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(Original Article)

Effectiveness of Bio-agent Formulations on Suppression of Lupine wilt Caused by *Fusarium oxysporum* f. sp. *lupini*

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Abstract

This study aimed to evaluate the bio-agent formulations of *Trichoderma harzianum, Bacillus subtilis* and *Pseudomonas aeruginosa* for the protection of lupine plants against wilt disease caused by *Fusarium oxysporum* f. sp. *lupini*. *T. harzianum* isolate (T1), *P. aeruginosa* isolate (P1) and *B. subtilis* isolate (B3) were the most effective ones in reducing the growth of *F. oxysporum* f. sp. *lupini in vitro*. Consequently, these isolates were used as bio-agent to control wilt on lupine plants in greenhouse experiments. The bio-agent isolates administered in the form of cells in broth media, powdered cells, and immobilized cells on sawdust as formulations were tested for their abilities to control lupine wilt in a greenhouse compared to Captan fungicide. Soil treatments with the three bio-agent formulations (broth medium or cells powder and sawdust) showed a markedly great reduction in wilt disease severity. Although all the formulations reduced the wilt disease severity compared to the water treatment, using the cells powder formulation of bio-agent reduced the wilt severity more than broth medium and sawdust formulations. In all formulations, *T. harzianum* (T1) exhibited the highest effect in the control of wilt compared with other bio-agent treatments. The sawdust and powder bio-agent formulations significantly reduced the disease severity of wilt on Lupine, as well as improved growth parameters (shoot, root, and plant length and seed yield/plant) of lupine plants compared with control treatment.

Keywords: *Fusarium oxysporum f. sp. lupini, Fusarium wilt, Lupine, Bio-agents, Formulations.*

Introduction

Lupine (*Lupinu stermis* Forsk) is one of important and most widely used crop, not only as a source of protein and fodder but also to improve soils due to its effectiveness in fixing nitrogen which correlated with the conventional cereal rotation (Abd El-Hai *et al*., 2016). One of the most significant pathogens reducing grain legume crop output and causing significant economic losses is soil-borne fungal infections is *Fusarium*. Numerous pathogens that live in the

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soil attack lupine, of which *Fusarium oxysporum* f.sp. *lupini* (Snyder and Hansen) may cause considerable losses in seed production and quality (Zian, 2005).

Chemical fungicides are a risky option for disease prevention since they are expensive, not economically feasible, and economically unacceptable as disturbing material. In addition, the lupine crop is utilized for human and animal use. Therefore, biological control presents a risk-free alternative approach to disease management and the creation of spotless plants. The potential of several microbes as bio-control agents has been investigated. To manage a variety of plant diseases, *Trichoderma* and *Bacillus* are frequently used. Numerous soil-borne fungi were controlled by *Trichoderma* spp. In addition, *Trichoderma* spp. treatment in greenhouse or field experiments was seen to improve plant growth as a result of biological control (Lubaina and Murugan, 2015).

Through a variety of processes, including the generation of antibiotics, siderophores, and lytic enzymes as well as the release of volatile antifungal chemicals into the environment, *Pseudomonas* can regulate phytopathogens (Kumari and Khanna؏ 2019). There have been several published researches examining how these bacteria affect phytopathogens. In order to stop the growth of *F. oxysporum* f. sp. *cumini* (FOC), *Pseudomonas luteola* and *P. fluorescens* were reported by Abed *et al.* (2016). The capability of *P. fluorescens* to prevent the development of FOC was also highlighted (Rathore *et al*. 2020). *P. aeruginosa* isolates ISO1 and ISO2, were antagonistic to *Fusarium solani* at substantial levels (Al-Ghafri *et al*, 2020).

Bacillus strains have been examined in the past for their antifungal abilities against a wide range of phytopathogenic fungi. Numerous antifungal strategies are combined by bacterial species, which have been generally acknowledged as promoting effective biocontrol (Liu *et al.,* 2017). The isolates CCIBP-A5 was chosen as the *Bacillus* strain with the greatest percentage values of radial growth inhibition activity against *F. oxysporum* out of 17 *Bacillus* strains (Leyva *et al*., 2017). Additionally, under *in vitro* conditions, this strain was able to create volatile and dispersed antifungal metabolites.

