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RESEARCH PAPER

Differences among three wheat cultivars in susceptibility to *Fusarium culmorum* and the effect of a number of bacterial strains on infested seed germination

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A B S T R A C T:

Wheat seedling blight caused by *Fusarium culmorum,* is one of the most common among wheat diseases. Last decades, use of resistant cultivars and biological control by antagonistic micro-organisms have received much attention. Resistance levels of three wheat cultivars (Saber Bag, Smito and Sham 5) to this disease was assessed under greenhouse conditions. None of the cultivars was high resistant but Saber Bag showed the highest germination rate 63.3%. This was 11% more than the two other cultivars.

In a dual culture three bacterial strains, two *Bacillus* and one *Pseudomonas* had very good effect in inhibition of the mycelium growth of this fungus. Bacterial strain *Bacillus sp* M1, *B. subtilis* K3 and *Pseudomonas sp* 53 inhibited the fungal mycelium growth by 100%, 97.5 %, and 98% respectively. In greenhouse experiments, bacterial strain M1 increased the number of seed germinations by 50% compared to untreated *Fusarium* control in cv. Sham5 and 43% in cv. Smito. Also strain 53 increased the number of germinations by 35% in cv. Smito.

KEY WORDS: bacteria, biological control, *Fusarium culmorum*, resistance, wheat cultivar**.**
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1. INTRODUCTION:

The present state of yield losses due to pathogenic diseases is upsetting, with an estimated up to 40% of crop yield losses caused by plant pathogens globally (Savary, S et al., 2012; Savary, S et al., 2019).

Fusarium species are worldwide vital pathogens of agricultural crops (Dunlap, C.A, et al., 2011). These pathogens are responsible for enormous economic losses of many main cereal food crops worldwide.

A number of *Fusarium* species are pathogenic for human and livestock and at the same time is responsible for plant diseases in numerous crops including cereals. They can be isolated from different plant parts, and soil (Summerell *et al.*, 2003).

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Leslie and Summerell (2006) stated that at least 80% of all cultivated plants are associated with one disease caused by a *Fusarium* species. These pathogens can coexist in the same plant causing complex diseases and able to produce secondary metabolites, mycotoxins which beside yield reduction and destroys the grain quality, are harmful to human and animals (Nelson *et al*., 1993; Parry *et al*., 1995; Logrieco *et al*., 2007).

Fusarium culmorum. is a global soil borne fungus able to cause Fusarium head blight, foot and root rot on different small-grain cereals, especially wheat and barley. It causes significant quantity and quality losses and results in grain mycotoxins contamination (Sherm *et al.*, 2013).

Fusarium pathogens are not reduced effectively by traditional disease control methods. Therefore, there is a dependency on chemical control in order to obtain reasonable level of yield. However, plant residue and soil infestation are potential problem with chemical usage. Besides, the organic sector in agriculture that does not use fungicide for control of plant diseases is increasing, therefore there is a crucial need for new areas and alternate control methods.

One of these areas is the application of resistant cultivars. Unfortunately, highly resistance cultivars are not available yet (Chrpová *et al*., 2007).

Another new strategy, which is the usage of micro-organisms as bio- means of controlling fungal diseases (Weller, 1988; Cook, 1993; Bouanaka *et al.*, 2021; Kowalska *et al.*, 2021). Naturally occurring antagonistic micro-organisms play a significant role in defeating plant diseases. Therefore, the use of bio-control agents either as an alternative or as a supplement to existing forms of plant disease control has attracted worldwide attention to be included in an integrated pest management strategy system.

Bacterial isolates used as bio- control agents have received excessive consideration because of their ability to suppress different plant diseases involving a combination of various mode of actions (Baehler *et al*., 2006; Cazorla *et al*., 2006). Numerous bacteria and fungi have been experienced for their effectiveness to control different plant pathogens. Many bacterial strains, including *Bacillus* and *Pseudomonas* have been used as seed treatments in wheat against *Fusarium* seedling blight caused by, *F. graminearum* , *F. culmorum* and *M. nivale* (Khan *et al*., 2006; Johnsson *et al*., 1998; Amein *et al*., 2008). Also some bacteria have been applied to the wheat heads (Khan et al., 2004).

The aim of the present research was: 1) to compare the susceptibility of three different wheat cultivars to *Fusarium culmor*. 2) to evaluate and compare the ability of some bacterial strains to control this pathogen.

2.Material and Methods:

2. 1. Fusarium isolate:

The *Fusarium culmorum* isolate used in this experiment was isolated from a wheat field belonging to Agriculture University experimental site (Gerdarasha).

