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RESEARCH PAPER

Assessing Microbial and Biochemical Quality Indicators of Tanos Treated Soils

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A B S T R A C T:

Different doses of tanos fungicide and two soil textures in combination (2×4: soil texture × fungicide doses) were studied in a pot experiment under chickpea cultivation to determine their effects on certain soil chemical, biochemical and microbiological properties. The main results showed that: clayey soil showed highest pH, enzymatic activities and microbial population during this study. Tanos doses showed significant changes in soil EC, reduced urease (R^2 = 0.9775), dehydrogenase (R^2 = 0.8806) and nitrate reductase (R^2 = 0.8541) activities. Whereas, significant increases in bacterial and fungal population in response to tanos doses were observed after two months. According to paired *t* test results, different tanos doses, as well as the combination effects between soil textures and tanos doses have significantly changed fungal population, urease, dehydrogenase and nitrate reductase activities after two months of experiment. The combined treatment S2D3 showed the lowest nitrate reductase activity, bacterial and fungal counts during the study. A significant correlation (R^2 = 0.8581) was observed between urease activity and bacterial population.

KEY WORDS: Soil Enzymes, Bacteria, Fungi, Fungicide, Tanos.
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1. INTRODUCTION

Soil is the vital component of natural environment and it is important as plants, animals, rocks, landforms and rivers that influences the distribution of plant species and provides habitat for a wide range of organisms. Soil controls the flow of water and chemicals between atmosphere and earth, acting as a source and storage of gasses in the atmosphere (Nortcliff *et al.*, 2006). Soil needs to be conserved and improved its performance and productive capacity. A wide range of pollutants, especially pesticides and heavy metals are stored in agricultural soils in several regions of the world (Bünemann *et al*., 2006).

Soil pollution by pesticides may relate to the accumulation of pesticides and chemical elements in the soil in a situation where they become more than natural (Usman *et al*., 2017).

The application of fungicides to soil to control plant diseases has become a common practice in crop production in many parts of the world. Fungicides are bio-toxicants which interfere not only with the biochemical and the physiological reactions of the target plant pathogens, but also influence population or activity of other non-target microorganisms in soil (Chen *et al*., 2001). Concern over the effects of fungicides on soil processes is because microbes mediate many of the reactions in nutrient cycling; there is also the possibility that fungicides can enter into the food chain and, thus, affect higher organisms including humans (Monkiedje and Spiteller, 2002).

Tanos 50WG is a double-component combined and systemic fungicide used to protect potatoes from potato blight and potato leaf spot caused by *Alternaria* sp., tomatoes from potato

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blight, cucumbers and zucchini from *Peronospora* cucumber, and vines from *Peronospora* soybean and chickpea. The active substance of Tanos is cymoxanil 250g.kg^{-1} [1-[(E/Z)-2-cyano-2-methoxyiminoacetyl]-3-ethylurea] and famoxadone 250g.kg^{-1} [(RS)-3-anilino-5-methyl-5-(4-phenoxyphenyl)-1,3- oxazolidine-2,4-dione]. It is formulated as: dispersible granule (Sasvary and Supinova, 2016).

Soil enzymes are considered as valuable parameters for assessing the side effects of pesticide treatments on soil microbial biomass and microbiological potential (Gundi *et al*., 2005). It has detected that some pesticides changed soil enzyme activity. Soil enzymes are essential in catalyzing reactions necessary for decomposition of organic matter and nutrient cycling because they are important in energy transfer, environmental quality and crop productivity. Enzymes are closely related to organic elements transformation which induced by microorganisms (Cycon *et al*., 2005).

Despite the good results of pesticides in agriculture and public health like increasing crop yield, economic boost, decreasing fatalities from pest-borne diseases; their use usually accompanied with undesirable environmental, health effects and crop production especially from repeated application. Thus, the present work aims to determine the effects of different doses of tanos fungicide on soil quality indicators regarding to enzymatic activities and microbial communities in two different textured soils under cultivated chickpea plant.

2. MATERIALS AND METHODS

2.1. Experimental layout and statistical analysis

Two different soil textures with no history of prior pesticide-use from Shaways (clayey) and Aski Kalak (sandy loam) were collected in May 2019 and brought to the greenhouse, where gravels and stones were separated, screened, crushed, air-dried, and 4-mm sieved. Tanos fungicide obtained from Dubbana Company was prepared on the base of its active ingredient as given by Hill (2008) at four different doses (D1, no tanos fungicide treatment; D2, recommended dose; D3, double and D4, ten-fold doses). The two soils were separately sprayed with tanos fungicide at its different doses, and left an overnight at room temperature.

Eight combined treatments were set up each with three replications in a factorial experiment (2×4, two soil textures and four different doses) combined with Completely Randomized Design (CRD).

