. before sowing) was obtained experimentally. The results of cowpeas seed germination indicate that generally the studied exposing times to DC electrical field had a good reflect compared with control treatments except the time of 20 min. and differences was observed among the studied cultivars. The results partially agree with results of Lynikien and Pozeliene (2003)

**Table 4, Response of *(Vigna unguiculata* L.) cultivars to seed exposing time to DC electrical field represented by root growth characteristics.**

| Cultivars | Exposing time (min.) | Root length (cm) | Root elongation speed/ plant | Root fresh weight (g) | Root dry weight (g) |
|---|---|---|---|---|---|
| **San martin** | **0** | 11.150 ab | 0.530 ab | 0.310 a | 0.020 abc |
| | **10** | 11.363 ab | 0.540 ab | 0.447 a | 0.030 a |
| | **20** | 11.363 ab | 0.540 ab | 0.447 a | 0.027 ab |
| | **40** | 10.303 ab | 0.490 ab | 0.413 a | 0.020 abc |
| **Gabs ton** | **0** | 11.457 ab | 0.510 ab | 0.347 a | 0.023 abc |
| | **10** | 10.153 ab | 0.483 ab | 0.313 a | 0.023 abc |
| | **20** | 10.733 ab | 0.513 ab | 0.377 a | 0.017 bc |
| | **40** | 10.650 ab | 0.437 b | 0.280 a | 0.012 c |
| **West hillier** | **0** | 12.163 a | 0.580 a | 0.400 a | 0.023 abc |
| | **10** | 11.573 ab | 0.550 a | 0.357 a | 0.020 abc |
| | **20** | 10.677 ab | 0.507 ab | 0.397 a | 0.027 ab |
| | **40** | 9.877 b | 0.473 ab | 0.287 a | 0.015 bc |

*Values within each column followed with the same latter are not significantly different from each other according to Duncan's Multiple Range test at the 0.05 level.

on barley and with (Gui *et al*., 2013) on coat seed germination; they concluded that the content of energy conveyed to plant seeds depends on the strength of the electrical field and electrical properties of seed, moreover seed vigor was obviously changed by exposure to electrostatic field. Electrostatic field represents a fast, effective and economic method for the pre treatment shallow dormancy of thin coat seeds as in cowpea. During the exposure the direction of electrostatic field does not change (Lynikien and Pozeliene, 2003).

As mentioned in previous sections, that most of growth parameters considering the spatially organized cell walls. Moreover, the position of sessile cells rather than their lineage has a predominant role in dictating their combination effects of exposing times to DC electric field with studied cultivars had significant effects comparing with control treatment this agree with the results of Sedighi *et al. (*2013) on maize seeds. Nevertheless, root parameters gave fewer values in the longest exposing time, it is recognized that cell polarity, characterized by asymmetric distribution of subcellular structures and molecules, is a fundamental attribute to the development of all eukaryotic organisms. The unique significance of cell polarity in plant development is confirm by the fact that plant cell morphogenesis is largely defined by developmental fates. (Yang, 1998), because of this polarity the electric current can affect the direction and movement of storage nutrients of

the seeds to the cell walls and accelerate meristem cells division which consists of undifferentiated, rapidly dividing cells and improve tissues and organs of the postembryonic plant, this indicates that the organizing centre for plant morphogenesis is meristem cells (Kerk and Feldman, 1995). The electrical stimulation may cause the formation of free radicals as ionizing radiation in the cells (Bratton and Haenry, 1977). Furthermore the results can be explained by the activating effect of DC on plant hormones in beans when DC treatment was increased germination viability of them (Gabdrakhmanova and Qussiny, 2011). Likewise may be DC electrical field affect on the direction of plant hormones when they had been tried with various concentrations *(*cytokinin (Thidiazuron) and auxin (Indol 3-Acetic Acid)*)* that stimulate a metabolism of seedlings, like change the auxin and cytokinin ratio in the seeds which leading to the germination of *Chlorophytum borivilianum* seeds (Trivedi and Tiwari, 2016). The hydraulic and electrical systems of a plant cell were therefore intimately coupled, with the both sensory and motor components (Shepherd, 2012).

## 5. CONCLUSION

The effect of exposure times of DC electric fields on cowpea seed germination and seedling growth have been investigated as a potential means to accelerate the germination and improve the growth of the seedlings. The following conclusions have been obtained:

1. Based on the findings of this trial generally, it was concluded that exposing cowpea seeds to weak voltage direct electrical current (12V) before sowing for 20 minutes was the best for seed germination.

2- Exposure for different times to direct electrical current had different effects on the cultivars regarding seedling growth parameters. 10 min. exposure was more effective for San martin for most seedling growth characteristics. Depending to exposing times to DC electrical field the other two cultivars were varied in recording growth values, with no significant differences with the control treatment.

Based on the results referred to, and to improve seedlings growth, good quality of seedlings before transplanting which is reflected on the cowpea production, the following recommendations are suggested:

1- More studies in this area need to be done, especially for more exposing times and for more intensity of direct electrical fields in wide ranges.

2- Continuous studies under different growth conditions ( as controlled conditions in glass or plastic houses ) are required to know the effects of San martin, Gabs ton and West hillier seeds exposure for different times to DC electrical field (12V) on the germination, seedling growth and yield quality and quantity of cowpea plant.