2. 2. Wheat cultivars:

Three common winter wheat write (Triticum aestivum) cultivars (Smito, Saber beg and Sham 5) were used in this study.

2. 3. Seed sterilization:

Seeds were surface disinfected to remove any unwanted organisms, by washing for 2 minutes in 20 mL L -1 (2 % v/v) sodium hypochlorite, following by three rinses for 5 minutes each in (SDW) sterile distilled water, and dried on filter paper in a plastic Petri dish at room temp. under natural indoor light for 24 hours.

2. 4. Pathogen inoculum preparation:

Fusarium spores were collected from lawn cultures grown for three weeks on (PDA), potato dextrose agar medium at room temperature (22 – 24 °C) by flooding the culture with sterile distilled water and removing spores from the hyphae with the aid of a sterile glass spreader. To remove any hyphal fragments present, the solution with spores was filtered through 2 successive sterile absorbent cotton wool plugs. Number of spores were counted using a hemocytometer, diluted to 10^5 spores/mL as a stock spore solution, and kept in refrigerator at 4 C, till use.

2. 5. Seed infestation:

Fifty surface sterilized seeds were putted in 50 ml. falcon tube and then coated with 5 ml *Fusarium* spore suspension and mixed by hand for five minutes then the excess suspension was drained then, the seeds were dried on filter paper under a fan overnight and saved until use.

2. 6. Soil sterilization:

One liter of formalin was added to 20 liter of water and mixed with 200 kg of soil. The soil was covered for one week then left open and mixed for three days before use.

2. 7. Cultivars experiment:

Seeds of each three cultivars were surface sterilised and infested with the pathogen *F. culmorum* as described above. Seeds were sown two cm deep in six pots (12 cm in diam. and 15 cm high), three seeds per pot. Each pot was filled with about 500 g. of commercial peat mixture soil, mixed with 20% (v/v) sand. Puts were placed in greenhouse as randomized block. The pots were watered twice per week.

Seed germination was counted after ten days and one month. The experiment was conducted twice.

2. 8. Screening of bacterial isolates:

Among a group of bacterial strains earlier isolated from different crops in Sweden, three strains were chosen. (*Pseudomonas*53) isolated from wheat root, (*Bacillus* sp M1*)* isolated from carrot seed and (*Bacillus subtilis* K3) isolated from oilseed rape seed were used in this study.

To formulate seeds for screening, bacteria were cultured in (NA) nutrient agar in plastic Petri dishes at 22–25 °C for 48 h. the bacterial strains were then grown on LB (Luria-Bertani medium) in a 250 ml. conical flask and incubated for 48 h on a rotary shaker (180 rpm) in the dark at 26 – 28 °C. Fifty infested seeds with *F. culmorum* prepared as described above in 50 ml. falcon tube and were coated with 5 ml bacterial suspension of an individual strains and after 15 minutes, excess liquid was drained, and seeds were sown in soil (mixture of silty clay loam and loam) next day.

2. 9. In vitro test: (dual culture):

Using micropipette and glass spreader, 50 ul bacterial suspension of each strain were spread over the PDA medium in each petri dish (90 mm diam.). A five mm fungal plug from a one-week-old culture on PDA were placed in the centre of the dishes. Ability of the bacterial strains to inhibit the fungal growth was assessed by measuring the diameter of mycelial colony growth (mm) after 4 days of incubation at 24 – 26 °C. Three replicate plates were used for each bacterial strain combination (treatment).

2. 10. In vivo experiment:

A germination test in Petri dishes showed about 85% seed germination for all three cultivars (data not shown). Infested seeds and individually treated by each bacterial strain was sown (2) cm deep in six pots (12 cm in diam. and 15 cm high), with 4 seeds. per pot. Each pot was filled with about 500 gram of commercial peat mixture soil, mixed with 20% (v/v) sand. Puts were placed in greenhouse as randomized block. The pots were watered twice per week.

Seed germination was counted after ten days and one month. The experiment was conducted twice.