The experiment last for two months and included two sampling periods. For this purpose 24 pre-labeled and similar plastic pots (with average diameter 27 cm and height 25 cm) were filled with 5 kg pesticide-treated soil and a sample was taken from each pot. The pots were provided by a below-container to collect the irrigation water and return-back to the pots as described by (Khudhur and Sarmamy, 2019). The pots were irrigated at 60% of field capacity. Statistical analysis was performed using SPSS version 23 and Microsoft Office Excel 2019 and the means were compared using Duncan's Multiple Range test at level of significant of 0.05. Paired t test was applied to find out the differences between the sampling periods.

2.2. Laboratory analyses

2.2.1. Soil chemical characteristics

Soil pH was assessed using standardized pH-meter with pH buffers (4, 7 and 9) and EC was detected by calibrated EC-meter with KCl solution in 1:1 (soil: water suspension). Soil organic matter was detected by Walkley-Black procedure as described by Ryan *et al*. (2001).

2.2.2. Estimation of soil enzymes

Urease activity was determined by the modified approach of Hoffmann and Teicher 1961 described by (Uzun and Uyanoz, 2011). After incubating urea-induced soils for 3 hours at 30°C, the formatted ammonium was observed spectrophotometrically at 636 nm (Bashour and Sayegh, 2007). Modified method of Casida 1977 given in Anjaneyulu *et al.*, (2011), was used for dehydrogenase estimation. Soils were introduced with 3% aqueous solution of triphenyl tetrazolium chloride (TTC) and incubated at 30°C for 24 hours. The produced triphenyl tetrazolium formazone was measured at 485 nm. Standard protocol for nitrite estimation in solutions was

followed for estimation of nitrate reductase as given by Nath and Samanta (2012). Soils were incubated in peptone water media with 1% KNO_3 at 30 °C for 3 hours and the supernatants were treated with sulphanilamide and the pink color solutions were estimated at 540 nm. The catalase enzyme was detected by $KMnO_4$ titration method as given by Kumar (2004). Soils were amended with H_2O_2 and the rest of the peroxide was balanced out by 3N H_2SO_4 and the aliquots were titrated with 0.1 N $KMnO_4$.

2.2.3. Microbial population counting

For bacterial counting from soil samples, 1g of soil was serially diluted in sterile distilled water and 1 ml of soil suspensions from 10^{-1} to 10^{-7} was spread on the nutrient agar plate. The growth of the bacterial colonies were observed after 24 hours of incubation at 30°C in inverted position (Khudhur *et al.*, 2016). For counting of total soil fungi, Sabouraud dextrose agar was prepared. Then 0.2 mg Chloramphinicol was added. Serial dilution was performed and 1 ml of 10^{-3} to 10^{-7} dilutions were poured in each Petri dish containing prepared medium (PDA + Chloramphinicol) by sterile pipette, each sample made by three replication plates and incubated at 25 °C for 10 days (Khudhur and Abdulla, 2016).

3. RESULTS AND DISCUSSION

Soil pH considered as an important soil quality indicator affecting on soil microorganisms and enzymatic activity (Martinez *et al.*, 2010). Soil pH values of clay-textured soil was highest during both sampling periods (8.48 ± 0.217^{a}) and (8.47 ± 0.074^{a}), however, soil pH showed no significant changes toward fungicide doses (Table 1) in the first sampling.

Table 1: Effect of soil texture, tanos doses and their combinations on soil chemical characteristics (Mean±S.D.).