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# Some Physiological and Nutritional Factors that affect the growth of Some Fungi

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## ABSTRACT

The present study is attempt to evaluated and compared the ability of different genera and species of fungus to utilize different nutrient sources and to grow under different environmental conditions. Four genera of molds were grown on different synthetic, semi-synthetic and natural media such as Potato Sucrose agar (PSA), Czapek's Dox agar (CDA) and Water agar (WA), which subjected to a range of temperatures (4, 15, 25, 37 and 50ºC) to investigate their influence on the performance of the mycelium of *Alternaria alternata, Aspergillus fumigatus, Penicillium citrinum* and *Trichoderma harzianum*. PSA and CDA were most favorable nutrients for fast radial growth of mycelium of all genera of fungi and at all temperatures and PH levels, while on WA, the radial mycelial growth was very little or there was no growth at all environmental conditions. In general, there was high growth at temperatures (15ºC-37ºC), while at 50ºC there was no growth or very little growth of fungi. On the other hand, a different growth of all fungal genera has been shown in all media with various pH levels, except at pH 11, which have seen little growth colonies or no growth. *Penicillium citrinum* have seen the highest growth in acidic pH, while *Aspergillus fumigatus* have seen in alkaline pH. On PSA media, all genera have shown the highest growth at 25°C, while no growth at 4°C and 50°C, except a little growth of *Alternaria alternata* and *Penicillium citrinum* at 4°C and *Aspergillus fumigates* at 50°C, while in pH ranges, the highest growth of all fungal genera have shown at pH 3-5, While no growth at pH11, except a bit growth of *Aspergillus fumigatus* and *Trichoderma harzianum*. On CDA media, all genera have shown the highest growth at 25°C, while no growth at 4°C and 50°C, except a little growth of *Penicillium citrinum* at 4°C, while in pH ranges, the highest growth of all fungal genera have shown at pH 3-7, While the lowest growth of *Alternaria alternata*, *Aspergillus fumigatus* and *Trichoderma harzianum* with no growth of *Penicillium citrinum*. While on WA media, all genera have shown a little growth at all temperatures, except *Trichoderma harzianum*, there was no growth at all temperatures, *Alternaria alternata*, and also no growth at 4°C and 50°C, *Aspergillus fumigatus*, no growth at 4°C, *Penicillium citrinum*,and no growth at 50°C, while in pH ranges, a little growth of all fungal genera have shown at pH 3-7, Whilst no growth of all genera at pH11, except *Aspergillus fumigatus*.

## 1. INTRODUCTION

Fungi are consisting of large numbers of organisms that are unique compared with plant and animals, among these are molds and yeasts. Despite the great variation in morphology and characteristics of the fungi, most of them share the following characteristics including: the presence of chitin in the cell wall, ergosterol in the cell membrane, lack of chlorophyll, asexual or sexual reproduction, their heterotrophic nature and lack of susceptibility to antibacterial antibiotics (Forbes *et al.*, 2007**).**

The fungal kingdom is arranged into four major phyla on the basis of differences in their sexual reproduction. These are chytridiomycota, zygomycota, ascomycota, and basidiomycota (Hogg, 2005).

The physiology of fungi means the growth, nutrition, metabolism, reproduction, and death of fungal cells and also refers to fungal interaction with their biotic and a biotic surroundings, which including cellular responses to stress. They impact significantly on human health and industry. The metabolism of fungi is responsible for bioremediation of heavy metals and for detoxification of organic pollutants in the environment (Walker and White, 2005).

The fungi have shown changes in their growth rate when grown on different types of nutrient media (Brock and Heymann, 2006). Temperature and pH are the significant factor for studying the spoilage of fungi ecologically (Ahmed and Naresh, 2009). The growth of fungi could be affected by pH in a medium, which it grows in, either directly by its action on the cell surfaces or indirectly by its effect on the nutrients availability. However, acid/alkaline requirement for growth of molds and yeasts is quite a wide spectrum, ranging from pH 3 to more than pH 8 and about 5 is the optimum pH (Pardo *et al.,* 2006). In general, *Penicillium* species are more tolerant to acidophiles or acid pH, while *Aspergillus* species are more tolerant to alkalophiles or alkaline pH (Wheeler *et al.,* 1991). A

neutral to the weak acidic condition was favourable for the growth of fungal mycelia, with optimum pH 5–7(Zhao *et al.,* 2010).

The effects of high temperature on the growth fungi depend on several factors, including the genus, species, and strain of the fungus**,** types of nutrients, and many other environmental factors. Temperature is very important for the fungal growth, most fungi will grow between 0 and 35$^{o}$C, but the optimum temperature lies in the range of 20-30$^{o}$C (Alexopoulos, 1962).

According to temperature requirements for optimal growth, fungal genera divided into three distinct groups such as Psychrophiles (less than 10°C), Mesophiles (18-22°C) and Thermophiles (at or above 37°C). There are also psychrotolerant and thermotolerant fungi, indicating that growth can occur at either low or high temperatures, but are not optimal.

The aim of the present research was to estimate the ideal media, temperature, and pH, which could be used to produce the maximum yield of *Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium citrinum* and *Trichoderma harzianum* mycelia and sporulation.

## 2. MATERIALS AND METHODS

### 2.1. Collection of Fungi

All genera of fungi, which used in this study were obtained from the University of Salahaddin, college of Science-Department of Biology, They activated by sub-culturing on

PSA in Petri dishes.

