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Research Article

Field Approaches of Chemical Inducers and Bioagents for Controlling Root Diseases Incidence of Pea (*Pisum sativum* L.) Under Field Conditions

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Abstract

Background and Objective: Pea plants (*Pisum sativum* L.) affected severely by several soilborne pathogenic fungi causing root diseases, damping-off, root rot and wilt resulted in serious losses in either plant stand and produced yield as well. The aim of the present study designed to assess the efficacy of some biotic and abiotic agents against the incidence of some roots diseases affects pea plants under field conditions. **Materials and Methods:** The causal agents of pea root diseases were isolated and identified as *Pythium* sp., *Rhizoctonia solani*, *Fusarium solani* and *F. oxysporum*, respectively. The efficacy of some bioagents as seed dressing as well as chemical inducers and alga extract of *Chlorella vulgaris* as foliar spray were evaluated against pea root disease incidence under field conditions throughout two growing seasons. **Results:** Announced reduction effect on root diseases incidence was recorded at (*Chaetomium globosum*+organic waste) as seed treatment and (Chitosan+Thyme oil) as foliar treatment followed by salicylic acid, (Potassium di-hydrogen phosphate+Thyme oil) and algal extract treatments, respectively. The fungicides Rizolex-T 50% and Topsin-M70 provided good protection to treated seeds from germination to pods formation stages of pea plants. The produced yield of green pods followed the same trend over untreated control treatment. **Conclusion:** The promising outcome in the present study demonstrated the activity of bioagents as a seed dressing or foliar spray with chemical inducers treatments which might be recommended for the future use for such management of soil-borne root diseases.

Key words: Biocontrol, chemical inducers, damping-off, root rot, wilt, pea

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pea plants (*Pisum sativum* L.) is one of the most common grain legumes grown in Egypt. Pea extremely susceptible to be attacked by several soilborne plant pathogens causing various root diseases such as damping-off, root rot and wilt diseases which considered an important limiting factor for pea production. Root rot was reported to cause up to 50% yield losses in heavily infested fields[1]. Soil-borne fungal pathogens have been accounted as a complex of more than twenty species which associated with foot and root rot of pea and much of them often infect a wide spectrum of other leguminous plants. The most important species of these pathogenic fungi are *Pythium* spp., *Aphanomyces euteiches*, *Fusarium solani* f. sp. *pisi*, *F. oxysporum* f. sp. *pisi*, *F. avenaceum*, *Phoma pinodella* and *Mycosphaerella pinodes*[1-4]. Biological control one of the potential means to control soil-borne diseases. In this concern, under greenhouse and field conditions it was reported that many fungal strains of *Trichoderma* significantly showed a suppressive effect against plant diseases caused by *Rhizoctonia solani*, *Sclerotium rolfsii*, *Pythium aphanidermatium*, *Fusarium oxysporum* and *F. culmorum*[5,6]. Furthermore, Fusarium wilts of various plant species had been successfully suppressed by application of fluorescent Pseudomonads[7]. Suppression of *Pythium* spp. as well as root rot incidence of many crops is referred to the production of siderophores by *Pseudomonas aeruginosa* during iron limited condition[8]. The purpose of the present study designed to assess the efficacy of some biotic and abiotic agents against the incidence of some roots diseases affects pea plants under field conditions.

MATERIALS AND METHODS

Isolation and Identification of the causal organism(s): Pea plant samples showing different symptoms, i.e., damping-off, root rot and wilt diseases were collected from a certain field located at Kafr-Eldawar district, Alexandria governorate. The collected samples were transferred to the laboratory for isolating and purification trails. The purified isolates were subjected to microscopic examination and then identified with the aid of Barnett and Hunter[9].

Plant materials: The used Pea seeds Master cv. in the present work were supplied by Vegetable Researches Department, Agricultural Research Centre, Giza, Egypt were used. Pea seeds were soaked individually for 1 h in each of the tested bioagents suspension before sowing. The control treatment was pea seeds soaked in water.

Tested bioagents: The candidate biocontrol fungal isolates, *Trichoderma harzianum* (TH112), *T. virens* (TV121), *Chaetomium globosum* (CG56) as well as antagonistic bacterial isolate *Pseudomonas fluorescens* (PF43) kindly obtained from Culture Collection Unit, Plant Pathology Department, National Research Centre (NRC), Egypt were used in present study. These microorganisms were isolated from the rhizosphere of various healthy and root rot infected leguminous crops, grown in the Delta and Middle Egypt regions and proved their antagonistic efficacy against a wide spectrum of plant pathogens *in vitro* as well as under greenhouses and field trials in previous work of the same Department[10-12]. Meanwhile the used extract of algae *Chlorella vulgaris* was kindly obtained from Algal Biotechnology Unit, National Research Centre, Giza, Egypt.