Treatments	Soil pH		Soil EC (µS.cm^{-1})		Soil organic matter (%)	
	1st sampling	2nd sampling	1st sampling	2nd sampling	1st sampling	2nd sampling
S1	**8.47 ± 0.217^{a}**	**8.47 ± 0.074^{a}**	**284.25 ± 64.59^{b}**	**387.75 ± 85.63^{a}**	**0.69 ± 0.326^{b}**	1.25 ± 0.297
S2	**8.34 ± 0.189^{b}**	**8.33 ± 0.086^{b}**	**385.00 ± 120.4^{a}**	**229.91 ± 64.14^{b}**	**1.44 ± 0.302^{a}**	1.46 ± 0.656
t value	**1.000 (NS)**		**0.200 (NS)**		**-1.074 (NS)**	
D1	8.44 ± 0.278	**8.47 ± 0.082^{a}**	**247.83 ± 47.40^{c}**	348.16 ± 166.9^{a}	**1.18 ± 0.228^{a}**	1.51 ± 0.390
D2	8.31 ± 0.143	8.40 ± 0.079^{ab}	293.66 ± 58.15^{c}	**264.00 ± 97.72^{b}**	1.11 ± 0.287^{ab}	1.34 ± 0.483
D3	8.45 ± 0.255	**8.36 ± 0.126^{b}**	358.00 ± 95.03^{b}	**353.50 ± 80.42^{a}**	**0.89 ± 0.481^{b}**	1.48 ± 0.249
D4	8.43 ± 0.156	**8.36 ± 0.105^{b}**	**439.00 ± 117.5^{a}**	269.66 ± 51.99^{b}	1.09 ± 0.834^{ab}	1.09 ± 0.781
t value	**0.236 (NS)**		**0.813 (NS)**		**-2.352 (NS)**	
S1D1	8.24 ± 0.260^{c}	**8.53 ± 0.060^{a}**	254.33 ± 70.06^{cd}	**495.66 ± 62.18^{a}**	1.03 ± 0.137^{bc}	1.17 ± 0.069^{cd}
S1D2	8.42 ± 0.113^{bc}	8.45 ± 0.072^{ab}	266.33 ± 78.23^{cd}	329.66 ± 32.81^{bcd}	0.89 ± 0.182^{c}	**0.91 ± 0.105^{d}**
S1D3	**8.68 ± 0.056^{a}**	8.46 ± 0.060^{a}	277.00 ± 43.27^{cd}	420.66 ± 28.01^{ab}	0.51 ± 0.210^{d}	1.44 ± 0.344^{abc}
S1D4	8.54 ± 0.136^{ab}	8.43 ± 0.102^{ab}	339.33 ± 59.91^{c}	305.00 ± 13.23^{bcd}	**0.34 ± 0.137^{d}**	1.49 ± 0.173^{abc}
S2D1	8.63 ± 0.110^{ab}	8.42 ± 0.072^{ab}	**241.33 ± 24.11^{d}**	200.66 ± 22.30^{d}	1.33 ± 0.210^{b}	**1.86 ± 0.137^{a}**
S2D2	**8.21 ± 0.085^{c}**	8.36 ± 0.072^{ab}	321.00 ± 9.643^{cd}	**198.33 ± 99.29^{d}**	1.33 ± 0.173^{b}	1.77 ± 0.173^{ab}
S2D3	8.22 ± 0.032^{c}	**8.26 ± 0.086^{b}**	439.00 ± 31.95^{b}	286.33 ± 43.02^{cd}	1.28 ± 0.278^{b}	1.51 ± 0.182^{abc}
S2D4	8.31 ± 0.057^{c}	8.29 ± 0.036^{ab}	**538.66 ± 33.62^{a}**	234.33 ± 53.26^{abc}	**1.83 ± 0.210^{a}**	1.69 ± 1.000^{bc}
t value	**0.101 (NS)**		**0.420 (NS)**		**-1.171 (NS)**	
	NS: paired t value non-significant. **** : paired t value highly significant at $p \leq 0.001$.**					

Although the second sampling, showed changes in pH as the double and ten-fold doses of tanos has caused slight reductions in soil pH (8.36), but, these changes were not significant (*t* value = 0.236). Hicks *et al.* (1990) reported that pH affects the athletic behavior of the pesticide molecule on clay and organic surfaces and thus the chemical speciation, mobility and bioavailability of the molecule and this seem to confirm the present findings. Fungicide doses have showed significant changes in soil EC during both sampling periods (Table 1). In treatments received double and ten-fold dose, the EC values were 353.50 ± 80.42^{a} and 439.00 ± 117.5^{a} µS.cm^{-1} respectively, and the combined treatments S2D4 and S1D1 revealed highest soil EC (538.66 ± 33.62^{a} and 495.66 ± 62.18^{a} µS.cm^{-1}) respectively. Soil organic matter has significant effects on microbial activity due to its nature as a nutrient sink and a source that can enhance soil physical and chemical properties and promote biological activity (Fontaine *et al.*, 2003). Soil organic matter have been increased in the combinant treatment S2D4 (1.83 ± 0.210^{a}).

Soil enzymatic activity is an indication for living cell activity as well as the concentration of soil colloids and humic substances can be used as soil contamination indicators (Bello *et al.*, 2013). Urease is a soil enzyme that catalyzes urea hydrolysis into CO_2 and NH_3 and is a key component of the soil nitrogen content (Sarathchandra *et al.*, 1984). Clayey soil showed highest and significant urease activity during both sampling periods 78.705 ± 10.986^{a} and 112.005 ± 21.564^{a} µg N-NH_4^+.g^{-1}.$3h^{-1}$ respectively (Table 2).

Table 2: Effect of soil texture, tanos doses and combinations on soil urease and dehydrogenase activities (Mean±S.D.).