Bacillus spp. produces metabolites with antifungal action that have a variety of chemical configurations that govern their biological activity. Lipopeptide-based antibiotics are among the most significant (Gond *et al.,* 2015). Other authors claimed that the antifungal activity of various *Bacillus* strains against *F. oxysporum* f. sp. *lycopersici* was mediated by lipopeptide compounds as well as hydrolytic enzymes such chitinases (Abdallah *et al*., 2017).

Biological control of the disease may be achieved directly by the addition of antagonists or indirectly by modifying the environment to selectively favor antagonists (Leggett 1982). A significant biocontrol of the disease was achieved by the application of the bacteria *Bacillus subtilis*, *B. globisporus, B. pumilus* and *Pseudomonas flurescens* (Abd-Elrazik *et al*. 1985; Hassan,1992, Sallam *et. al*, 2009). The application of the powder formulations of *Bacillus subtilis* and

Trichoderma. harzianum to infested soil at the time of planting and two weeks before transplanting, significantly reduced the incidence of white rot onion (Sallam *et. al*, 2009).

This study was assigned to ascertain the efficiency of *B. subtilis*, *P. aeruginosa*, and *T. harzianum* as formulations of sawdust, powder, and broth medium biocontrol agents preventing wilt of lupine, as well as to investigate the interactions between the antagonists and to describe how they affected the wilt disease under greenhouse conditions.

Materials and Methods

1-Isolation and identification of the causal pathogen of lupine wilt disease

Lupine plants that showed symptoms of apparent Fusarium wilt, were collected from different districts of Assiut governorate. Diseased roots were sliced into small pieces, submerged for two minutes in a solution of 0.1% mercuric chloride, and repeatedly rinsed in sterile water to sterilize their surfaces. Diseased root samples were placed on Petri plates containing potato dextrose agar (PDA) medium, which has a 40 mg streptoamphenicol concentration per 100 ml of medium. The samples were then incubated at 28 ºC for 72 hours. Pure cultures were prepared outlined hyphal tip isolation process, and they were then kept at 5ºC on PDA slants in test tubes for further investigation. The isolates were identified according to Barnett and Hunter (1986), at the Assiut University Mycological Centre (AUMC), Assiut, Egypt.

2-Pathogenicity tests

Under greenhouse conditions, pathogenicity tests were conducted on the lupine cultivar Giza 1. The eleven isolates were inoculated individually into sterilized milk bottles containing Barley medium (75g Barley grains + 25g pure sand + 2g sucrose + 0.1g yeast extract + 100 ml water) and cultured at 28 °C for two weeks to create the inoculum. Each isolate's inoculum was added to the potted soil at a rate of 5% (w/w), and the soil was well mixed with each pot. A 5% formalin solution was used to sterilize soil and pots (25 cm in diameter) for 15 minutes, and then the soil and pots were allowed to dry for two weeks. For seven days, the soil was covered with plastic sheeting to preserve the gas. The soil wasn't planted until there were no longer any signs of formaldehyde (after 2 weeks). The process of seed disinfestation involved soaking the seeds in sodium hypochlorite (0.1%) for 2 minutes, followed by a sterile water wash (3 times). Five seeds were planted in each pot, and three pots were utilized to reproduce each isolate. As a control, pots with soil free of infestation were used. The disease severity index (DSI %) was calculated 90 days after planting. The parameters for disease severity were determined using the following equation.

$$DSI\ (\%) = \sum (0A+1B+2C+3D+4E)/4Tx100$$

Where, A, B, C, and D, are the number of plants corresponding to the numerical grade 0, 1, 2, 3, and 4 respectively; 4T is the total number of plants (T=5) multiplied by the maximum discoloration grade 4, T=A+B+C+D+E. To detect

the different degrees of disease, plants were classified into five categories according to Subbarao *et al*., (1999) as follows:

(0) = no symptoms; (1) =light wilt; (2) = moderate wilt; (3) = highly wilt and (4) = plant death.

