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Biological and Chemical Resistance Inducers as Seed priming for Controlling Faba Bean Root rot Disease under Field Conditions

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Abstract- ***Under field conditions different alternative approaches of antagonistic bio-agents, Trichodermaharzianum and Bacillus subtilis and plant resistance inducers, Calcium chloride and Benzoic acid applied as seed coating sown in soil with or without compost were evaluated for their efficacy against Faba bean root rot disease. The disease incidence at both pre-, and post-emergence stages was significantly reduced at all treatments either alone or in combinations comparing with fungicide and control treatments. Calcium chloride as Seed coating with either T. harzianum*or *B. subtilis followed by seed coating with either of the two bioagents sown in composted soil showed significant reduction in diseases incidence comparing with the other applied treatments. The present findings demonstrate that plant resistance inducers in combination with bio-agents may have important implications for the future use of antagonistic microorganisms on a commercial scale for controlling such diseases.***

Index Terms**: Faba bean, root rot, Calcium chloride, Benzoic acid, Compost, Trichodermaharzianum and Bacillus subtilis**

I. INTRODUCTION

Faba bean (*Viciafaba*L.) is one of the earliest domesticated food legumes and is now cultivated on large areas in many countries. Production of the crop is, however, constrained by several disease infections including fungal diseases. Root rot disease became serious in recent years and it is considered one of the main constraints for faba bean production in Egypt, especially in the new reclaimed soil. Several pathogenic soilborne fungi such as *Fusariumoxysporum* Schl. f.sp.*fabae* Yu & Fang, *F. solani* (Mart.) Sacc. f. sp. *fabae* Yu & Fang, *F. avenoceum* (Fr.) Sacc.,*F. inflexum* Schneider and *F. equiseti* (Corda) Sacc. Were reported on faba bean [1,2,3,4]. Root rot disease occurs during the growing season from seedling to flowering stages, and may cause pre-emergence infection. So far, apart from scientific and practical difficulties, there is no economic way to control the crop diseases. The management of soil-borne plant pathogens is particularly complex because these organisms live in or near the dynamic environment of the rhizosphere, and can frequently survive a long period in soil through the formation of resistant survival structures.

The exceptionally long survival of root diseases pathogens along with the susceptibility of different cultivated cultivars rendered the issue of control of a paramount importance. The wide and indiscriminate use of chemical fungicides has been the cause of the appearance of resistant plant pathogens, leading to the occurrence of serious diseases. Therefore, there is an increasing interest to obtain alternative antimicrobial agents for using in plant disease control systems. The current management strategy relies on the intensive use of fungicides. In addition, chemical control does not give satisfactory control of the root disease.

Application of biological control using antagonistic microorganisms has proved to be successful for controlling various plant diseases [5]. However, it is still not easy and costly in application. The concern of pesticides use with respect to human health and environment has brought increasing interest in alternatives use by avoiding negative effect on the environment. In addition, fungicide alternatives that have fungicidal effect on disease incidence and development are safety of application and environmental pollution concern. In this regards, Calcium chloride as $CaCl_2$ was reported to suppress growth of the citrus mould pathogen *Penicilliumdigitatum*[6]. Calcium chloride effectively reduced silver scurf lesions on potato tubers, but not sporulation of *Helminthosporiumsolani*. Also, it is known that addition of calcium chloride can also improve the activity of biocontrol agents [6,7]. Moreover, certain strategies such as pre- or postharvest application of calcium salts, hydrogen peroxide and chitosan against fruit decay are proposed [8,9]. Furthermore, chemical elicitors (inducers) seem to predispose the original defence mechanisms in plants against diseases or produce some new compounds supporting it. Many others [10,11,12] used several chemical or natural compounds known to induce plant resistance including salicylic, benzoic, citric and oxalic acids. Other investigators [13] stated that there are positive relationships between peroxidase (enzymes and isozymes) and resistance development in plants. Also, Resulted faba bean chocolate spot disease reduction was accompanied with a gradual increase in peroxidase activity during experiment period. Among the inducers tested, citric and benzoic acids were the most effective one, since they recorded the lowest percentages of disease severity and the highest levels of peroxidase activities.