Treatments	Urease (µg N-NH_4^+.g^{-1}.$3h^{-1}$)		Dehydrogenase (TPF µg.g^{-1}.$24h^{-1}$)	
	1st sampling	2nd sampling	1st sampling	2nd sampling
S1	**78.705 ± 10.986^{a}**	**112.005 ± 21.564^{a}**	**609.792 ± 18.086^{a}**	19.722 ± 4.214
S2	**42.420 ± 11.372^{b}**	**88.915 ± 28.132^{b}**	**160.077 ± 73.210^{b}**	13.561 ± 6.374
t value	**-6.047 (NS)**		**1.661 (NS)**	
D1	**72.350 ± 20.917^{a}**	105.450 ± 10.612^{ab}	**450.187 ± 237.041^{a}**	17.182 ± 6.519^{ab}
D2	67.270 ± 18.864^{a}	97.450 ± 10.573^{ab}	424.852 ± 218.627^{b}	**20.208 ± 4.780^{a}**
D3	55.010 ± 21.583^{b}	**89.870 ± 38.629^{b}**	333.219 ± 258.362^{c}	16.326 ± 2.411^{ab}
D4	**47.620 ± 20.299^{c}**	**109.070 ± 37.724^{a}**	**331.479 ± 271.805^{c}**	**12.851 ± 8.359^{b}**
t value	**-5.504****		**12.381****	
S1D1	**90.380 ± 9.983^{a}**	96.600 ± 4.160^{cde}	**666.285 ± 18.086^{a}**	22.230 ± 1.459^{a}
S1D2	84.040 ± 4.014^{a}	88.680 ± 4.151^{de}	624.384 ± 4.057^{b}	**24.133 ± 2.792^{a}**
S1D3	74.300 ± 5.765^{b}	124.940 ± 4.607^{ab}	568.962 ± 12.352^{c}	15.928 ± 2.614^{b}
S1D4	66.100 ± 0.510^{b}	**137.800 ± 8.990^{a}**	579.538 ± 1.204^{c}	16.597 ± 2.464^{b}
S2D1	54.320 ± 4.350^{c}	114.300 ± 4.160^{bc}	234.089 ± 7.020^{c}	**12.133 ± 5.258^{b}**
S2D2	50.500 ± 5.466^{c}	106.220 ± 4.151^{bcd}	225.321 ± 6.311^{c}	16.283 ± 1.763^{b}
S2D3	35.720 ± 3.875^{d}	**54.800 ± 4.435^{e}**	97.476 ± 0.811^{d}	16.725 ± 2.687^{b}
S2D4	**29.140 ± 2.311^{d}**	80.340 ± 31.63^{e}	**83.420 ± 9.693^{d}**	21.87 ± 11.248^{a}
t value	**-2.416****		**4.264****	
	NS: paired t value non-significant. **** : paired t value highly significant at $p \leq 0.001$.**			

Tanos doses significantly affected urease activity (R^2 = 0.9775), it was observed that with increasing tanos dose will decrease urease enzyme activity (47.620 ± 20.299^{c}) µg N-NH_4^+.g^{-1}.$3h^{-1}$ as supported by (Figure 1).

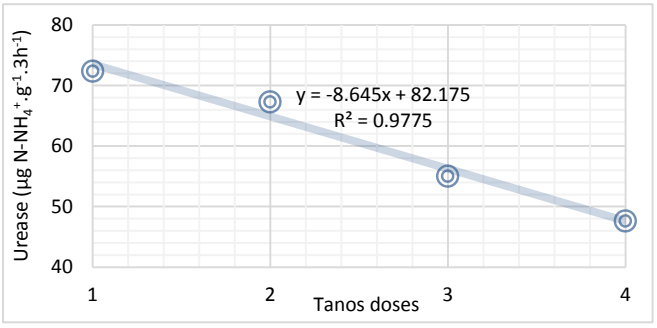

Figure 1: Correlation between tanos doses and urease activity during the first sampling.

Floch *et al.* (2011) stated that pesticides may have a direct impact on enzymes by inhibiting their catalytic ability, or by involving in the alteration of microbial activity and this may confirm this situation. Moreover, different tanos doses have significantly changed urease activity after two months of experiment according to *t* value (-5.504^{**}, $p \leq 0.001$) (Table 2). The combination effects of both soil texture and fungicide doses were significantly affected urease activity, the highest urease was observed in S1D1 (90.380 ± 9.983^{a}) and S1D4 (137.800 ± 8.990^{a}), whereas the lowest activities were detected in S2D4 (29.140 ± 2.311^{d}) and S2D3 (54.800 ± 4.435^{e}) during both sampling periods respectively. These decreases in urease activities may refer to reducing urea hydrolysis due to tanos fungicide use in accordance with (Antonious, 2003). Dehydrogenase activities were decreased as tanos doses increased during the studied periods (Table 2 and Figure 2).

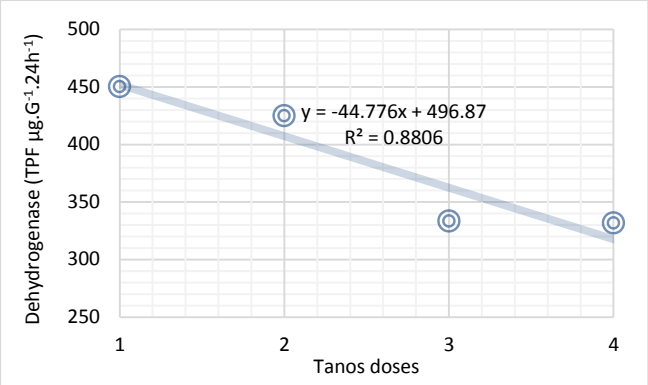

Figure 2: Correlation between tanos doses and dehydrogenase activity during the first sampling.

Its activity was sharply decreased during the second sampling period with the highest recorded *t* value (12.381**) (Figure 3).