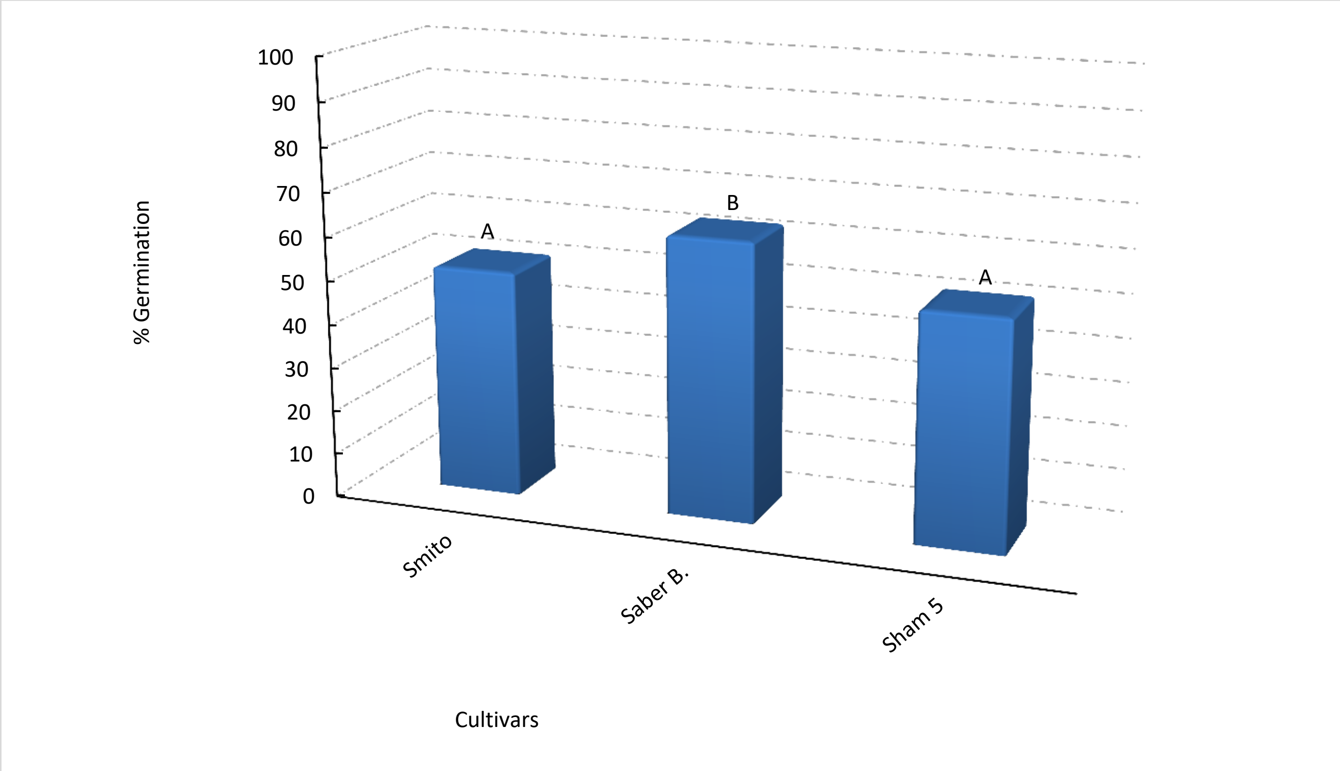
Statistics:

Data were subject to (ANOVA) analysis of variance, and Fisher's protected least significant difference test, ($P < 0{\cdot}05$), were applied for analyses of the results.

3. Results:

3. 1. Wheat cultivar's reaction to the pathogen:

The resistance level of wheat cultivars to *F. culmorom* was assessed. Cultivar Saber beg had 63.3% germination rate and was better than the two other cultivars Sham 5 and Smito which had germination rates of 51.9% and 51.7% respectively (Figure 1).



Different letters within columns indicate statistically significant differences, according to Duncan's Significance Level test (P < 0.05).

Fig. 1. Results of susceptibility of three different wheat cultivars to *Fusarium culmorum.*

Experiment was conducted in pots in greenhouse with six pots each with four seeds. The experiment was conducted twice.

3. 2. In vitro test: (dual culture):

Bacterial strain *Bacillus subtilis* M1 inhabited the fungal mycelium growth by 100%, *B. subtilis* K3 inhabited the *Fusarium* mycelium growth by 97.5 %, and *P. fluorescens* 53 inhabited the mycelium growth by 98%. Results of this experiment are present in (Tab.1 and Fig. 2).

Table 1. inhibition of *F. culmorum´s mycelium* growth in PDA medium by three different bacterial strains in dual culture experiment. The results are mean of three replicates.