Preparation of bioagents inoculums: Inoculums of tested bioagents were prepared following the method described by Abdel-Kader *et al.*[3]. On the other hand, suspensions of both fungal spores and bacterial cells grown individually on organic waste medium contain wheat bran+soybean powder+bagas at the rate of 1:1:1 (w:w:w) and 40% water were also prepared according to El-Mougy and Abdel-Kader[11]. All concentrations of both fungal and bacterial suspensions were adjusted to 10^8 mL^{-1} CFU using a hemocytometer slide. To obtain a sticky feature, CMC at the rate of 1% was added to each of the tested fungal spores or bacterial cells suspension.

Chemical inducers: The chemical inducers, organic acids (Salicylic acid) and organic salts (Potassium sorbate, Potassium dihydrogen phosphate) were purchased from Al-Gamhoria Company Ltd., for chemicals and medicinal instruments, Cairo, Egypt. Meanwhile, the essential oil used in the present study was obtained from CID Company, Egypt. Thyme oil (*Thymus vulgaris* L.) was kept in dark-colored bottles at 4°C for until use.

Fungicides: The fungicides Rixolex-T 50% (3 g kg^{-1} seeds) and Topcin-M70% (2 g L^{-1}) were used as a seed dressing and foliar spray for comprising treatments.

Field experiments: The field experiment was conducted during two successive growing seasons 2018 and 2019 at a field located at Kafr-Eldawar, Alexandria governorate where the diseased pea samples were previously collected. This field characterized as high infestation with various soilborne pathogens the causal agents of damping-off, root rot and wilt diseases. These experiments were performed to evaluate the efficacy of bioagents and chemical resistance inducers against pea disease incidence. All the same applied procedures were done at the two successive growing seasons. All above-mentioned material were used as a seed dressing or foliar spray treatments for controlling pea root diseases (damping-off, root rot and wilt) incidence.

The applied treatments were designed as follows:

Seed dressing (S):

- ST1 : *T. harzianum* (10^8 CFU mL^{-1})
- ST2 : *T. harzianum* (grown on organic waste) at concentration of 10^8 CFU mL^{-1}
- ST3 : *T. virens* (10^8 CFU mL^{-1})
- ST4 : *T. virens* (grown on organic waste) at concentration of 10^8 CFU mL^{-1}
- ST5 : *Chaetomium globosum* (10^8 CFU mL^{-1})
- ST6 : *Chaetomium globosum* (grown on organic waste) at concentration of 10^8 CFU mL^{-1}
- ST7 : *P. fluorescens* (10^8 CFU mL^{-1})
- ST8 : *P. fluorences* (grown on organic waste) at concentration of 10^8 CFU mL^{-1}
- ST9 : Organic waste only at the rate of 3 g kg^{-1} seeds
- ST10 : The fungicide Rizolex-T 50% (3 g kg^{-1} seeds)

Foliar spray (F):

- FT1 : Chitosan (0.5 g L^{-1})
- FT2 : Chitosan+Thyme oil (0.5 g L^{-1} +5.0 mL L^{-1})
- FT3 : Potassium dihydrogen phosphate, 20 mM (2.7 g L^{-1})
- FT4 : Potassium dihydrogen phosphate, 20 mM (2.7 g L^{-1})+Thyme oil (5.0 mL L^{-1})
- FT5 : Salicylic acid, 20 mM (3.6 g L^{-1})
- FT6 : Algal extract (*Chlorella vulgaris*), 2 g L^{-1}
- FT7 : Fungicide Topcin-M70, 2 g L^{-1}
- Control

The field trail contains 90 plots, 7×6 m (42 m^2) each comprised of 12 rows where 30 holes existed per row which were conducted in a completely randomized block design with 5 plots as replicates for each particular treatment as well as untreated manner. Three pea seeds per hole were sown in all plots at September 1st of each season. During the growing season, all conventional agricultural practices were followed. The spore suspension at the rate of 10^8 CFU mL^{-1} was used for seed dressing before cultivation. Meanwhile, for each particular foliar spray tested treatment, 10 L water contains certain weights of used chemical inducers individually to obtain the proposed concentration. At the time of first true leaf appearance, all foliar spray procedures were applied twice with consideration to 15-day interval. At the maturity stage, the whole plot areas in each treatment were harvested and total accumulated pod yield (kg/plot) was calculated.

Disease assessments: The disease occurrence was determined using the following equation:

$$DI\ (\%) = \frac{\text{Number of infected plants}}{\text{Total Number of examined plants in each plot}} \times 100$$

Pre-emergence damping off was calculated after 15 days of sowing date. While observations for the different occurrence of target diseases throughout the experimental plots were performed 10 days after each spray treatment. The cumulative disease incidence was calculated after the pod's formation stage.