3-Bio-agent test

From the Assiut University Mycological Centre (AUMC) bacterial collection, five *B. subtilis* (AUMC b-63, AUMC b-101, AUMC b-153, AUMC b-190, and AUMC b-203) and four *P. aeruginosa* (AUMC b-64, AUMC b-72, AUMC b-90, and AUMC b-116) isolates were obtained, in addition to three *T. harzianum* isolates (T1, T2, and T3) previously isolated and identified (Ahmed Hoda, *et al*. 2010). This test looked at the *in vitro* antagonistic effects of *B. subtilis*, *P. aeruginosa*, and *T. harzianum*, strains on the linear growth of *Fusarium oxysporum* f.sp. *lupini*. The pathogen was taken from a 4-day-old culture, and equal discs (4 mm in diameter) were positioned in the center of each plate. On either side of the plate's edge, at the same distance from the disc holding the pathogen, two identical discs of each antagonistic fungus were placed.

To test the impact of the antagonistic effect of *B. subtilis* and *P. aeruginosa*, plates were streaked with bacterial growth from a 2-day-old culture at the plate's edge on the opposite sides. A 4-day-old culture disc of *Fusarium oxysporum* f. sp. *lupini* was then individually inoculated into each plate after the plates had been incubated at 28±2 ºC for two days. In the control treatments, only *Fusarium oxysporum* f.sp. *lupini* mycelia growing discs were used to inoculate the plates. For 5 days, all plates were incubated at 28±2 ºC. For any given treatment, three plates were employed. The following formula was used to obtain the growth inhibition percentage:

Growth inhibition % = Growth in control – Growth in treatment /Growth in control x100

4-Preparation of bio-agent formulations

4.1-Preparation of fungal inoculum

On PDA medium at 25 °C for 12 days, *F. oxysporum* f.sp. *lupini,* and *T. harzianum* were cultivated. The conidia were then collected with a sterile needle in sterilized distilled water and combined. The resulting suspension concentration was adjusted to 1 x 10^6 CFU/ml (Sharma *et al*., 2005).

4.2-Preparation of *Pseudomonas* and *Bacillus* inoculum

P. aeruginosa and *B. subtilis* isolates were cultivated separately in 250 ml flasks contains 50 ml of nutritional broth medium under a shaker incubator at 150 rpm for 48 hours at 28°C, and then a cell suspension of each bacterial isolates was adjusted to contain 1x10^9 CFU/ml.

4.3-Preparation of free and immobilized cells of the biocontrol agents

B. subtilis and *P. aeruginosa* were cultured in a nutrient broth (NB) medium and *T. harzianum* was grown in a potato dextrose broth medium. All organisms were incubated under 150 rpm shaking conditions. *T. harzianum* was incubated at 25 ºC for 7 days whereas bacterial cultures were incubated at 27 ºC for 48 hours. Following incubation, 500 g of fine sawdust powder was separately added to the medium contents (two liters for each organism), which were then individually mixed under septic conditions. The mixture was subsequently dried to a fine powder using a Freeze drier (VirTis, model #6KBTES-55, NY, USA), at the Assiut University Mycological Centre, to produce what we called Sawdust formula. In addition to the sawdust preparation, the three bio-agents (*B. subtilis*, *P. aeruginosa*, and *T. harzianum*) were prepared as lyophilized cells using a solution of 10 % skimmed milk and 5 % inositol to produce free cells as powder formula. The obtained two formulas and the broth medium and water were used in the biocontrol experiments.

5-Seed treatment with Captan fungicide

This study's usage of the fungicide Captan included 50 % Trichloromethyl thiocyclohexene- 1,2-dicarboxymide as its active component. Captan was applied to seedlings at a rate of 2 g/km.

6-Greenhouse experiment

All the experiments were carried out under a greenhouse condition of Plant Pathology Dept. Faculty of Agriculture, Assiut University, Egypt. *In vitro, T. harzianum* isolate (T_1), *P. aeruginosa* isolate (P_1) and *B. subtilis* isolate (B_3), which were most inhibited effective on the growth of *F. oxysporum* f.sp. *lupini* pathogen than other isolates, were selected to us as bio-agent formulations. The effect of three forms of bio-agent formulations of broth medium, powdered cells, and immobilized cells on sawdust was tested for their abilities to protect lupine plants from wilt disease caused by *F. oxysporum* f. sp*lupini* in greenhouse. The experiment used the following treatments:

1) *T. harzianum* (T)
2) *P. aeruginosa* (P)
3) *B. subtilis* (B)
4) *B+T* (*B. subtilis* + *T. harzianum*)
5) *P+T* (*P. aeruginosa* +*T. harzianum*)
6) *P+B (P. aeruginosa* + *B. subtilis*)
7) *P+B+T* (*P. aeruginosa*+ *B. subtilis* + *T. harzianum*)
8) Captan
9) Control (Water)

All treatments were applied by three forms bio-agent formulations and water of each treatment.