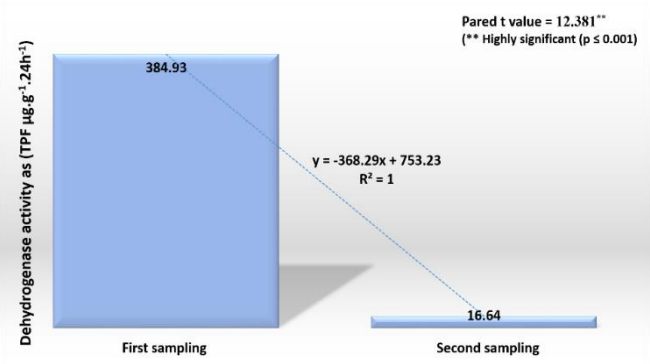

Figure 3: Dehydrogenase responses to different tanos doses during both sampling periods.

The reported sensitivity of dehydrogenase to the adverse effects of pesticides is consistent with many studies (Cycon *et al.*, 2005). Significant changes have observed between the sampling periods (*t* value of -5.132**) regarding to nitrate reductase activity (Table 3). During the second sampling, significant reduction has observed in responses to tanos doses as supported by (Figure 4).

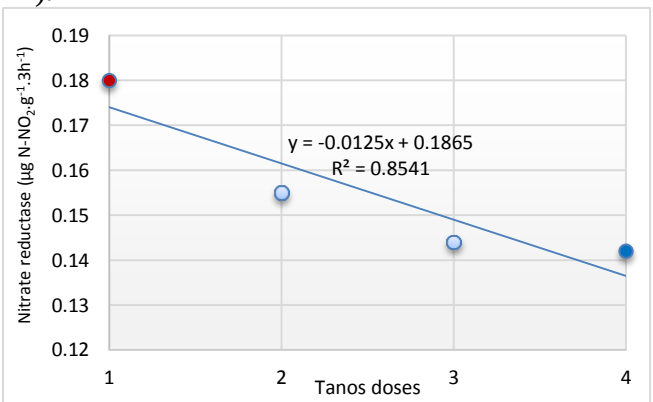

Figure 4: Correlation between tanos doses and nitrate reductase activity during the second sampling.

The combined treatment S2D3 showed the lowest value of nitrate reductase activity 0.061 ± 0.005^{f} and 0.106 ± 0.009^{e} (µg N-NO_2.g^{-1}.$3h^{-1}$) during both sampling periods respectively. While the highest activity was in control S2D1 (0.226 ± 0.0327^{a} µg N-NO_2.g^{-1}.$3h^{-1}$) during the second sampling. Catalase is an intracellular enzyme found in all aerobic bacteria and most facultative anaerobic bacteria, but not present in binding anaerobic agents (Trasar-Cepeda *et al.*, 2000). During the second sampling, catalase has increased slowly and the lowest value was recorded in control S2D1 (9.933 ± 0.351^{c} ml $KMnO_4$.g^{-1}.$20min^{-1}$) (Table 3).

Table 3: Effect of soil texture, tanos doses and combinations on nitrate reductase and catalase (Mean±S.D.).

Treat-ments	Nitrate reductase (µg N-NO_2.g^{-1}.$3h^{-1}$)		Catalase (ml $KMnO_4$.g^{-1}.$20min^{-1}$)	
	1st sampling	2nd sampling	1st sampling	2nd sampling
S1	**0.135±0.032^{a}**	0.151±0.023	**9.616±0.032^{a}**	11.941±0.023
S2	**0.073±0.012^{b}**	0.159±0.051	**11.708±0.012^{b}**	11.541±0.051
t value	-1.457 (NS)		-0.866 (NS)	
D1	0.110±0.028^{b}	**0.180±0.055^{a}**	**10.116±1.149^{b}**	**10.566±0.794^{b}**
D2	**0.132±0.056^{a}**	0.155±0.028^{b}	10.216±1.227^{b}	**12.533±1.318^{a}**
D3	0.089±0.031^{c}	0.144±0.044^{b}	**11.266±1.148^{a}**	12.233±1.543^{a}
D4	**0.085±0.021^{c}**	**0.142±0.015^{b}**	11.050±2.044^{ab}	11.633±0.999^{a}
t value	-5.132**		2.528 (NS)	
S1D1	0.135±0.014^{b}	0.133±0.012^{de}	9.633±1.563^{d}	11.200±0.500^{bc}
S1D2	**0.183±0.017^{a}**	0.138±0.014^{cde}	**9.200±0.754^{d}**	13.400±1.081^{a}
S1D3	0.117±0.005^{c}	0.182±0.021^{b}	10.333±0.702^{cd}	11.033±0.611^{bc}
S1D4	0.105±0.001^{c}	0.152±0.004^{bcd}	9.300±1.081^{d}	12.133±1.258^{ab}
S2D1	0.086±0.009^{d}		10.600±0.400^{cd}	**9.933±0.351^{c}**
S2D2	0.082±0.007^{de}	0.173±0.028^{bc}	11.233±0.305^{bc}	11.666±0.960^{b}
S2D3	**0.061±0.005^{f}**	**0.106±0.009^{e}**	12.200±0.435^{ab}	**13.433±1.123^{a}**
S2D4	0.065±0.000^{ef}	0.131±0.016^{de}	**12.800±0.0300^{a}**	11.133±0.404^{bc}
t value	-2.566**		-1.640 (NS)	
	NS: paired t value non-significant.			
	**** : paired t value highly significant at $p \leq 0.001$.**			