Description of pea root diseases: In present investigation, under field conditions, pea root diseases were surveyed and recorded according to diagnostic symptoms reported by several researchers as follows:

Damping-off: Typical symptoms occur soon after plant germinates at the base of the seedling. Seedling stems become water-soaked and thin, almost threadlike, where infected. Young leaves wilt and turn green-gray to brown. Roots are absent, stunted or have grayish-brown sunken spots. Sometimes the causal fungi can attack the germinating seedling before they emerge on soil surface resulting in pre-emergence damping-off. Several fungi can cause decay of seeds and seedlings including species of *Rhizoctonia*, *Fusarium* and *Phytophthora*. However, species of *Pythium* are most often the main causal[13,14].

Root rot: Affected plants may turn from solid and white to black/brown and soft with brown lesions that appeared on the infected site. Affected roots may also fall off the plant when touched. The leaves of affected plants may also wilt, become small or discolored. Affected plants may also look stunted due to poor growth. Species of the *Pythium*, *Phytophthora*, *Rhizoctonia*, or *Fusarium* fungi are the usual causals agents[15].

Wilt: Affected plants first started as yellowing and wilting appearance. Leaf wilting, Leaves develop a yellow then brown color, often leaves eventually die and fall. Plant stunting, browning of the vascular system, leaf death and lack of fruit production also occur. Wilt disease caused by many forms of the soil-inhabiting fungus *Fusarium oxysporum*[6,16].

Statistical analysis: IBM SPSS software version 14.0. was used for analyzing the obtained results. Analysis of variance was performed and comparing the mean values was followed by Duncan's multiple range test at $p<0.05$.

RESULTS

Isolation and Identification of the causal organism(s): The present isolated fungi from infected pea plants showing damping-off, root rot and wilt symptoms were identified as *Pythium* sp., *Rhizoctonia solani*, *Fusarium solani* and *F. oxysporum*.

Diseases incidence under field conditions: Data presented in Table 1 and 2 and Fig. 1 and 2 showed that high effective response against the target diseases incidence was observed at whole utilized treatments that differed than check control throughout the two cultivated seasons 2018 and 2019.

Data in Table 1 showed that seed dressing with the fungicide Rhizolex T50 (ST10) showed a protective effect against pathogens reflected in low root diseases incidence recorded as 0.0, 0.0, 0.3 and 1.6% for pre-, post-emergence damping-off, root rot and wilt, compared with control treatment which was 12.9, 3.8; 7.1 and 8.3%, in respective order (Table 1).

Regarding bioagents treatments, it is interesting to observe that growth suspensions of bioagents stored on the organic waste used for seed coating revealed superior and effective protection effect against pea root pathogens compared with the same bioagents used as fresh growth suspension. Presented data in Table 1 for the first growing season (2018) revealed that the lowest percentages of pre and post-emergence damping-off were recorded as 0.0, 0.0% at seed dressing with at treatments of (ST6) followed by 0.0, 0.4% at (ST2) treatment as well as 1.8, 1.0, 2.5 and 1.5% at treatments of (ST5) and (ST8), in relevant respective order. Likewise, (ST6) revealed the lowest root rot incidence as 0.3% followed by 1.1, 1.1 and 1.5% at seed treatments with (ST2), (ST5) and (ST8), respectively. Also, the same treatments of (ST6), (ST2), (ST5) and (ST8), showed the lowest wilt incidence in ascending order which recorded as 1.3, 1.6, 2.0 and 2.0%, respectively.

Other applied seed treatments with (ST4), (ST3), (ST1), (ST7) and (ST9) showed lesser effect on pea root disease incidence.

As for foliar spray treatments, all applied foliar spray treatments proved to be effective against the incidence of pea root diseases (Table 2) compared with untreated control. Presented data showed that foliar spray with (FT2) revealed the highest drastic effect on root rot and wilt diseases whereas they recorded 0.0% and 0.6% incidence for the two diseases, respectively. Also, salicylic acid and (FT4) indicated high effect against root diseases that percentages of 0.8, 1.3 and 1.3%, 1.3% were recorded for root rot and wilt incidence,

Table 1: Efficacy evaluation of seed dressing with bioagents against root diseases of pea (*Pisum sativum* L.) under field conditions during growing season 2018