Treatments	***Average growth (mm)***
A. Control (Fus)	40
B. Strain (K3)	1
C. Strain (M1)	0
D. Strain (53)	1

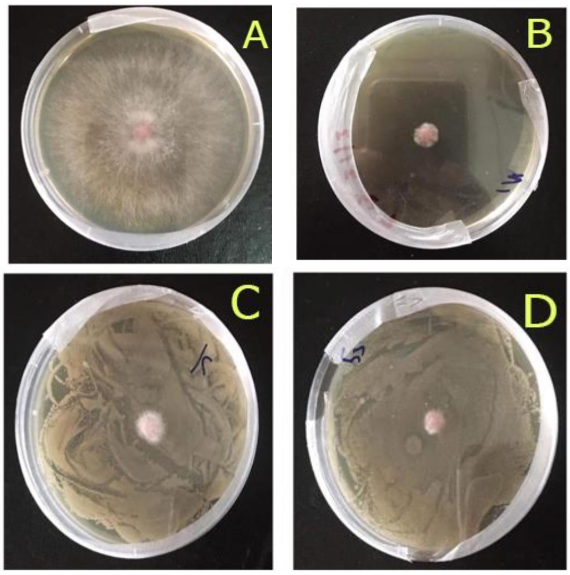


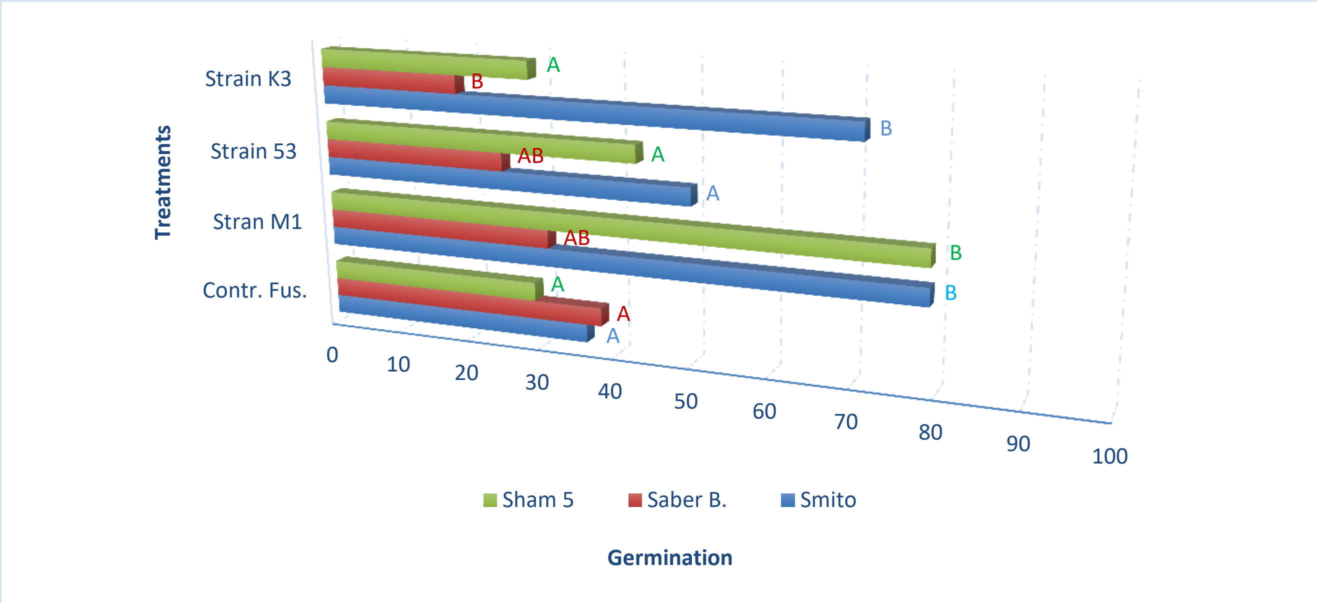
Fig. 2. Inhibition of *F. culmorum* mycelium in dual cultures by antagonistic bacteria of *Bacillus* strains (M 1, K3) and the *Pseudomonas* strain (53) grown on Petri dishes on (PDA medium). The results are mean of three replicates.

3. 3. In vivo test:

The germination rate in Fusarium control (cv. Smitto) was 36%. These rates were increased to 79% when seeds were treated with bacterial strain M1 (*Bacillus sp)*, 71% with strain 53 (*Pseudomonas spp)* and 50% with strain K3 (*B. subtilis)*.

In cv. Saber bag the germination rate was 38% in control. These rates were decreased to 31%, 25% and 19% when seeds were treated with strains M1 (*Bacillus sp)*, 53 (*Pseudomonas sp)* and K3 (*B. subtilis)* respectively.