### 2.2. Preparation of media

**2.2.1. Potato Sucrose Agar (PSA)**: The chemical compound of this media includes: 250 gm of Potato, 20 gm of Sucrose, 15 gm of Agar and 1 Liter of Distilled water (D.W.) (Samson, 2004).

**2.2.2. Czapek (Dox) Agar (CDA)**: The chemical compound of this media includes: 2gm of $NaNO_3$, 1gm of $KH_2PO_4$, 0.5gm of KCl, 0.5gm of $MgSO_4.7H_2O$, 0.01gm of $FeSO_4.7H_2O$, 30gm of Sucrose, 20gm of Agar and 1 liter of D.W.(Samson, 2004).

**2.2.3. Water agar (WA):** It consists of 20 gm of agar and 1L of tab water (Johnston and Booth, 1983). Potato sucrose agar, CDA and WA were prepared (1litter for each medium), then the prepared media were distributed in sterile 250ml conical flasks. Finally autoclaved at 121°C, by 1.5 bar for 17 minutes (Samson, 2004).

### 2.3. The influence of temperatures on the fungal growth

Effect of different synthetic and semi-synthetic media and different temperatures (4, 15, 25, 37 and 50 ºC) on the colony growth of *Alternaria alternata, Aspergillus fumigatus, Penicillium citrinum* and *Trichoderma harzianum* were estimated. Fungal media, such as PSA, CDA and WA were poured into Petri plates (9cm diameter).

Petri plates containing 20 ml of each of media were inoculated with five mm diameter mycelial discs from seven-day-old cultures of different isolates. The agar plugs were removed with a sterile cork holer from the edges of colonies and one such plug was placed in the center of each 9 cm Petri plate in each of the three media. The inoculated plates were incubated at different temperatures: 4, 15, 25, 37 and 50ºC. For each medium, there were three replicate plates. The diameter of mold colony in each plate was measured at a **7**-day interval along two axes perpendicular to one another. Then the radial growth rates of each mold genera were calculated.

### 2.4. The influence of pH on the growth of fungi

The pH levels that selected for this study were 3, 5, 7, 9 and 11. One hundred ml of each of medium PSA, CDA and WA were prepared and distributed in sterile conical flasks (250ml). The pH levels were adjusted to 3, 5, 7, 9 and 11, by adding hydrochloric acid (HCl) or sodium hydroxide (NaOH) to each of the flasks that contain media. The electrical pH meter measured the pH before sterilization in an autoclave at 121$^o$C. Then pour each of the sterilized media into sterilized Petridishes; allow the plate to become solidified. Use sterilized cork hole to cut disks of agar and mycelium from 7 days old molds culture, transfer an inoculum into poured solidified media in upside situation that the mycelium becomes contact with the medium, incubate the inoculated medium at 25ºC for 5-7 days. Finally measure the diameter of the mycelium, and compare the growth at each pH (Carlos and Joseph, 2012). Each treatment was in triplicate.

## 3. RESULTS

The result presented in this study show the radial mycelial growth rates of *Alternaria alternata, Aspergillus fumigatus, Penicilliumcitrinum* and *Trichoderma*

*harzianum*, were significantly affected by culture media, temperature and pH.

In PSA and CDA, there were highest growths at temperatures 15ºC-37ºC after 5-7 days of inoculation, while at 50ºC there was no growth or very little mycelia growth of fungi. On the other hand, there has been shown that different growth rate of all fungal genera in all media with various pH level, except at pH11, which have seen a little growth or no growth. On WA, the radial mycelial growth was very little or no growth at all temperatures and pH levels. In general, *Penicillium citrinum* have has seen the highest growth in acidic pH while *Aspergillus fumigatus* have seen in alkaline pH.

In Table 1, Figure 1 (a) and (b), showed the effects of temperature and pH on the radial fungal growth on PSA media. In which, all genera have shown the highest growth at 25°C, while no growth at 4°C and 50°C, except a little growth of *Alternaria alternata* (1.1cm) and *Penicillium citrinum* (1.2cm) at 4°C and *Aspergillus fumigates* (2.1cm) at 50°C. While in pH ranges, the highest growth of all fungal genera has shown at pH 3-5, with no growth at pH11, except a bit growth of *Aspergillus fumigates* (2cm) and *Trichoderma harzianum* (0.5cm).

In general it has shown that radial growth of mycelia of all genera on PSA media at 4ºC were: (1.1, 0, 1.2 and 0) cm, but at 15ºC were (7, 2.9, 4.5 and 9) cm, whilst at 25°C were (9, 9, 8 and 9) cm, nevertheless at 37°C were (2.9, 4.3, 6.1and 3.3) cm, and at 50°C were (0, 2.1, 0 and 0) cm, while at pH 3 were (4.5, 3, 6 and 5.5), but at pH5 were (4, 5, 6.5 and 6), and also at pH7 were (2, 5.5, 2 and 3.5), whilst at pH9 were (1.5, 6, 1 and 3), and at pH11 were (0, 2, 0 and 0.5), for each (*Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium citrinum* and *Trichoderma harzianum*,) respectively.