	Average root diseases incidence (%)				
	Damping-off				
Treatments	Pre-emergence(ii)	Post-emergence(iii)	Root rot(iv)	Wilt(v)	Accumulated yield kg/plot(vi)
ST1	7.4±1.32c(i)	1.6±0.23d	2.8±0.62e	4.3±0.28c	33.4±0.6de
ST2	0.0±0.00f	0.4±1.16ef	1.1±0.27e	1.6±0.56e	40.7±1.4b
ST3	7.0±0.63c	1.2±0.67e	3.6±0.29d	4.6±0.64c	38.6±0.4c
ST4	2.2±0.64d	1.6±0.43d	1.9±0.97e	2.6±0.79d	35.6±1.0d
ST5	1.8±0.91de	1.0±0.14d	1.1±0.23e	2.0±0.67de	34.8±1.0d
ST6	0.0±0.00f	0.0±0.00f	0.3±0.09f	1.3±0.67e	48.6±0.7a
ST7	8.5±0.29c	2.4±0.88c	4.5±0.41c	5.6±0.22b	26.8±1.5
ST8	2.5±0.54d	1.5±0.33d	1.5±0.54e	2.0±0.41d	31.6±0.9f
ST9	10.7±0.63b	4.6±0.48b	5.7±0.74b	5.8±0.47b	28.4±1.0g
ST10	0.0±0.00f	0.0±0.00f	0.3±0.04f	1.6±0.39e	40.8±1.9b
Control	12.9±1.23a	3.8±0.50a	7.1±0.32a	8.3±9.11a	22.8±1.0h

Means±Standard Deviations within each column followed by the same letter are not significantly different by Duncan multiple range test at $p<0.05$, Pre-emergence damping-off calculated as percentage of emerged seedlings in relative to the sown seeds, post-emergence damping-off calculated as percentage of damping seedlings in relative to emerged seedlings, root rot calculated as percentage of seedlings showed root rot disease symptoms in relative to emerged seedlings, Wilt calculated as percentage of seedlings showed wilt disease symptoms in relative to emerged seedlings, average accumulated yield kg/plot, ST1: *T. harzianum* (10^8 CFU mL^{-1}), ST2: *T. harzianum* (grown on organic waste) at concentration of 10^8 CFU mL^{-1}, ST3: *T. virens* (10^8 CFU mL^{-1}), ST4: *T. virens* (grown on organic waste) at concentration of 10^8 CFU mL^{-1}, ST5: *Chaetomium globosum* (10^8 CFU mL^{-1}), ST6: *Chaetomium globosum* (grown on organic waste) at concentration of 10^8 CFU mL^{-1}, ST7: *P. fluorescens* (10^8 CFU mL^{-1}), ST8: *P. fluorences* (grown on organic waste) at concentration of 10^8 CFU mL^{-1}, ST9: Organic waste only at the rate of 3 g kg^{-1} seeds, ST10: Fungicide Rizolex-T 50% (3 g kg^{-1} seeds)

Table 2: Efficacy evaluation of foliar spray with chemical fungicide alternatives against root diseases of pea (*Pisum sativum* L.) under field conditions during growing season 2018

	Average root diseases incidence (%)				
	Damping-off				
Treatments	Pre-emergence(ii)	Post-emergence(iii)	Root rot(iv)	Wilt(v)	Accumulated yield kg/plot(vi)
FT1	-	-	2.7±0.94[c(i)]	2.0±0.31[b]	38.8±2.0[d]
FT2	-	-	0.0±0.00[f]	0.6±0.10[d]	44.4±2.2[a]
FT3	-	-	3.6±0.96[b]	2.3±0.37[b]	36.8±1.0[e]
FT4	-	-	1.3±0.09[d]	1.3±0.21[c]	43.2±0.6[ab]
FT5	-	-	0.8±0.20[e]	1.3±0.10[c]	40.6±1.8[c]
FT6	-	-	1.8±0.42[d]	2.0±0.75[b]	36.6±0.6[e]
FT7	-	-	0.0±0.00[f]	0.6±0.22[d]	42.4±0.7[ab]
Control	12.9±1.23[a]	3.8±0.50[a]	7.1±0.32[a]	8.3±9.11[a]	22.8±1.0[f]

Means±standard deviations within each column followed by the same letter are not significantly different by Duncan multiple range test at p<0.05, Pre-emergence damping-off calculated as percentage of emerged seedlings in relative to the sown seeds, Post-emergence damping-off calculated as percentage of damping seedlings in relative to emerged seedlings, Root rot calculated as percentage of seedlings showed root rot disease symptoms in relative to emerged seedlings, Wilt calculated as percentage of seedlings showed wilt disease symptoms in relative to emerged seedlings, average accumulated yield kg/plot, FT1: Chitosan (0.5 g L^{-1}), FT2: Chitosan+Thyme oil (0.5 g L^{-1}+5.0 mL L^{-1}), FT3: Potassium di-hydrogen phosphate, 20 mM (2.7 g L^{-1}), FT4: Potassium di-hydrogen phosphate, 20 mM (2.7 g L^{-1})+Thyme oil (5.0 mL L^{-1}), FT5: Salicylic acid, 20 mM (3.6 g L^{-1}), FT6: Algal extract (*Chlorella vulgaris*), 2 g L^{-1}, FT7: Fungicide Topcin-M70, 2 g L^{-1}