A 5% formalin solution was used to sterilize soil and pots (25 cm in diameter) for 15 minutes, and then the soil and pots were allowed to dry for two weeks. The soil was covered with plastic sheeting to preserve the gas. Each bio-agent formulation material as soil treatment was added separately two weeks before inoculation with pathogen (FOL). Bio-agent formulations were added to the potted soil at a rate 240 g of sawdust, 16 g of powder and 50 ml of broth medium or water as control treatment, and then mixed well thoroughly with the soil. For inoculation of the pathogen, barley medium of pathogen inoculums of isolate F3 was added to the potted soil at a rate of 5% (w/w) and mixed thoroughly with soil to each pot. For cultivation, each pot was seeded with 5 surface-sterilized seeds of Giza 1 lupine cultivar and 4 pots were used for each treatment as replicates. Plants received daily and as needed irrigation. After 90 days following sowing, wilt disease severity index (DSI%) was observed and recorded as mentioned above. The effect of the treatments on lupine plant growth and seed production was evaluated at the end of the experiment. The plants from different treatments were removed, washed thoroughly with running water, blotted with tissue paper then shoot length, root length, plant height and seed yield per plant were measured.

7-Statistical Analysis

The whole experiment was repeated twice, and the data was pooled. This study was performed for each parameter studied in factorial experiments based on randomised complete block design model with four replications. To study the effect of some bio-agent formulations on disease severity of lupine wilt in greenhouse, factor A was four types of application (sawdust, powder, broth medium and water) and factor B was bio-agent isolates (*T. harzianum*, *P. aeruginosa* and *B. subtilis*). The data were statistically analysed with the Statistical Analysis System MSTAT-C (Version 2.1) and subjected to ANOVA. Comparison among treatment means was done by the least significance difference (LSD) test at $P \leq 0.05$ according to Gomez and Gomez (1984).

Results

1-Isolation and identification of lupine wilt causal pathogen

Thirty-one *Fusarium* isolates were isolated from diseased lupine samples collected from various regions in Assiut governorate, Egypt. Among all Fusarium isolates, eleven isolates were identified as *Fusarium oxysporum* f. sp. *lupini* (Snyder and Hansen) according to Barnett and Hunter (1986). The identification of fungal isolates was confirmed by Assiut University Mycological Center (MUMC). The pathogenic capability of the collected isolates was evaluated, and it clarified that eleven isolates showed significant disease severity to lupine plants, ranging from 12.50 in isolate F4 to 85% in isolate F3 (Table 1). The isolates caused symptoms of wilt on lupine plants. Isolates F3 caused the highest percentage of disease severity (85.00%) followed by isolates F9, F11,

F10 and F1. On the other hand, isolates F5, F2, F7 and F6 caused moderate disease severity of wilt. The isolates F8 and F4 showed the lowest disease severity.

Table 1. Pathogenicity test of eleven *F. oxysporum* f. sp. *lupini* isolates and disease severity on lupine (Giza 1 cultivar)

Isolates number	Disease severity (%)
F1	56.25[d]
F2	40.00[f]
F3	85.00[a]
F4	12.50[h]
F5	47.50[e]
F6	32.50[g]
F7	38.75[f]
F8	12.50[h]
F9	71.25[b]
F10	53.75[d]
F11	62.50[c]
Control	2.50[i]
L.S.D. at %5	5.21

[a,b…f]: within each column, values in a column followed by the same letter are not significantly different at p = 0.05 based on a LSD test.