Moreover, the highest catalase values were in the combined treatments S2D3 (12.800±0.0300[a]) and S2D4 (13.433±1.123[a] ml $KMnO_4.g^{-1}.20min^{-1}$) respectively during both sampling periods, it is therefore difficult to detect a clear response of this enzymatic activity to pesticides, as this enzyme has received little attention in the last 10 years (Trasar-Cepeda *et al.*, 2000). According to the presented data in (Table 4), bacterial and fungal population were higher in clayey soil than the sandy loam soil during both sampling periods.

Table 4: Effects of soil texture, tanos doses and their combinations on soil total microbial population (Mean).

Treat-ments	Total bacteria×10^6 cfu.g^{-1} dry soil		Total fungi×10^5 cfu.g^{-1} dry soil	
	1st sampling	2nd sampling	1st sampling	2nd sampling
S1	**35.259[a]**	**35.490[a]**	**35.259[a]**	**35.490[a]**
S2	**2.011[b]**	**27.053[b]**	**2.011[b]**	**27.053[b]**
t value	**-1.019 (NS)**		**-1.614 (NS)**	
D1	27.027	31.027[b]	7.336[a]	**58.712[b]**
D2	28.058	**27.790[b]**	5.101[a]	59.835[b]
D3	4.818	30.780[b]	**1.180[b]**	117.613[ab]
D4	14.636	**35.488[a]**	**5.741[a]**	**152.510[a]**
t value	**-1.985 (NS)**		**3.926[**]**	
S1D1	**52.92[a]**	27.88[c]	**14.08[a]**	97.86[b]
S1D2	50.56[a]	24.96[c]	8.678[b]	98.01[b]
S1D3	9.448[b]	43.76[a]	2.319[c]	**225.5[a]**
S1D4	28.11[ab]	**45.35[a]**	11.34[b]	213.1[a]
S2D1	1.135[b]	34.173[b]	0.597[c]	19.56[b]
S2D2	5.561[b]	30.619[bc]	1.524[c]	21.66[b]
S2D3	**0.189[b]**	**17.797[d]**	**0.042[c]**	**9.702[b]**
S2D4	1.160[b]	25.622[c]	0.145[c]	91.90[b]
t value	**-1.475 (NS)**		**-3.200[**]**	
	NS: paired t value non-significant. **[**] : paired t value highly significant at $p \leq 0.001$.**			

Significant increase in bacterial population in response to tanos doses was observed during the second sampling, so the highest tanos dose (D4) showed highest bacterial population (35.488×10^6 cfu.g^{-1} dry soil) and fungal population (5.741×10^5 cfu.g^{-1} dry soil). This situation, as stated by (Kalia and Gosal, 2011), may refer to the ability of microorganisms to use pesticides as growth C source, a fraction of the used pesticide, as demonstrated in (Figure 5).

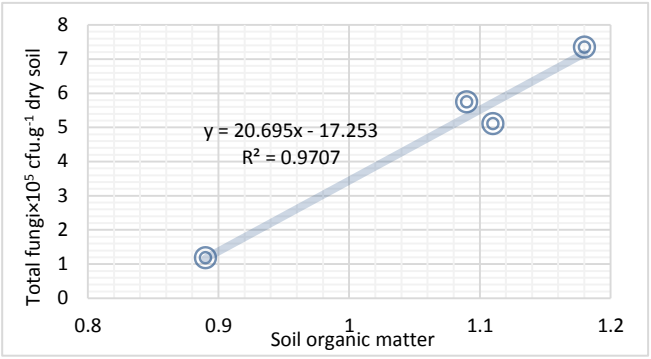

Figure 5: Correlation between soil organic matter and total fungal population during the study.

Moreover, it is well documented that with certain pesticides, repeated applications can promote microbial populations capable of selectively degrading that fungicide and various pesticides may degrade by bacterial isolates (Kanekar *et al.*, 2004). Furthermore, soil enzyme activities are greatly affected by organic matter content of the soil and often are used as indices of microbial activity, soil fertility and pollution (Cycon *et al.*, 2005). According to the observed data, a significant correlation (R^2 = 0.8581) was observed between urease activity and bacterial population (Figure 6).