The present research focuses on finding compounds that are safe to humans and the environment, *e.g.* Calcium chloride and Benzoic acid as well as biocontrol agents which may provide an alternative control of many soil and seed-borne pathogens. The objective of the present work was to evaluate fungicide alternatives and /or bio-agents against root rot incidence when used as seed treatment under field conditions.

II. MATERIALS AND METHODS

Integration of bio-agents, *T. harzianum*and/or *B. subtilis* and calcium chloride or benzoic acid as seed dressing against root rot incidence of faba bean was evaluated under field conditions. The used antagonistic microorganisms, *i.e.T. harzianum* and *Bacillus subtilis*, were kindly obtained from the Plant Pathology Department of the National Research Centre, Giza, Egypt.

Fungal inoculum (*Trichodermaharzianum*) was grown on PDA medium at 28±1oC until an abundant heavy growth of conidia was evident. Conidia were harvested by scraping the surface of the colonies with a spatula, transferred to sterilized distilled water and filtered through nylon mesh. All spore solutions were adjusted with sterile water to give a spore concentration of 104-105 spores per milliliter. Meanwhile, antagonistic bacterium (*Bacillus subtilis*) was grown on Nutrient broth medium and incubated in a rotary shaker at 200 rpm for 24 h at 28±1oC. The bacterial cells were harvested by centrifugation at 6,000 rpm for 10 min, washed twice with 0.05 M phosphate buffer at pH 7.0, and re-suspended in distilled water. The concentrations of bacterial cells in the suspensions were adjusted to 105-106 cells per milliliter. Concentrations of both bacterial cells and fungal spores suspensions were adjusted with the aid of a haemacytometer slide. A few drops of the emulsifier Tween 20 (Sigma Co.) were added to the prepared bio-agents to obtain distributed separated spores/cells suspensions.

Faba bean seeds (cv. Giza 3) were surface disinfected by immersing in sodium hypochlorite (2%) for 2 min, and washed several times with sterilized water, then dried between two sterilized layers of filter paper.

Seeds of faba bean (at the ratio of 500 g/L) were imbibed in each of the prepared priming solutions for 16 h (Jensen *et al*. 2004). The bio-primed seeds were then air-dried on filter paper for 1 h in a laminar flow hood and packed into glass jars sealed with a 45-mmmembrane and stored in a refrigerator at 5 ^{0}Cuntil required.

The bio-agents primed faba bean seeds were coated with either calcium chloride or benzoic acid at the rate of 3g/kg seeds. Seed dressing was carried out by applying the tested materials to the gum moistened seeds in polyethylene bags and shaking well to ensure even distribution of the added materials. The treated seeds were then left on a plastic tray to air dried. The fungicide Rizolex-T 50 WP at the recommended dose (3 g/kg) was applied as the seed dressing as stated before. In addition, disinfected, untreated faba bean seeds were sown as a comparison treatment. Meanwhile, tested compost was applied at the rate of 20m ^{3}/Fadden (0.005 m^2/plot) to the abovementioned prepared field plots, one week before sowing [14]

This study was performed in a naturally infested field with faba bean root rot pathogens, at the Experimental and Production Station, National Research Centre, Beheira Governorate, Egypt during the growing season 2014/2015. A field experiment was established which consisted of (3.5x6.0 m) plots, composed of 12 rows and a 25 cm spacing between plants within a row. Three replicates (plots) per each relevant treatment were used in a completely randomized block design. Three seeds of faba bean per hole were used in all the treatments. Plots received the usual agricultural practices, *i.e.* NPK fertilizer and irrigation *etc*. Percentage of root rot incidence at the pre- and post-emergence of growth stages was investigated and calculated 15 and 45 days after the sowing date.