2-Antagonistic effect of *T. harzianum, B. subtilis* and *P. aeruginosa isolates* against *F. oxysporum* f. sp. *lupini in vitro*:

The current findings showed *in vitro* inhibition of *F. oxysporum* f. sp. *lupine* (isolate F3) by the antagonistic ability of *B. cereus*, *P. aeruginosa*, and *T. harzianum* isolates, with *B. cereus* AUMC b-153, *P. aeruginosa* AUMC b-64, and *T. harzianum* T1 having the most effective ability (Table 2). The data indicate that *Trichoderma*, *Pseudomonas* and *Bacillus* isolates inhibited the growth of *Fusarium oxysporum* f. sp. *lupini in vitro*. *T. harzianum* isolate (T1), was the most effective than other isolates of *Trichoderma*. The results also reveal that *T. harzianum* overgrew mycelial growth of the causal pathogen. On the other hand, *P. aeruginosa* isolate (P1) and *B. subtilis* isolate (B3) were the most effective ones in reducing growth of the pathogen *in vitro*. Consequently, these isolates (T1, P1 and B3) were used as bio-agent to control the Fusarium wilt on lupine plants in greenhouse experiments.

Table 2. *In vitro* antagonistic effect of some fungal and bacterial on the linear growth of *F. oxysporum* f. sp. *lupini*.

Isolates number	AUMC accession number	% Growth inhibition
B. subtilis B1	B-63	44.44[c]
B. subtilis B2	B-101	38.88[cd]
B. subtilis B3	B-153	61.85[b]
B. subtilis B4	B-190	32.22[de]
B. subtilis B5	B-203	25.77[ef]
P. aeruginosa P1	B-64	59.25[b]
P. aeruginosa P2	B-72	32.07[de]
P. aeruginosa P3	B-90	39.99[cd]
P. aeruginosa P4	B-116	19.24[f]
T. harzianum T1	T1	74.81[a]
T. harzianum T2	T2	55.55[b]
T. harzianum T3	T3	61.11[b]
L.S.D. at %5		10.86

[a,b…f]: within each column, values in a column followed by the same letter are not significantly different at p = 0.05 based on a LSD test.

3-Greenhouse experiment

3.1-Effect of bio-agent applications on Fusarium wilt severity on lupine under greenhouse conditions.

The effect of bio-agent applications of *T. harzianum, P. aeruginosa* and *B. subtilis* isolates and Captan fungicide on disease incidence as disease severity of wilt on lupine caused by *F. oxysporum* f. sp. *lupini* was investigated under greenhouse conditions. Soil treatment with the three bio-agent isolates alone or in combination with them and Captan fungicide significantly reduced the disease severity of lupine wilt incidence under artificial infection with the Fusarium pathogen. Data in Table 3 presented the soil treated with bio-agents material as different formulations (broth medium or powder and sawdust) showed a markedly great reduction in disease severity of wilt incidence in lupine plants compared with those grown in soil treated with water application (control). In all isolates, although all the used formulations reduced the incidence of wilt disease in lupine plants compared to control, the powder formulation of bio-agent reduced the infection more than broth medium and Sawdust formulations. In all formulations, the *T. harzianum* (T1) exhibited the highest effect in control of wilt compared with other bio-agent or other treatments. The lowest incidence of wilt as well as highest percentage of survival plants were obtained when plants treated alone with *T. harzianum* as powder formulation in both seasons (Table 3).

Table 3. Effect of formulation application of certain bioagent on the lupine wilt incidence under greenhouse conditions.

Bio-agents	Disease severity index (DI%) of lupine wilt disease				
	Broth medium	Powder	Sawdust	Water	Mean
Control	82.50[a]	83.75[a]	83.75[a]	85.00[a]	**83.75[a]**
B. subtilis (B)	16.25[gh]	11.25[ij]	18.75[fg]	82.50[a]	**32.18[f]**
P. aeruginosa (P)	21.25[f]	16.25[gh]	21.25[f]	85.00[a]	**35.93[e]**
T. harzianum (T)	12.50 [hi]	7.50 [j]	16.25[gh]	83.75[a]	**30.06[g]**
B+T	31.25[cd]	26.25[e]	33.75[bc]	82.50[a]	**43.44[d]**
P+T	33.75[bc]	27.50[de]	27.50[de]	83.75[a]	**43.12[d]**
B+P	33.75[bc]	31.25[cd]	31.25[cd]	86.25[a]	**45.62[c]**
B+P+T	37.50[a]	36.25[b]	37.50[b]	83.75[a]	**48.75[b]**
Captan	36.25[b]	31.25[cd]	35.00[bc]	83.75[a]	**46.56[c]**
Mean	**33.88[b]**	**30.13[c]**	**33.88[b]**	**84.02[a]**	

L.S.D. at 5 %, Application (A) = 1.53; Bio-agent (B) = 1.91, Interaction (A×B) = 4.61

[a,b…f]: within each column, values in a column followed by the same letter are not significantly different at p = 0.05 based on a LSD test.