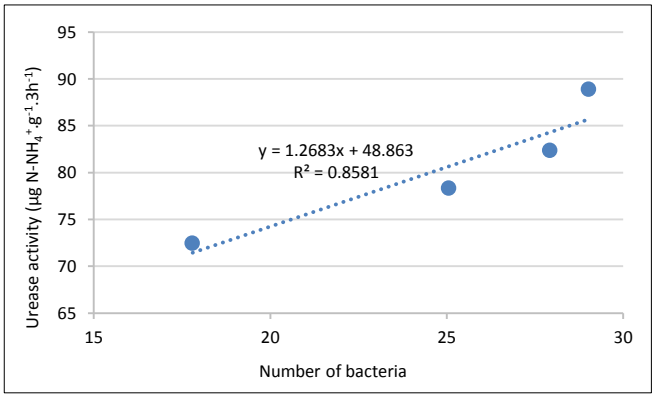

Figure 6: Correlation between bacterial population and urease activity during the second sampling.

However, the numbers of bacteria were directly proportional with urease enzyme activity in the soil. In this regard, Sarathchandra *et al*. (1984) stated that urease is producing by large number of bacteria. The highest tanos dose (D4) revealed the highest fungal population during both samplings (5.741 $\times 10^5$ cfu.g^{-1} dry soil) and (152.51 $\times 10^5$ cfu.g^{-1} dry soil) respectively (Figure 7) and the differences between the two sampling periods was significant (*t* value = 3.926** at $p \leq 0.001$).

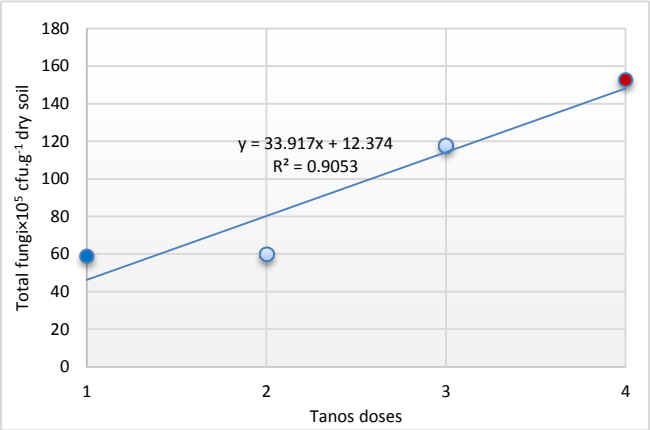

Figure 7: Correlation between tanos doses and soil fungi during the second sampling.

The statement of (Rajbongsh *et al.*, 2014) who state that the fungal population has typically decrease following fungicide application, but it slowly recover from the deleterious effect of fungicides applied to the soil over time, may confirm our findings. Although there were increases in bacterial and fungal population during the second sampling, but the combined treatment S2D3 showed the lowest bacterial number (0.189 and 17.797 $\times 10^6$ cfu.g^{-1} dry soil) and fungal count (0.042 and 9.702 $\times 10^5$ cfu.g^{-1} dry soil) during both sampling periods respectively. While, S1D3 showed the highest fungi number (225.5 $\times 10^5$ cfu.g^{-1} dry soil) during the second sampling. The combined effect of soil texture and pesticide doses showed significant differences between the two sampling periods (*t* value = -3.200** at $p \leq 0.001$).

4. CONCLUSIONS

Microbial and biochemical soil parameters are identified soil quality assessment indicators. From the results of this investigation we concluded that long-term and repeated fungicide applications interfere with soil biochemical balance, which can reduce soil fertility and productivity by affecting local metabolism and enzymatic activity especially the dehydrogenase, as shown by tanos doses especially double and tenfold doses. Tanos fungicides affect non-target and beneficial microorganisms and their behaviors, which are important for preserving soil fertility, as revealed during the first sampling, while a recovery of soil bacteria and fungi population was observed after two months of experiment.

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Conflict of Interest

The authors have no Conflict of Interest.

REFERENCES

Anjaneyulu, E., Ramgopal, M., Narasimha, G. and Balaji, M. 2011. Effect of pig iron slag particles on soil physico-chemical, biological and enzyme activities. *Iranica J. Energy & Environment*, 2(2): 161-165.

Antonious, G.F. 2003. Impact of soil management and two botanical insecticides on urease and invertase activity. *J. Environ. Sci. Health B*, 38, 479-488.

Bashour I.I. and Sayegh, A.H. 2007. Methods of Analysis for Soils of Arid and Semi-Arid Regions. Food and Agriculture Organization of the United Nations, Rome.

Bello, D., Trasar-Cepeda, C., Leirós M.C. and Gilsotres, F. 2013. Modification of enzymatic activity in soils of contrasting pH. *Soil Biol Biochem*, 56:80-86.

Bünemann, E., Schwenke, G., and Van Zwieten, L. 2006. Impact of agricultural inputs on soil organisms-a review. *Australian Journal of Soil Research*, 44(4), 379-406.

Chen, S.K., Edwards, C.A. and Sulber, S. 2001. Effects of the fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations. *Soil Biology and Biochemistry*, 33: 1971-1980.

Cycon, M., Kaczyñska, A. and piotrowska-Seget, Z. 2005. Soil enzyme activities as indicator of soil pollution by pesticides. *Pestycydy*, (1-2), 35-45. ISSN 0208-8703.