Statistical Analysis

All experiments were set up in a complete randomized design. One-way ANOVA was used to analyze differences between treatments. A general linear model option of the analysis system SAS [15] was used to perform the ANOVA. Duncan's multiple range test at $P \leq 0.05$ level was used for means separation [16].

III. RESULTS AND DISCUSSION

The obtained results in Table (1) and Fig (1) showed the efficacy of applied compost as soil drench or Calcium chloride, benzoic acid, and /or bio-agents as seed treatment against faba bean root rot disease incidence. Presented data revealed that all applied treatments reduced significantly root rot incidence at both pre-, and post-emergence growth stages of faba bean plants comparing with fungicide Rizolex and untreated check control. Data also showed that combination treatments of compost, Calcium chloride, benzoic acid with bio-agents reduced significantly root rot incidence comparing with the application of each of them alone. At pre-emergence growth stage individual treatment with compost, *T. harzianum* and *B. subtilis* showed significant root rot incidence recorded as 14.2, 13.4 and 11.2% comparing with calcium chloride, benzoic acid and Rizolex which were 16.6, 16.2 and 16.6%, respectively. Similar trend was also observed concerning post-emergence root rot incidence. It is clear from presented data in Table (1) that application of the bioaghents enhanced the efficacy of compost, calcium chloride and benzoic acid as combined treatments. This fact was observed in the recorded disease incidence either at pre-, or post-emergence root rot of faba bean.

Illustrated data in Fig. (1) Showed application of individual treatments of calcium chloride, benzoic acid and compost resulted in reduction in disease incidence estimated as 20.1, 22.1 and 31.7%. More reduction in root rot incidence was recorded when the previous treatments combined with *B. subtilis* causing reduction calculated as 63.4, 27.0 and 50.0%, respectively. Lesser reduction was observed in combined treatments with *T. harzianum* which calculated as 60.0, 21.1 and 39.4%, respectively. Data also showed that the most significant treatment was (calcium chloride plus *B. subtilis*) followed by (calcium chloride plus *T. harzianum*) and (compost plus *B. subtilis*), respectively. They caused root rot reduction as 63.4, 60.0 and 50.0% compared with the fungicide treatment which was 20.1%, in respective order.

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Table 1. Root rot incidence of faba bean in response to seed dressing with different salts and/or bio-agentsunder field conditions

Seed treatment	Root rot disease incidence %	
	Growth stage	
	Pre-emergence	Post-emergence
Calcium chloride	16.6 b	14.8 d
Benzoic acid	16.2 b	16.2 c
Compost	14.2 c	12.8 e
T. harzianum	13.4 d	12.2 e
B. subtilis	11.2 f	10.4 f
Calcium chloride + *T. harzianum*	8.3 h	8.6 g
Calcium chloride + *B. subtilis*	7.6 i	6.8 h
Benzoic acid + *T. harzianum*	16.4 b	14.4 d
Benzoic acid + *B. subtilis*	14.6 c	14.2 d
Compost + *T. harzianum*	12.6 e	8.8 g
Compost + *B. subtilis*	10.4 g	8.6 g
Fungicide (Rixolex-T)	16.6 b	20.2 b
Untreated	20.8 a	28.6 a

Figures with the same letter for each column are not significantly different (P≤ 0.05).