3.2-Effect of application of the bio-agent formulations on plant growth and seed production of lupine infected by *F. oxysporum* f. sp. *lupini*.

Data in Table (4) demonstrate clearly that using bio-agent application materials of *T. harzianum, P. aeruginosa* and *B. subtilis* produced an increase in shoot, root, and plant length and seed production of lupine plants than water treatment (control). Alone or in companion of bio-agent isolates, the powder formulation enhanced the parameters plant growth. Powder formulation of *T. harzianum* bioagent significantly increased shoot, root, and plant length and seed production of lupine plants than in untreated plants. The shoot and root lengths were 66.45 cm and 28.5 cm, respectively, and were 1.77 and 2.71 times longer than the control. Lupine plant height reached 100.66 cm with 2.1 times longer than the control yielding 32.31 g seeds/plant**.**

Table 4. Effect of application the bio-agent formulations on shoot, root length, plant height and seed yield of lupine infected by *F. oxysporum.* f. sp. *lupini.*

Formulation application	Shoot Length (cm)	Root Length (cm)	Plant height (cm)	Seed yield/plant (g)
Control	37.50[e]	10.50[f]	48.00[e]	17.94[e]
B. subtilis (B)	58.50[abc]	23.25[b]	81.75[b]	28.89[ab]
P. aeruginosa (P)	55.50[bcd]	24.50[b]	80.00[b]	26.82[bc]
T. harzianum (T)	66.45[a]	28.50[a]	100.66[a]	32.31[a]
B+T	51.00[cd]	16.42[cde]	67.42[cd]	25.00[cd]
P+T	47.50[d]	19.59[bc]	67.10[cd]	24.43[cd]
P+B	48.45[d]	17.73[cd]	66.18[cd]	22.76[d]
P+B+T	52.50[cd]	12.00[ef]	64.50[d]	21.61[de]
Captan	62.25[ab]	13.50[def]	75.75[bc]	23.04[d]
L.S.D. at %5	8.94	4.92	10.20	3.68

[a,b…f]: within each column, values in a column followed by the same letter are not significantly different at p = 0.05 based on a LSD test.

Discussion

Fusarium oxysporum f. sp. *lupini*, a widespread fungal infection on lupine plants, produces wilt disease, which can result in large financial losses. Using chemical fungicides to prevent infection is risky. Therefore, biological control offers a risk-free alternative approach to managing diseases. The potential of several microorganisms as biocontrol agents for plant diseases has been studied. The pathogen was identified as *F. oxysporum* causes wilt disease, which is one of the most serious and prevalent conditions affecting lupine growth in Egypt. These results are consistent with those of other investigators (Shaban *et al.,* 2011; Zian *et al.*, 2013; Khalifa *et al.,* 2020).

In this study, *B. subtilis*, *P. aeruginosa*, and *T. harzianum* minimized the Fusarium radial growth by 61.85, 59.25, and 74.81%, respectively, using the three applied strains. The pathogen's mycelial development was also shown to be overgrown by *T. harzianum*, *P. aeruginosa*, and *B. subtilis*.

The study conducted by Rajeswari and Kannabiran (2011) revealed that the volatile chemicals found in *T. harzianum*, known as alkyl pyrones, had antifungal characteristics that hindered the pathogenic fungus's pathogenic germination and mycelial growth *in vitro*. Furthermore, it is widely acknowledged that *Trichoderma* spp. exhibit remarkable abilities and efficaciousness in the biological regulation of numerous commercially noteworthy phytopathogens (Whipps and Lumsden, 2001; McLean *et al*., 2004; Ahmed *et al*., 2013). Nonetheless, it is commonly recognized that some *Trichoderma* species generate a range of antibiotics, such as trichodermin, trichodermol A, and arzianolide (Claydon *et al.,* 1991). Nawar (2007) states that most fungal phytopathogens are suppressed by these compounds.