Floch, C., Chevremont, A.C., Joanico, K., Capowiez, Y. and Criquet, S. 2011. Indicators of pesticide contamination functional diversity of bacterial

communities via Biolog Ecoplates. *Eur J Soil Biol* 47: 256-263n.

Fontaine, S., Marotti, A. and Abbadie, L. 2003. The priming effect of organic matter: a question of microbial competition. *Soil Biology & Biochemistry*, 35: 837-843.

Gundi, V., Narasimha, G. and Reddy, B.R. 2005. Interaction Effects of soil insecticides on microbial populations and dehydrogenase activity in a black clay soil. *J. Environmental Science and Health, Part B*, 40(2): 269-283.

Hicks, R.J., Stotzky, G. and Voris, P.V. 1990. Review and evaluation of the effects of xenobiotic chemicals on microorganisms in soil. *Adv Appl Microbiol*, 35:195-253.

Hill, D.S. 2008. Pests of Crop in Warmer Climates and their Control. Springer Netherlands. XII Edition. 708pp.

Kalia, A. and Gosal, S.K. 2011. Effect of pesticide application on soil microorganisms. *Agronomy and Soil Science*, 57(6):569-596.

Kanekar, P.P., Bhadbhade, B., Deshpande, N.M. and Sarnai, S.S. 2004. Biodegradation of organophosphorus pesticides. *Proc. Indian Natl. Sci. Acad.*, Part B70 (1): 57-70.

Khudhur, N.S. and Abdulla, N.Q.F. 2016. Soil fungal population study related to oil pollution along different distances from Kawrgosk Oil Refinery of Erbil-Iraq. *Al-Anbar J. of Agr. Sci.*, 14(2): 1e-15e.

Khudhur, N.S. and Sarmamy, A.O.I. 2019. Determination of diazinon residues in artificially polluted soils. *ZANCO Journal of Pure and Applied Sciences*, 31(5): 1-8.

Khudhur, N.S., Khudhur, S.M. and Ameen, N.O.H. 2016. A Study on soil bacterial population in Steel Company and some related area in Erbil City in relation to heavy metal pollution. *ZANCO Journal of Pure and Applied Sciences*, 28(5): 101-116.

Kumar, A. 2004. Industrial pollution and management, In, Study of correlation of physical, chemical and biological characteristics with catalase activity in industrially polluted and unpolluted soils of Warangal (D.T.) A.P. by Kumari B.L. and Charya M.A., APH Publishing. New Delhi, pp. 134-138.

Martinez, M.M., Gutiérrez, V., Jannsens, M. and Ortega, R. 2010. Biological soil quality indicators: a review. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, pp. 319-328.

Monkiedje, A. and Spiteller, M. 2002. Effects of the phenylamide fungicides, mefenoxam and metalaxyl, on the microbiological properties of a sandy loam and a sandy clay soil. *Biol Fertil Soils*, 35: 393-398.

Nath, R. and Samanta, R. 2012. Soil pH, microbial population, nitrate reductase and alkaline phosphatase activities of different environment of Dibrugarh district, Assam. *Advances in Applied Science Researc*h, 3(3): 1772-1775.

Nortcliff, S., Hulpke, H., Bannick, C.G., Terytze, K., Knoop, G., Bredemeier, M. and Schulte-Bisping, H. 2006. Soil, definition, function, and utilization of soil. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Vol. 33: 309-420. *DOI: 10.1002/14356007.b07_613.pub3*

Rajbongshi, P., Devashree, Y. and Kumardutta, B. 2014. A study on the effect of some fungicides on the population of soil microflora. *Journal of international academic research for multidisciplinary*, 1(12).

Ryan, J., Estefan, G. and Rashid, A. 2001. Soil and Plant Analysis Laboratory Manual. Second Edition. International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria. 172 pp.

Sarathchandra, S.U., Perrott, K.W. and Upsdell, M.P. 1984. Microbiological and biochemical characteristics of a range of New Zealand soils under established pasture. *Soil Biol. Biochem*. 16, 177-183.

Sasvary and Supinova 2016. Accidental Cymoxanil (TANOS) pesticide poisoning (Case report). *Clinical Social Work and Health Intervention*, 7(3): 27-31.

Trasar-Cepeda C., Leirós M.C., Seoane S. and Gil-Sotres F. 2000. Limitations of soil enzymes as indicators of soil pollution. *Soil Biology and Biochemistry*, 32: 1867–1875.

Usman, S., Kundiri, A.M. and Nzamouhe, M. 2017a. Effects of organophosphate herbicides on biological organisms in soil medium- A mini review. *Journal of Ecology and Toxicology*, 1(1):1-5.

Uzun, N. and Uyanoz, R. 2011. Determination of urease catalase activities and CO_2 respiration in different soils obtained from in semi-arid region Konya, Turkey. *Trends Soil Sci. Plant Nutr. J*., 2(1): 1-6.