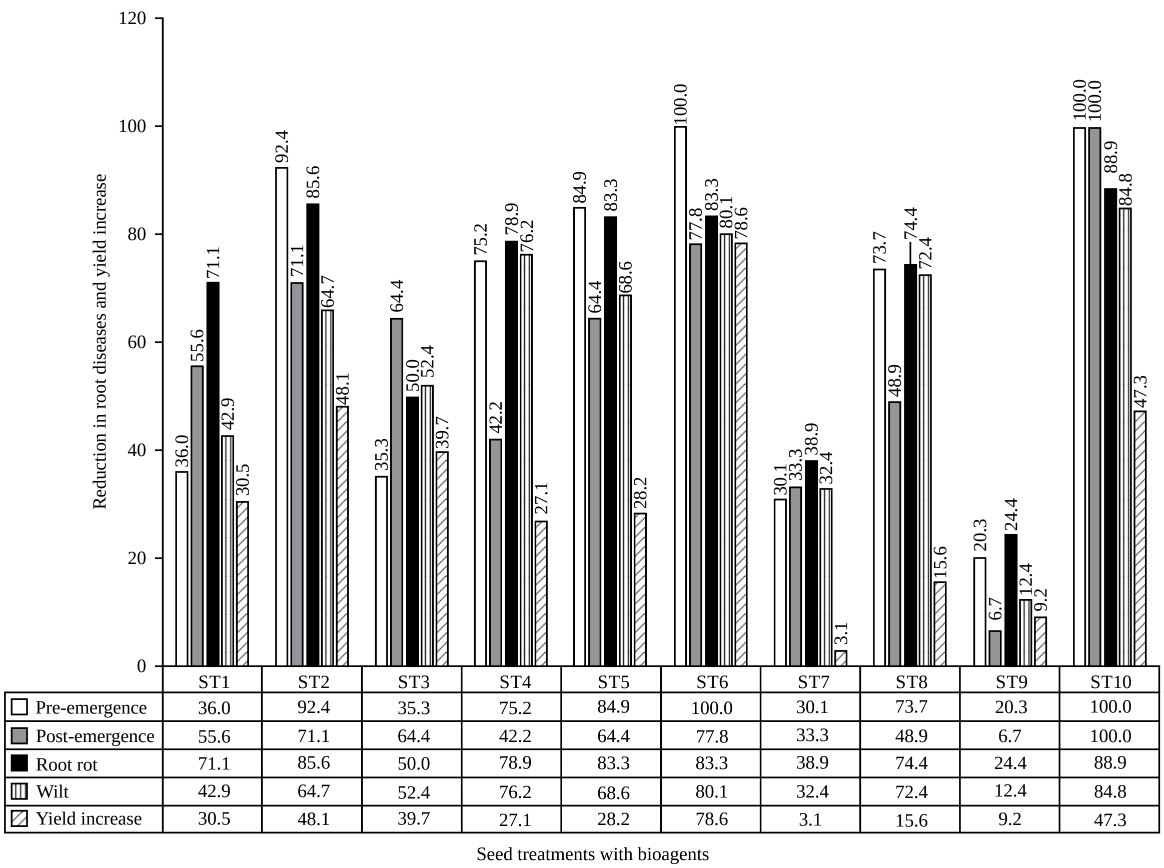
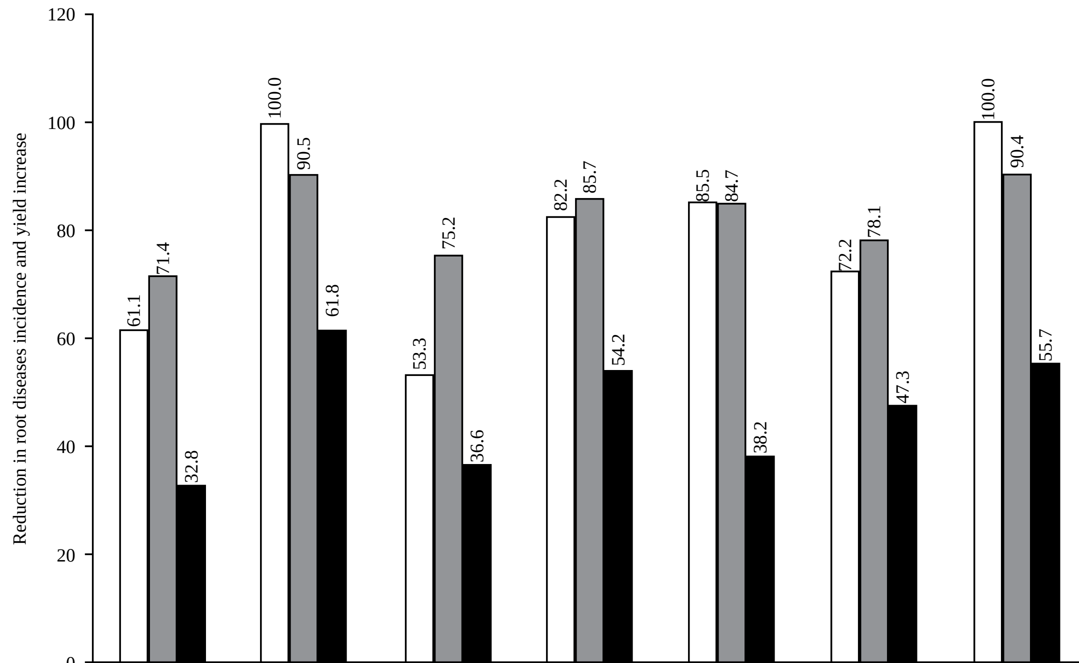