Numerous investigations have exhibited the capability of *Pseudomonas* strains to provide defense against phytopathogens. The best siderophore synthesis was documented by *Pseudomonas* which had a high antagonistic impact against *Pyricularia oryzae*. The *Pseudomonas* isolate E1FP13 tested positive for the synthesis of lipase, an enzyme that breaks down the fungal cell wall, in addition to siderophores (Zouari *et al*. 2020). Since isolate E1PP6 failed to produce any of the examined diffusible lytic enzymes, it is possible that alternative processes, including the cyclic dipeptide cyclo synthesis, or enzymes like β-glucanase, were involved in the fungal cell wall destruction process (Dewi *et al.,* 2016). The presence of Fe-chelating siderophores and hydrogen cyanide, which is harmful to pathogenic fungus, may be the cause of *P. aeruginosa* antifungal activity. Furthermore, our findings concur with those of Al-Ghafri *et al*. (2020) and Rathore *et al.* (2020), who demonstrated that particular *Pseudomonas* strains might regulate Fusarium wilt of chickpea, which is brought on by *F. oxysporum* f. sp. *ciceris*. The findings indicated that the isolates of *Pseudomonas* generated diverse antifungal chemicals and inflicted some harm on the *F. oxysporum* f. sp. *ciceris* hyphae. Additionally, the isolates enhanced chickpea growth by producing a number of metabolites that are crucial for the uptake and provision of nutrients.

To combat fungal infections, several species of *Bacillus* have been employed (Kamali *et al*. 2019, Martín *et al.* 2021). Numerous processes, including the creation of systemic resistance, the stimulation of host growth, and/or antibiosis, can be responsible for this. Numerous investigators have detailed modifications to the *F. oxysporum* f. sp. *ciceris* mycelium's shape in various phytopathogenic agents. These outcomes also line up with Tang *et al.* (2014) findings. Li *et al.* (2012) reported that two species of *Fusarium* experienced lysis of their conidia and mycelium when lipopeptides were present around the minimum inhibitory concentrations. Comparable outcomes were reported by Liao *et al.* (2016), who showed that lipopeptide substances like fengicinas can distort the germ tube of fungus conidia. The current investigation indicates that *B. subtilis* used antibiosis as a regulatory mechanism.

In this investigation, the use of the three bioagents, *T. harzianum*, *P. aeruginosa*, and *B. subtilis*, decreased the disease severity in the greenhouse. These results corroborate those of Shaban and El-Bramawy (2011) and Rajeswari and Kannabiran (2011), who revealed that certain fungal pathogens were inhibited by extracellular lytic enzymes produced by *Trichoderma* species. According to previous reports, the inhibitory potential of *Trichoderma* species in this study may be caused by volatile chemicals, non-volatile antibiotics, and extracellular enzymes.

Bacillus subtilis and *P. aeruginosa* were likewise successful in raising the proportion of healthy plants and decreasing the disease severity (Khan *et al*. 2018). Captan, a chemical fungicide, was less successful than the biocontrol candidates. These findings are consistent with the current investigation, which shows that the application of *B. pumilus* and *P. alcaligenes* enhanced the development of plants inoculated with *F. oxysporum*. These outcomes could have resulted from pathogens directly opposing one another, from the manufacture of antibiotics, or from pathogens competing with one another for vital nutrients (Khan *et al.* 2018). It is known that *Bacillus* species lower the wilting index in plants inoculated with *F. udum*. The potential cause of the observed enhancement in plant development could be the pathogen-inhibiting properties of Bacillus species (Martín *et al.,* 2021).