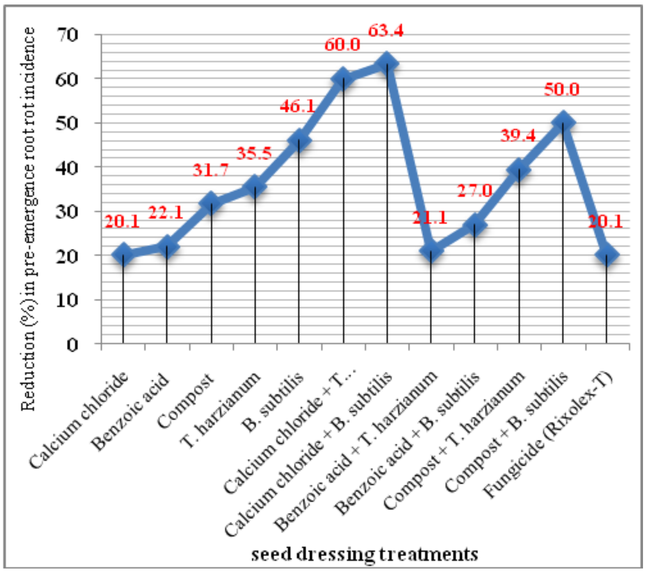

Fig. 1. Reduction (%) in pre-emergence root rot incidence of faba bean in response to seed dressing with different salts and/or bio-agentsunder field conditions

Similarly, at post-emergence growth stage combined treatments enhanced disease reduction comparing with individual one. Illustrated data in Fig. (2) revealed that the bioagent*T. harzianum* caused more reduction in root rot incidence from 48.2 to 69.9%, 43.3 to 49.6% and 55.2 to 69.2% when combined with calcium chloride, benzoic acid and compost, respectively. Also, increase in disease reduction was observed at the same applied treatments to reach 76.2, 50.3 and 72.0% when combined with *B. subtilis*, in respective order. Only 29.3% disease reduction was recorded in seed dressing with the fungicide Rizolex over untreated check control treatment.

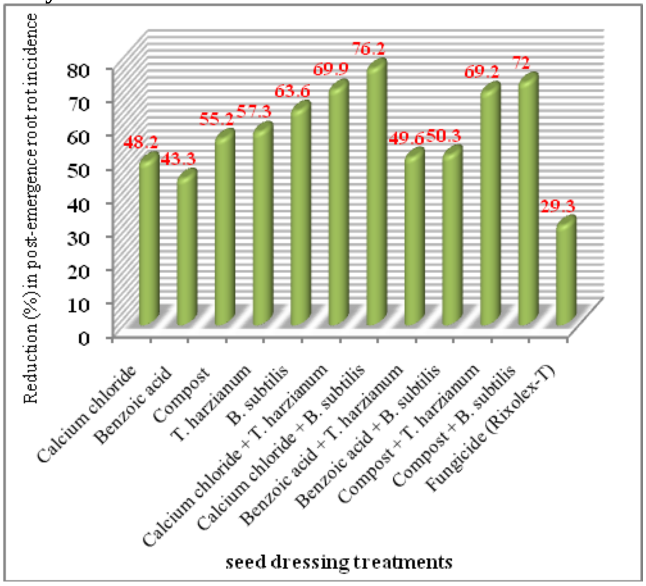

Fig. 2. Reduction (%) in post-emergence root rot incidence of faba bean in response to seed dressing with different salts and/or bio-agentsunder field conditions

In the present study the coating faba bean seeds with only the tested bioagents, *T. harzianum*, *B. subtilis*, could reduce root rot incidence comparing with other treatment as well as control. This reduction was increased when these bioagents combined with calcium chloride and decreased with benzoic acid. This observation could be due to synergistic effect of calcium chloride and antagonistic effect on the tested bioagents. Inthis regards, many researchers have shown that calcium plays an important role in the inhibition of postharvest decay of fruits [17,18], and in enhancing the efficacy of postharvest bio-control agents [19,20,21].Similarly, calcium chloride at 2% (20 mg/ml)obviously inhibited spore germination and germ tube growth of *R. stolonifer*PDA medium [22].This result further supports by [23,24] who reported that addition of calcium chloride canalso improve the activity of biocontrol agents. On the other hand, several inorganic salts and organic lipophilic acids and their salts, some of which are used , in the food – processing industry, have antimicrobial properties and could be useful as postharvest treatment for decay control. The food preservatives potassium sorbate or sodium benzoate had antifungal activities against postharvest decaying fungi [25,26]. They added that sorbic acid and its salts derivatives are the most widely used antimicrobial agents for food preservation worldwide. Also, in study of [27] recorded that potassium sorbate, sodium benzoate at 2.5 % completely inhibit the linear growth and spore germination of both pathogenic fungi, *Penicilliumdigitatum* and *P.italicum*. Inhibition of microorganisms by sorbic acid and its salts might caused by alternation of cell transport function, inhibition of enzymes involved in the glycolytic pathway or tricarboxylic acid cycle by inhibition of RNA, DNA, and protein synthesis, and by uncoupling of the oxidative phosphorylation in mitochondria [28].