Table 2, Figure 2(a) and (b), illustrate the effect of temperature and pH on radial growth of each mold on CDA media. It shows that all genera have the highest growth at 25°C, while no growth at 4°C and 50°C, except a little growth of *Penicillium citrinum* (1.5cm) at 4°C.While in pH ranges, the highest growth of all fungal genera has shown at pH 3-7, While, at pH11, it has shown the lowest growth of *Alternaria alternata* (0.5cm), *Aspergillus fumigatus* (3) and *Trichoderma harzianum* (1cm), while the growth of *Penicillium citrinum*. The radial mycelium growth of all fungi on CDA media at 4ºC were (0, 0, 1.5 and 0) cm, but at 15ºC were (5, 2.7, 2.7 and 9) cm, and at 25°C were (9, 6.2, 8 and 9) cm, whilst at 37°C were (0, 4.3, 1.4 and 5) cm, nevertheless at 50°C was (0, 0, 0 and 0) cm, while at pH3 were (5, 3.5, 6 and 7), and also at pH5 were (4.5, 4, 6.5 and 7.5), but at pH7 were (3, 6, 4 and 5), whilst at pH9 were (2, 6.5, 2 and 4),while at pH11 were (0.5, 3, 0 and 1), for each (*Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium citrinum* and *Trichoderma harzianum*,) respectively.

Table 3, Figure3 (a) and (b), give information about the effect of temperature and pH on radial growth of each mold on WA media. Its how that all genera have a little growth at all temperatures, except *Trichoderma harzianum*, there was no growth at all temperatures, *Alternaria alternata*, no growth at 4°C and 50°C, *Aspergillus fumigatus*, no growth at 4°C, *Penicillium citrinum*, no growth at50°C. While in pH ranges, a little growth of all fungal genera has shown at pH 3-7, While no growth of all genera at pH11, except *Aspergillus fumigatus*(1cm).

In this table the radial mycelium growth of all fungi on WA media at 4ºC were (0, 0, 1.5 and 0) cm, at 15ºC were (1.5, 1.2, 2 and 0) cm, and at 25°C were (2, 1.5, 2 and 0) cm at 37°C were (1.5, 1.3, 1.5 and 0) cm, and also at 50°C

were (0, 1.4, 0 and 0)cm, while at pH3 were (0.5, 0, 2 and 1.5), nevertheless at pH5 were (1, 1, 1.5 and 1), but at pH7 were (1.5, 2, 1 and 1), whereas at pH9 were (1, 2.5, 0.5 and 0.5), and at pH11 were (0, 1, 0 and 0) for each (*Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium citrinum* and *Trichoderma harzianum*,) respectively.

## 4. DISCUSSION

The results of our research were in close agreement with those of Mustafa *et al.,* (2009), who examined mycelial growth and conidial production of *Trichoderma harzianum*, *T. viride*, *T. longibrachiatum*, on five different culture media including Potato Dextrose Agar, Waksman agar, Agar-agar, CDA and Corn Meal agar. The medium had a high effect on growth rate and the population of *Trichoderma* spp. Potato Dextrose Agar was the best medium for growth spore and biomass production. Alam *et al*., (2001), who recorded the highest growth of *Botryodiplodia theobromae* mycelium on PDA and maximum percentage of pycnidia on CDA. Similarly, Quroshi and Meah, (1991), who observed fastest liner growth of *Botryodiplodia theobromae* and the highest number of pycnidia on PDA. O'Brian *et al*., (2007), have reported that the temperature ranges of 25-30°C as the best-favoring proliferation as well as toxin production in *Aspergillus* sp..Our results also in agreement with Sabalpara *et al*., (1991), who have found very little or no growth of fungi at low temperatures such as at 10 and 15ºC, however, as temperature increased up to 25ºC, the mycelial growth of fungi increased and then decreased rapidly with further increase in temperature. Optimum growth occurred at 25-37ºC. Also, there are close agreements that reported by Saha *et al*., (2008), who reported that optimum temperatures for the colony growth of fungus were 25-30ºC. Khanzada *et al*., (2006), who found that most suitable media for mycelial growth of the fungi were PSA, Yeast extract mannitol agar and Corn meal agar. Cao, *et.al*., (2007), who focus on growth phases of *Penicillium* sp., upon the effects of physiological factors and show that all could grow at a wide temperature range (8.0–39.8°C), but growth was inhibited at 40°C, dramatically. Johnson, *et al*., (1987), who isolated *Trichoderma* spp. from Tennessee and Alaska soils on a selective medium at 10, 12 and 25°C.Tatiana, *et.al*.,(2010), who found that best results were obtained when *Alternaria* sp. was grown in a medium at 25$^{o}$C for seven days. Basu and Bhattacharyya, (1962), who found that the growth and sporulation of several strains of *Penicilliulm* spp. affected by certain carbohydrates, nitrogen compounds, and accessory growth factors. Allen *et al*., (1982), showed that the germination of conidia of *Alternaria* sp. was favored by a temperature between 25 and 28°C, and the colony growth of *Alternaria* sp. on PDA was greatest at 25°C. Upadhyay and Rai, (1978), have showed that *Trichoderma* spp. has an ability to utilize a variety of nutritional factors as well as they have a broad range of pH and temperature tolerance for their growth and sporulation, except. *viride* which was unable to grow at pH 9 and above. Ogunledun, (2007), showed that acidic pH significantly influence the growth of fungi, while the least growth as observed at neutral pH.