Similar to how *B. pumilus* is used as a biocontrol agent for Fusarium wilt, *Pseudomonas* species can likewise inhibit parasitic root infections by producing compounds that have biological activity (Akhtar and Siddiqui, 2007). Additionally, they produce siderophores, which limit the amount of iron available, the enzyme that regulates hormone levels, and antibiotics, which destroy pathogens (Martín *et al.* 2021). According to Siddiqui *et al.* (2007), applying *Pseudomonads fluorescens* and *Bacillus* spp. to pigeon pea reduces the wilting index. Furthermore, the current results are consistent with those of Mohamed *et al.* (2013), who demonstrated that soaking lupine seeds in *P. fluorescens* suspension effectively induced a suppressive response against *F. oxysporum* f. sp. *lupini*. In addition to minimizing the negative effects of diseases on plants and speeding up the biocontrol process, increasing plant development

makes plants more resistant to infections. The overall impact of all three routes, when the three bioagents in this investigation were applied as seed treatments, explains the significant reduction in disease severity. Rajeswari and Kannabiran (2011) findings are likewise consistent with ours.

In our study, the tested bio-agents as powder and sawdust formulations showed interesting highly significant effect giving great reduction of wilt disease severity on lupine plant comparing with the broth medium treatment. Accordingly, this hopeful applicable technique could be suggested on the light of the results obtained in the present research. Using the formulated bio-agents might be considered as safe, cheep and easily applied biological method against Fusarium wilt pathogen. Overall, from our study we can deduce that the formulation process leads to a final product by mixing the microbial component with different carriers and suitable materials for better protection from environmental conditions, long term survival of the bio-agents, as well as improved bioactivity and storage stability. The application of bio-agents as powder or sawdust formulations fits the modern strategy of control plant diseases which combines the suitable and sustainable control methods to suppress pathogen populations while maintaining the integrity of the ecosystem, this is as it explained and confirmed recently by Bharti and Ibrahim (2020). In conclusion, our study demonstrated that Sawdust and powder bio-agent formulations can be used as safety for long-term biological control of Fusarium wilt disease in lupine plants.

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فعاليــة المستحضــرات الحيويــة فــي مكافحــة ذبــول التــرمس المتســبب عــن الفطــر *Fusarium oxysporum* f. sp. *lupini*

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هــذه الدراســة تهــدف إلــى تقيــيم المستحضــرات الحيويــة للكائنــات الدقيقــة *Trichoderma harzianum* و *Bacillus subtilis* و *Pseudomonas aeruginosa* لحمايــة نباتات الترمس من مرض الذبول المتسبب عن فطر *Fusarium oxysporum* f. sp. *lupini* فــى المعمل كانـت العزلـه T1 مـن عـزلات الترايكوديرمـا و P1 مـن عـزلات البيسـدوموناس B3 مـن عزلات الباسلس هي الأكثر فعالية في تقليل نمو فطر المسبب المرضى. ونتيجة لذلك، تم اسـتخدام هذه العزلات فى مكافحه مرض ذبـول نبـات التـرمس فـى تجـارب الصـوبه. تـم تطبيـق العـزلات الحيوية على شكل خلايا في وسط البيئة السائلة، وخلايا مجفدة في صورة مسحوق، وخلايا مثبتـة على نشارة الخشب حيث تم اختبار المستحضرات لقدرتها على مكافحة ذبول الترمس في الصـوبه مقارنة بالمبيد الفطري الكابتان. أظهرت معاملات التربـة بالمستحضـرات البيولجيـه الثلاثـة (بيئـه المرق أو مسحوق البودره ونشاره الخشب) انخفاضًا كبيرًا بشكل ملحوظ في شدة مـرض الـذبول. وعلـى الـرغم مـن أن جميـع المستحضـرات المسـتخدمة خفضـت حـدوث مـرض الـذبول مقارنـة بالكنترول، إلا أن استخدام مسحوق قلل من الإصابة أكثر من مستحضرات وسـط المـرق ونشـارة الخشب. في جميع المستحضرات المستخدمه، أظهرت عزله الترايكودرما أعلى تأثير فـي مكافحـة الذبول مقارنة مع المعاملات الحيوية الأخرى. وأدت مستحضرات العوامل الحيوية لنشارة الخشب والمسحوق للعزلات الحيويه للكائنات الدقيقه السابقة إلى تقليل شدة الاصابة بمـرض الـذبول علـى الترمس بشكل كبير، بالإضـافة إلـى تحسين معـايير النمـو (طـول المجمـوع الخضـرى والجـذور وطول النبات وإنتاجية البذور/النبات) من نباتات الترمس مقارنة بمعامله الكنترول.