Fig. 1: Reduction (%) in root diseases of pea (*Pisum sativum* L.) and yield increase (%) in response to seed dressing with bioagents under field conditions during growing season 2019

ST1: *T. harzianum* (10^8 CFU mL^{-1}), ST2: *T. harzianum* (grown on organic waste) at concentration of 10^8 CFU mL^{-1}, ST3: *T. virens* (10^8 CFU mL^{-1}), ST4: *T. virens* (grown on organic waste) at concentration of 10^8 CFU mL^{-1}, ST5: *Chaetomium globosum* (10^8 CFU mL^{-1}), ST6: *Chaetomium globosum* (grown on organic waste) at concentration of 10^8 CFU mL^{-1}, ST7: *P. fluorescens* (10^8 CFU mL^{-1}), ST8: *P. fluorences* (grown on organic waste) at concentration of 10^8 CFU mL^{-1}, ST9: Organic waste only at the rate of 3 g kg^{-1} seeds, ST10: Fungicide Rizolex-T 50% (3 g kg^{-1} seeds)



	FT1	FT2	FT3	FT4	FT5	FT6	FT7
Root rot	61.1	100.0	53.3	82.2	85.5	72.2	100.0
Wilt	71.4	90.5	75.2	85.7	84.7	78.1	90.4
Yield increase	32.8	61.8	36.6	54.2	38.2	47.3	55.7

Foliar spray treatments

Fig. 2: Reduction (%) in root diseases of pea (*Pisum sativum* L.) and yield increase (%) in response to foliar spray with fungicide alternatives under field conditions during growing season 2019

FT1: Chitosan (0.5 g L^{-1}) FT2: Chitosan+Thyme oil (0.5 g L^{-1}+5.0 mL L^{-1}), FT3: Potassium di-hydrogen phosphate, 20 mM (2.7 g L^{-1}), FT4: Potassium di-hydrogen phosphate, 20 mM (2.7 g L^{-1})+Thyme oil (5.0 mL L^{-1}), FT5: Salicylic acid, 20 mM (3.6 g L^{-1}), FT6: Algal extract (*Chlorella vulgaris*), 2 g L^{-1}, FT7: Fungicide Topcin- M70, 2 g L^{-1}

respectively. Percentages of 1.8 and 2.0%, as a moderate effect, was recorded at Algal extract (*Chlorella vulgaris*) foliar treatments (FT6) for root rot and wilt diseases incidence. Data also, indicate that treatments of Chitosan (FT1) followed by (FT3) represent the lowest suppression effect on root diseases whereas percentages of 2.7, 3.6, 2.0 and 2.3% were recorded for root rot and wilt incidence, respectively. The fungicide Topsin M70 (FT7) showed a high protective effect on root disease incidence. Percentages of root rot and wilt incidence were recorded as 0.0 and 0.6% compared with 7.1 and 8.3% at untreated control, in respective order.

These results were confirmed with a similar trend obtained in the second growing season, 2019. Illustrated data in Fig. 1 and 2 showed that applied treatments of either seed dressing or foliar spray affected positively on pea root disease incidence. At seed dressing with bioagents (Fig. 1) an announced reduction in both root rot and wilt diseases was recorded. Treatment of (ST6) showed a superior effect on diseases incidence followed by (ST2), (ST5) and (ST8) in descending order. They recorded pre- and post-emergence damping-off disease reduction calculated as 100-77.8%, 92.4-71.1%, 84.9-64.4% and 73.7-48.9%, in relevant respective order. Also, at the same treatments percentages of 83.3, 85.6, 83.3 and 74.4% reduction in root rot incidence were recorded.