Cultivar Sham5 had 29% seed germination in control. These rate were increased to 79% and 43% with strains M1 (*Bacillus sp)* and 53 (*Pseudomonas sp)* respectively. Strain K3 (*B. subtilis)* had no effect on the germination rate (Figure 3).



Different letters within columns indicate statistically significant differences, according to Duncan's Significance Level test (P < 0.05).

Fig. 3. The effect of three different bacterial strain on wheat seed germination infested with *F. culmorum* in greenhouse experiment. Six pots (12 cm in diameter and 15 cm high), with 4 seeds per pot were used for each treatment. The experiment was conducted twice.

4. Discussion:

Host cultivar effect, is one of most important factors on biocontrol characteristics (De Souza et al., 2003; Meyer et al., 2010; Weller, 2007). However. there are no immune wheat cultivars to *F. culmorum*, many cultivars have good resistance levels (Gosman, et al., et al., 2007; ŠÍP, et al., 2011).

When comparing these three cultivars used in this study in susceptibility to *F. culmorum*, the Saber bag was less susceptible than the two other cvs and had 12% more germination than Sham 5 and Smito cultivars.

Wiśniewska H. and Kowalczyk K. (2005) found differences in resistance to *F. culmorum* among 30 spring wheat cultivars.

In an experiment by (Orakci *et al*., 2018), out of the 141 phenotype wheat genotypes from 19 different countries only 17 genotypes ranked as moderately resistant.

The exploitation of host response to beneficial microorganisms. carries excessive potential, that let breeders to select characters that cause positive effect on plant-beneficial interaction. Diverse factors, such as biocontrol agent, genotype, plant genotype, environmental aspects, (e.g., temperature, moisture and soil texture) and metabolites have a significant effect on the biocontrol ability of bacterial antagonists (Wissuwa *et al*., 2009).

Species of *Bacillus* and *Pseudomonas* among a diversity of bacterial genera, have been widely used as biological control agents (Dean *et al*., 2012; Djordje *et al*., 2018). The effect on seed germination of tested bacteria showed that the strain *Bacillus* sp. M1 had better results than the other two strains. The germination rate increased significantly in both sham5 and Smito cvs. The strain *Bacillus* K3 increased the germination rate by 14% on both Sham5 and Smito cvs. Bacterial strain 53 *Pseudomonas* sp. gave good result in Smito cv. and increased the rate of germination by 35%. All the strains had negative effect on Saber bag cv. and decreased the germination rates.

Our results showed that these bacterial strains remarkably increased the plant seedling emergence in two of three tested cultivars and very effective in inhibition of *F. culmorum* mycelium germination in dual culture.

(Rebib *et al*., 2012) reported the activity of *B. subtilis* strain SR146 against several species of Fusarium including *F. culmorum* and noticeably increased the plant seedling emergence and also complete inhibition of spore's germination.

Many Strains of *P. fluorescens* were effective in improving the negative effects of *F. culmorum* on seedling germination of different wheat cultivars (Narayanasamy P. 2013). The host genotype-dependent nature of the biocontrol efficacy of other bacteria in the seed germination tests proposes that potential biocontrol agents should be tested against a variety of host cultivars (Narayanasamy P. 2013).

Biocontrol agents use different strategies to weaken their targets, as shown by Bacillus species, which adopt various mechanisms, to inhibit the growth of *F. graminearum* including the production of bioactive compounds, (Khayalethu *et al*., 2019).

Some members of the genus *Bacillus* are among the most widely used bacterial species as biocontrol agents. These strains influence plant and fungal pathogen interactions by a number of mechanisms such as competing for crucial nutrients, antagonizing pathogens by producing toxic metabolites, or inducing systemic resistance in plants (Noor Khan *et al*., 2017). Strains of *Bacillus* has shown positive effect in controlling different diseases in different crops. For ex., the formulated microorganisms, *B. subtilis* provided the best protection from anthracnose on legumes when used as seed treatment (Tinivella *et al*., 2009).

In research by Guo et al. (2014), the antagonistic effect of the *B. subtilis* strain NCD-2, was strong against *R. solani* in vitro and suppressed cotton damping- off disease in vivo.

Conclusions:

1. There are differences in susceptibility/ tolerance among wheat cultivars to infection by *Fusarium colmorum* the causal agents of root and crown rot disease.
2. The research results showed the potential of some bacterial strain as biocontrol agents to control wheat root and crown rot disease, but it needs more experiments especially in field.

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