The results in the current study disagree with those of Sibounnavoung *et al.,* (2009), who found that the growth and sporulation of *Aspergillus* spp. is tolerant of acidic and neutral conditions while suppressed by an alkaline condition. Rosfarizan *et al*., (2000), who confirmed that most of the molds have an optimum pH between 5 and 6 for growth and

metabolic activities and they are generally tolerant to acidic pH. This study has obtained a result similar to the report by Kaiser *et al*., (2005) at pH 10.3 on *Alternaria solani* (42.8%), *Phytophthora capscici* (17.4%) and *P. cinnamomi* (12.6%) respectively, while at pH 11.7, the growth of them was completely inhibited. Our results also in agreement with Swe *et al*., (2009), who have revealed that the diameter of colony decreased in the media with the higher pH level, also it shows that, although certain alkaline medium favors the formation of *Aspergillus* sp. spore, higher pH values from 10 tend to inhibit its sporulation.

It has also been found that increased severity of several diseases in soil due to an elevation of soil pH (Cook and Baker, 1983; Simon and Sivasithamparam, 1988). The suitability of *Trichoderma* spp. as a biocontrol agent is due to that this antagonist has a broad range of temperature tolerance for growth and sporulation. The biocontrol formulations developed from *Trichoderma*, may be used in a wide range of geographical locations because of its ability to utilize a large number of carbon and nitrogen sources and its broad spectrum of pH and temperature tolerance (Jayaswal *et al.*, 2003).

## 5. Conclusions

Four genera of molds were grown on different synthetic, semi-synthetic and natural media, which subjected to several range of temperatures and with various pH levels to investigate their influence on the performance of the mycelium of some fungi. PSA and CDA were most favorable nutrients for fast radial growth of mycelium in all genera of fungi and at all temperatures and PH levels, while the mycelial growth on WA, was very little or there was no growth at all environmental conditions.

## Acknowledgment

I would like to thank University of Salahaddin-college of Science/Biology department, for helping us and providing space and laboratory facilities to carry out the research work in Mycology research laboratory.

**Table 1:** Effect of temperature and pH on the radial fungal growth on PSA media

| | | Radial fungal growth (cm) | | | |
|---|---|---|---|---|---|
| | | ***Alternaria alternata*** | ***Aspergillus fumigatus*** | ***Penicillium citrinum*** | ***Trichoderma harzianum*** |
| Temperatures | **4°C** | **1.1** | **0** | **1.2** | **0** |
| | **15°C** | **7** | **2.9** | **4.5** | **9** |
| | **25°C** | **9** | **9** | **8** | **9** |
| | **37°C** | **2.9** | **4.3** | **6.1** | **3.3** |
| | **50°C** | **0** | **2.1** | **0** | **0** |
| pH | **3** | **4.5** | **3** | **6** | **5.5** |
| | **5** | **4** | **5** | **6.5** | **6** |
| | **7** | **2** | **5.5** | **2** | **3.5** |
| | **9** | **1.5** | **6** | **1** | **3** |
| | **11** | **0** | **2** | **0** | **0.5** |