Similarly, these treatments showed reduction in wilt disease incidence recorded as 80.1, 64.7, 68.6 and 72.4%, in respective order. The other applied seed dressing with bioagents revealed diseases reduction calculated in range as 20.3-75.2%, 6.7-42.2% for pre- and post-emergence damping-off as well as 24.4-78.9 and 12.4-76.2% for root rot and wilt diseases, respectively.

On the other hand, at foliar spray application (Fig. 2) the highest disease reduction in root rot and wilt incidence was recorded as 100%, 90.5% at (FT2), followed by 82.2%, 85.5% at (FT4) and (FT5) treatments, respectively. Meanwhile, lower reductions as 72.2%, 78.1% and 53.3%, 75.2% were recorded at treatments of Algal extract (FT6) and (FT3), respectively.

Data also showed that the fungicide seed dressing with Rizolex-T50 (ST10) and Topsin M70 (FT7) as foliar spray showed superior effect on root diseases incidence compared with bioagents and fungicide alternatives. Rizolex-T50 (ST10) could reduce pre- and post-emergence damping-off by 100%, root rot by 88.9% and wilt by 84.8% (Fig. 1). Meanwhile, in Fig. 2 reduction in root rot and wilt incidence were recorded as 100 and 90.4% at Topsin M70 (FT7), respectively.

In the present study throughout the 2 growing seasons, the applied treatments either as a seed dressing or foliar spray obviously demonstrate a dramatic effect on root disease incidence of pea plants compared with untreated control and subsequently increase the numbers of plant stand and resulted in an increase the produced yield as well. At the growing season (2018) data in Table 1 and 2 revealed an increase in the produced yield calculated as 48.6 and 40.7 kg plot^{-1} at treatments of seed dressing with (ST6) and (ST2) as well as 44.4, 43.2 kg plot^{-1} at foliar spray with (FT2) and (FT4), respectively. Likewise, in 2019 season an increase of produced yield calculated as 78.6 and 48.1% over untreated control was recorded at seed dressing with (ST6) and (ST2) treatments (Fig. 1), respectively. Foliar spray treatments of (FT2) and (FT4), recorded 61.8 and 54.2% over untreated control (Fig. 2), in respective order. Also, the illustrated data in Fig. 2 revealed that fungicidal treatments (ST10) as a seed dressing and (FT7) as foliar spray showed 47.3 and 55.7% yield increase over untreated control, respectively.

DISCUSSION

In the present investigation, the obtained results proved the high effectivity of all applied treatments against the pea root disease incidence compared to control throughout the two cultivated seasons under natural field conditions. In this regard, in the case of seed dressing it was observed that stored bioagents on the organic waste had a superior and more effective protective effect against pea root pathogens compared with the same bioagents when used as fresh growth suspension. These results revealed a higher suppressive effect of the growth suspension of bioagents carried on organic waste than those of fresh cultures. In contrast, in a study of Abdel-Kader *et al.*[11] sawdust, CMC, talc powder and chitosan were used as carriers for storing bioagents, *T. harzianum*, *B. subtilis* and *P. fluorescens*. They tested their viable antagonistic ability against some soilborne plant pathogens under laboratory and greenhouse conditions. Referring to the recorded results of Abdel-Kader *et al.*[11], they used inert materials as carriers for the tested bioagents which are not suitable for their growth and therefore it thought that this is why the stored bioagents loosed their antagonistic viability by prolonging the storage period. Meanwhile, in the present study the formulated organic waste containing wheat bran, soybean and bags which considered suitable for enhancing the growth of carried bioagents and produce their metabolism which contains biological weapons, i.e., lytic enzymes, toxins, antibiotics etc. These components might be dissolved or emulsified in the water when the growth suspension is prepared before seed treatment. Likewise, several investigators proved the successful application of antagonistic microorganisms for controlling various plant diseases. In a study of El-Mougy and Abdel-Kader[10] they established an active process for providing seeds with protective effects against soilborne root pathogens through using bio-primed faba bean seeds with fungal and bacterial antagonists which significantly reduce the incidence of root disease. Furthermore, Usharani *et al.*[17] recorded very effective management of fusarium wilt (*F. oxysporum* f. sp. *lycopersici*) of tomato using seed treatment and soil application delivery systems for *P. fluorescens* formulated on various carriers. The obtained results in the present investigation are confirmed by these previously stated reports. Furthermore, data presented in Table 1 and 2 and Fig. 1 and 2 revealed that along with the two cultivating seasons treatments of either chitosan or Potassium di-hydrogen phosphate had a superior positive effect against disease incidence when combined with Thyme oil compared to each alone. This observation might be attributed to the enhancement of the Thyme oil role as an antifungal compound. It was notified that Thyme oil has a great constituent of biologically active compounds, i.e., Thymol, carvacrol, geraniol, thymol methyl ether, α –pinene which responsible for a few antifungal activities (https://www.holisticonline.com/Herbal-Med/_Herbs/ h280.htm). In this concern, similar reports of several investigators confirmed the obtained results in present work. Essential oils had been reported to have an inhibitor activity on the growth of several plant pathogens. In this concern, similar reports of several investigators confirmed the obtained results in present work. Essential oils had been reported to contain an inhibitor action against the growth of several plant pathogens. The influence of essential oils on the disease is known through direct competition with the target organism as a dual mechanism and deactivation the enzymes produced by the pathogen[18,19]. Also, it was found that essential oil treatment cause disarray in the plasma membrane and mitochondrial structure disarrangement of the pathogen[20].