Moreover, several works which conducted with bio-control applications against plant diseases control reported the efficacy of biological control of vegetable foliar diseases by different microorganisms. Biological control using natural products or antagonistic microorganisms in order to reduce the pesticides use and decline of their residues on agriculture products proved to be successful for controlling various plant pathogens in many countries [29]. It is still not expensive and is easy in application however it can serve as the best control measure under restricted conditions. In addition, its application is safe, un-hazardous for human and avoids environmental pollution [30]. In this regard, [31] reviewed that during the past ten years, over 80 bio-control products have been marketed worldwide. A large percentage of these have been developed for greenhouse crops. Products containing *Trichoderma, Ampelomycesquisqualis, Bacillus, Ulocladium*and *Pseudomonas* are being developed to control the primary foliar diseases, Botrytis and powdery mildew in greenhouses could predominate over chemical pesticides.Similar results were reported [32] who stated that *T. harzianum*i introduced to the soil was able to reduce root rot incidence of faba bean plants significantly. Moreover, the application of biological con-trols using antagonistic microorganisms, has proved to be successful for controlling various plant diseases in many countries [33,34,35]. Furthermore, the ability of biocontrol agents, fluorescent *Pseudomonas*, non-pathogen *Fusarium*stra in and *Trichodermaharzianum*T-22 when applied in combination and alone, to control of *Fusariumoxysporum*f. sp. *Lycopersici*the causal of tomato wilt in the greenhouse was studied [31]. They added that combination of *T. harzianum*T-22 + fluorescent *Pseudomonas* isolate gave the best control (70.2%).

Furthermore, in the present study the role of composted soil against disease incidence is obviously recorded. A number of investigations have demonstrated theeffectiveness of composts of various origins in suppressingsoil-borne plant pathogens [36,37,38], and theirapplication to soil has been proposed to control manydifferent diseases. Amendment of compost with*Trichodermaharzianum* also was reported to accelerateagricultural wastes composting and improved its diseasessuppressive effect [39].In this regards, several researchers have been recordedthat bio compost application as soil amendment couldsuppress diseases caused by *R. solani*and Fusarium spp.on many economic crops [40,41].Although some information is available on themechanisms responsible for the suppressive action ofcompost and compost extracts on plant pathogenic fungi[42] very limited information exists on the relationshipbetween chemical properties of HS and HS-like fractionsand fungal suppressive capacity. In this regards, severalresearchers have been recorded that bio compostapplication as soil amendment could suppress diseasescaused by *R. solani*and *Fusarium*spp. on many economiccrops [40,43,44]. Using agricultural wastes, domestic foodwastes or some grains as substrates for *T. harzianum* growth formulation and directly delivery in soil forcontrolling soil borne pathogens on some crops were alsorecorded [40,43,44].

IV.CONCLUSION

The present findings demonstrate that plant resistance inducers in combination with bio-agents may have important implications for the future use of antagonistic microorganisms on a commercial scale for controlling such root diseases. Hence, the objective of this study was to determine if plant resistance inducers and bioagents could provide enhancement effect against root rot disease. Considering their attribute and broad-spectrum activities, successful development of such compounds as antifungal would not only provide a potent tool for control of root diseases, but also could promise success in multipurpose biorational alternatives to conventional fungicides for the management of other plant diseases.

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