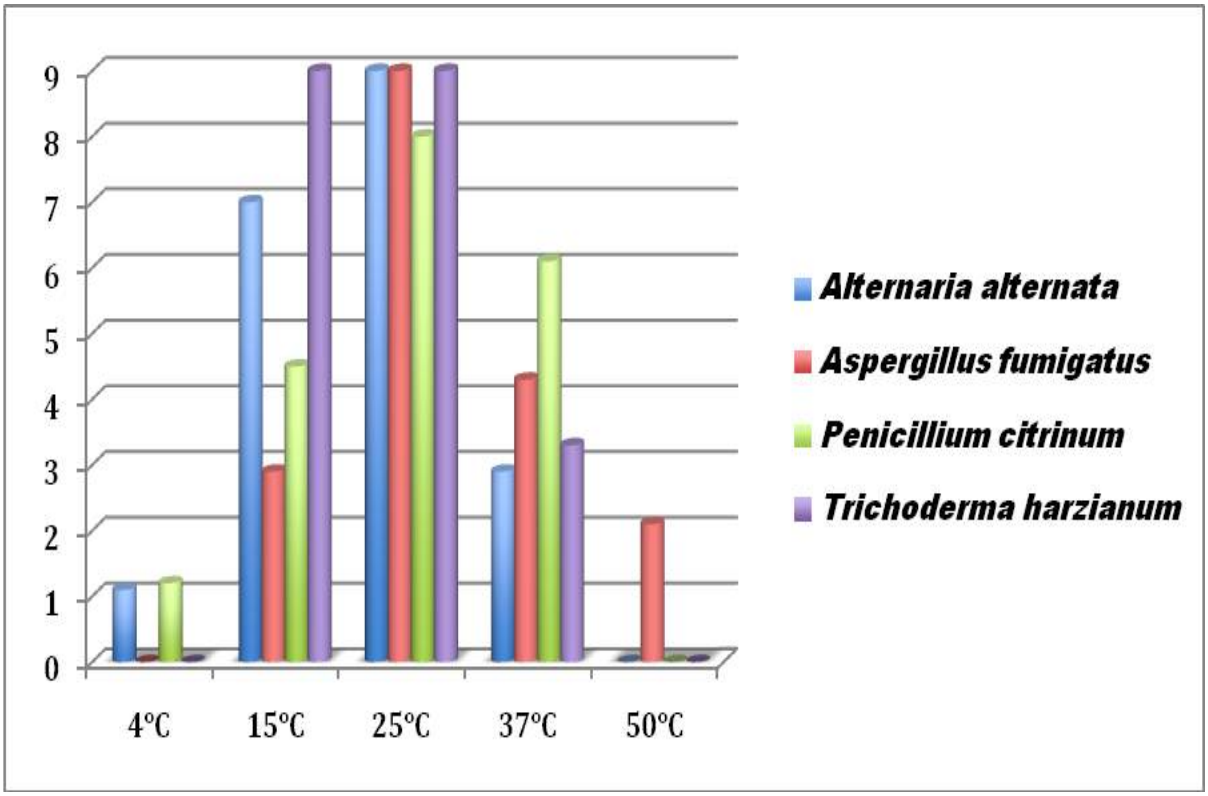


**Figure 1 (a):** Effect of temperature on the radial fungal growth on PSA media

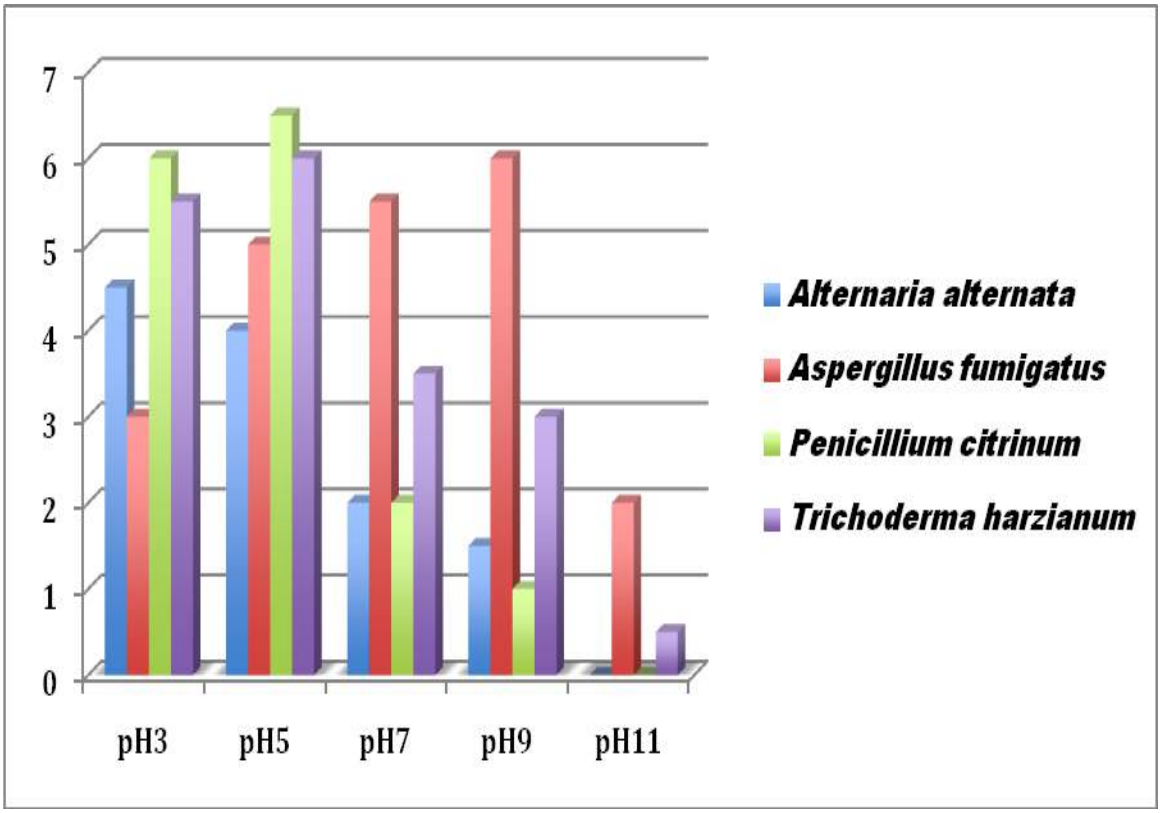


**Figure 1 (b):** Effect of pH on the radial fungal growth on PSA media

**Table 2:** Effect of temperature and pH on radial fungal growth on CDA media

| | | Radial fungal growth (cm) | | | |
|---|---|---|---|---|---|
| | | ***Alternaria alternate*** | ***Aspergillus fumigatus*** | ***Penicillium citrinum*** | ***Trichoderma harzianum*** |
| Temperature | **4°C** | **0** | **0** | **1.5** | **0** |
| | **15°C** | **5** | **2.7** | **2.7** | **9** |
| | **25°C** | **9** | **6.2** | **8** | **9** |
| | **37°C** | **0** | **4.3** | **1.4** | **5** |
| | **50°C** | **0** | **0** | **0** | **0** |
| pH | **3** | **5** | **3.5** | **6** | **7** |
| | **5** | **4.5** | **4** | **6.5** | **7.5** |
| | **7** | **3** | **6** | **4** | **5** |
| | **9** | **2** | **6.5** | **2** | **4** |
| | **11** | **0.5** | **3** | **0** | **1** |