Under field conditions, essential oils and chitosan as seed coating proved their high capability for decreasing bean root rot disease incidence[21]. Likewise, in the present study chitosan seed treatment found to have a suppressing effect against pea root disease incidence. Also, the suppressing effect against the growth of different plant pathogenic fungi as well as the capacity to be effective signals of plant defense reactions that due to the biological properties of Chitosan oligomers have attracted great attention of many investigators[22]. Chitosan has its antifungal efficacy which could be referred to the capability to overlap with the action of the plasma membrane of fungal cells[23] and it reacts with the fungal DNA and/or RNA[24].

Likewise, considering salicylic acid, it has been reported to be effective, as antimicrobial, in many trials acts as disease resistance inducers against various bacterial diseases, soft rot[25] wilt[26] as well as soilborne fungal diseases, root rot and wilt[27] and fungal foliar diseases[28,29]. Additionally, spray with potassium dihydrogen phosphate individually or combined with thyme oil had a significant positive effect on pea root diseases. In agreement with our findings, various investigators reported similar results. Both potassium phosphate compounds monopotassium phosphate (KH_2PO_4) and dipotassium phosphate (K_2HPO_4) could become natural alternatives to synthetic fungicides and induce plant resistance against various phytopathogenic fungi[12,30,31]. As a further consideration, Dipotassium phosphate is intended to control various fungal plant diseases. This salt contains active component seems to have a varied mode of action tools include the direct toxicity to the pathogen, enhance the plant defense mechanisms consequent to the fertilizing properties of the compound[32].

Algae are considered as one of the recent strategies used as biocontrol agents to control fungal plant diseases. It was reported that blue-green algae (Cyanobacteria) and eukaryotic algae produce biologically active compounds that have antifungal activity antibiotics and toxic activities against plant pathogens. Kulik[33] reported that the application of foliar plant spray with extracts from seaweeds (macroalgae) significantly reduced disease incidence of gray mold on strawberries caused by *Botrytis cinerea* and powdery mildew on turnips caused by *Erysiphe polygoni* as well as damping-off on tomato. Also, Jimenez *et al*.[34] stated that foliar treatment of organic extracts from the brown-alga *Lessonia trabeculata*. Also, Weed-Max and Oligo-X algal, the commercial blue-green algae extracts, could suppress various root rot fungal pathogens, and promote the antagonistic capacity of fungal and bacterial antagonists[35].

Furthermore, in a study of Abdel-Kader and El-Mougy[35] they demonstrated that in numerous tested vegetable crops significant reduction in root rot disease incidence and increase of plant health and the improvement of the yield were achievement by integrated approaches of soil drench with commercial algae extracts, Oligo-X and Weed-Max, combined with bioagents, *T. harzianum* and *B. subtilis* under greenhouse and plastic houses trails. they suggested that the application of commercial algae products combined with active bioagents is considered an easy and method against soil-borne root diseases.

SIGNIFICANCE STATEMENT

This study discovers the seed treatments with bioagents followed by foliar spray with fungicide alternatives can be beneficial for controlling root diseases caused by various soil-borne plant pathogens. The applied treatments utilized the soil inhabitant antagonistic microorganisms which considered the act as natural enemies against several soilborne plant pathogens. Furthermore, the used organic acids and salts are taken into account as plant resistant inducers throughout producing elicitors which could enhance the plant self-defense mechanisms in addition to its recorded direct effect on the growth of various plant pathogens. Therefore, the present study is considered as one of several cited investigations conducted with the field of plant disease control using fungicide alternatives and will help other researchers to uncover the critical areas of plant disease suppression. As a future vision, a new theory is needed for integrating between bioagents and chemical inducers in one formula easily applicable for controlling plant diseases may be achieved.

CONCLUSION

In the present work, the achieved results may suggest that such approaches considered easily applied, characterized as safe and cost-effective methods and could be recommended to control various soil-borne plant pathogens.

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