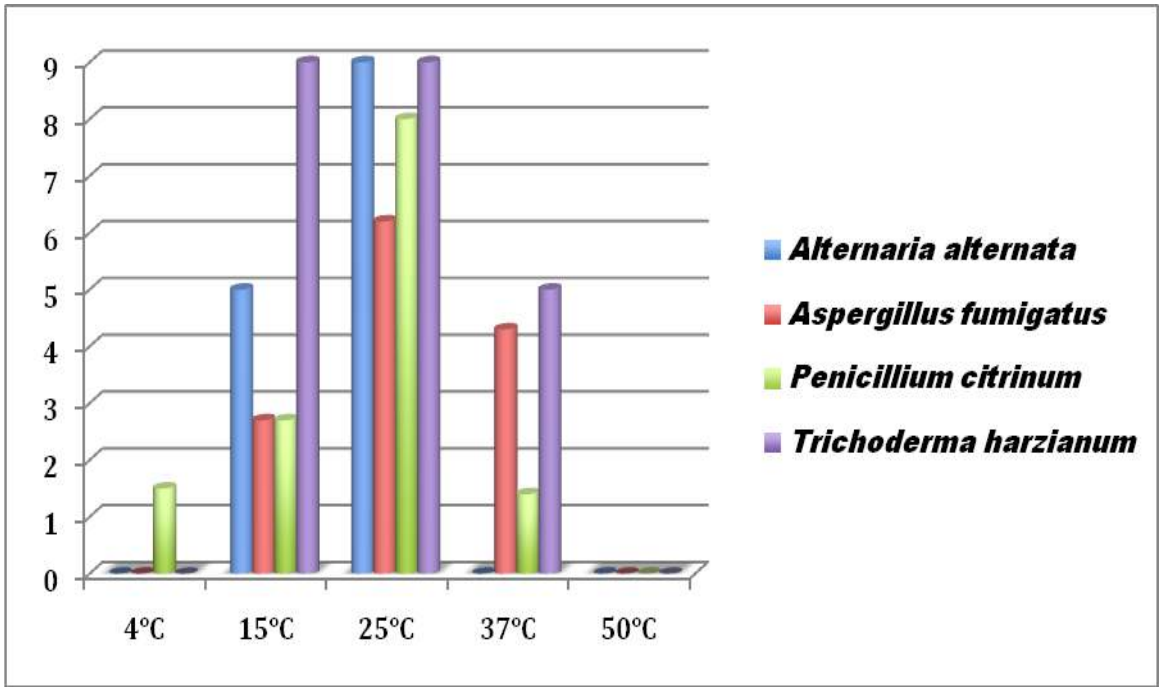


**Figure 2(a):** Effect of temperature on radial fungal growth on CDA media

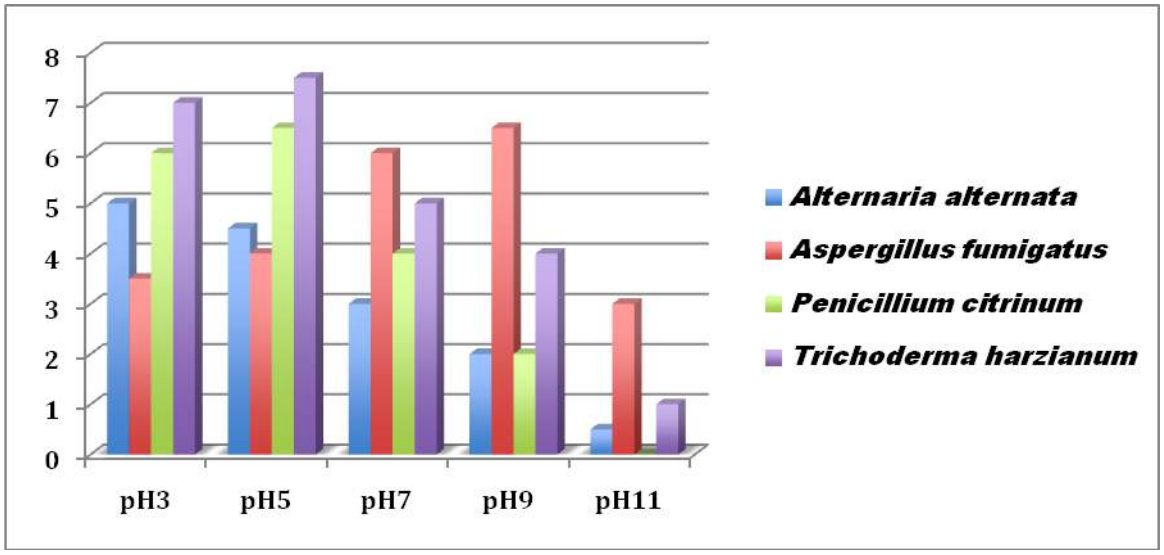


**Figure 2(b):** Effect of pH on radial fungal growth on CDA media

**Table 3:** The influence of temperatures and pH on radial fungal growth on WA media

| | Radial fungal growth (cm) | | | | |
|---|---|---|---|---|---|
| | **Fungi** | ***Alternaria alternate*** | ***Aspergillus fumigatus*** | ***Penicillium citrinum*** | ***Trichoderma harzianum*** |
| Temperatures | **4°C** | **0** | **0** | **1.5** | **0** |
| | **15°C** | **1.5** | **1.2** | **2** | **0** |
| | **25°C** | **2** | **1.5** | **2** | **0** |
| | **37°C** | **1.5** | **1.3** | **1.5** | **0** |
| | **50°C** | **0** | **1.4** | **0** | **0** |
| pH | **3** | **0.5** | **0** | **2** | **1.5** |
| | **5** | **1** | **1** | **1.5** | **1** |
| | **7** | **1.5** | **2** | **1** | **1** |
| | **9** | **1** | **2.5** | **0.5** | **0.5** |
| | **11** | **0** | **1** | **0** | **0** |

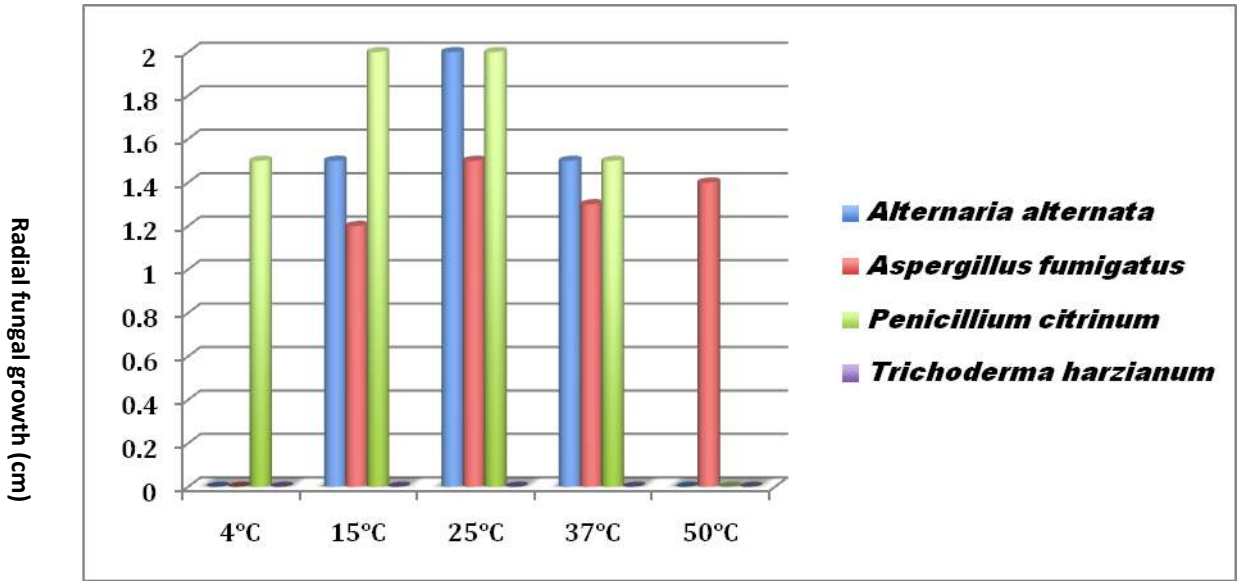


**Figure 3(a):** Effect of temperature on radial fungal growth on WA media

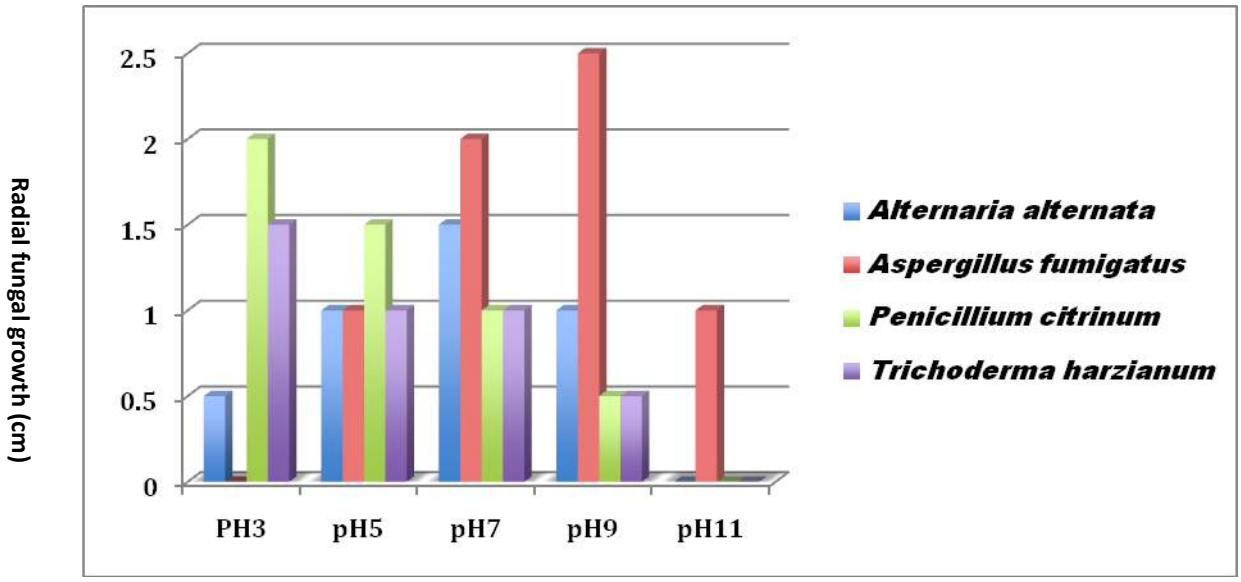


**Figure 3(b):** Effect of pH on radial fungal growth on WA media

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