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Evaluation of Seed Coating with Some Essential oils and Bio-agents against Root Rot Disease of Faba Bean

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Abstract*- the efficacy of Lemongrass, Thyme, peppermint essential oils and /or bio-agents as seed dressing against faba bean disease incidence was evaluated in naturally infested field with root rot causal organisms under field conditions. All applied treatments reduced significantly root rot incidence at both pre- and post-emergence growth stages comparing with untreated check control. The obtained results showed that combination treatments of Lemongrass, Thyme and peppermint oils with bio-agents reduced significantly root rot incidence of all grown faba bean plants comparing with the application of each of them alone as well as the fungicide Rizolex-T. Obtained results, in the present study, lead to suggest that integration between essential oil and bio-agents is considered an applicable, safe and cost-effective method for controlling such soil-borne diseases.

Index Terms: **Lemongrass, Thyme, peppermint, root rot, faba bean, Trichdermaharzianum, Bacillus subtilis.**

I. INTRODUCTION

Faba bean (Viciafabae) is one of the most important winter leguminous crops in Egypt its grown mostly to fulfill food requirements of human and dry seeds of high quality are used for meals and in the food industries. Low quality seeds, in addition to plant wastes, are used for animal feed. Several fungi are recorded as causal pathogens of faba bean root rot disease such as Rhizoctoniasolani, Fusariumsolani, Sclerotiumrolfsiiand Macro phomina phase olinacausing root rot diseases. This disease appears during the growing season at the seedling stage of plant growth. Many soil borne fungi attack earlier at the pre-emergence stage [1]. Plant diseases continue to cause severe damage to most agricultural crops during different stages of plant growth resulting in heavy losses of both yield and quality. Considering the regularity with which most serious diseases of crop plants appear in an area year after year, the rapid spread of most plant disease, and the difficulty of curing a disease after it has begun to develop. So, it is easy to understand why almost all control methods are aimed at protection plant from becoming diseased rather than at curing them after they have become diseased. As a matter of fact, few infectious plant diseases can be satisfactory controlled in the field by therapeutic means, although certain diseases can be cured under managed environmental conditions. The regional problem being addressed is the development of a scientifically and environmentally sound scheme for the sustainable production of pathogen-free crops to meet domestic requirements and export market opportunities. From an historical perspective, to date, production of pathogen-free crops seedlings and commercial vegetable crops have been heavily dependent upon the use (often overuse) of pesticides and other inputs which cause important adverse effects on the environment.

A successful disease-control program could involve generally requires the application of several control measures [2]. Generally, IPM is regarded as the use of environmentally safe practices to reduce the disease incidence and development or use of multiple control tactics integrated into a single pest control strategy [3]. The use of different approach in plant disease control, i.e., bio-control agents, plant extracts and natural compounds were used as an IPM program to control powdery mildew of greenhouse crops [2,4]. Among other control measures the use of compound that induce a systemic plant resistance which were successfully used against several plant diseases incidence affected either plant root or shoot systems. There are several fungicides alternatives commercially used for induction of plant resistance against viruses, bacteria, and fungal infections.

The application of biological controls using antagonistic microorganisms has proved to be successful for controlling various plant diseases in many countries [5]. However, this is not an easy method, and it is costly to apply. It is possible to use biological controls as the best control measure under greenhouse conditions. It was reported that Trichodermaharzianumintroduced to the soil was able to reduce root rot incidence of faba bean plants significantly more than the fungicide Rizolex-T [1]. In recent years, several attempts have been made to overcome this obstacle by applying antagonistic microorganisms. Trichodermaspp. are well documented as effective biological control agents of plant diseases caused by soil borne fungi [6,7,8]. As for antagonistic bacteria,[9] found that seed treatment with Bacillus spp. was actively controlled three fungal root diseases of wheat. Also, Pseudomonas cepaciaor Pseudomonas fluorescens applied to pea seeds act as biological control agent against Pythium damping-off and Aphanomyces root rot and was able to reduce diseases incidence [10,11]. Also, [12] recorded that, Bacillus sp. gave a highly antagonistic effect against some pathogenic fungi including Fusariumsolani.

Also, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use [13]. In this regard, lemongrass (CymbopogoncitratusL.) oil was reported to be antifungal activity against several plant pathogens. Fungal spore production, spore germination and germ tube length of C. coccodes, B. cinerea, C.

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herbarium and R. stolonifer was inhibited with lemongrass oil treatments [14]. Moreover, using lemongrass essential oils by spraying or dipping fruits for controlling postharvest diseases of several fruits has been reported [14,15]. Also, thymol is an essential oil component from thyme (Thymus capitates L.) and has been used as medicinal drug, food preventative, and beverage ingredient (Mansour et al., 1986) as well as plant diseases of several fruits and vegetables [16,17,18].

The objective of the present work was aimed to determine the efficacy of application of essential oils as seed treatments in combination with the bio-agent T. harzianum and B. subtilis was evaluated against root-rot incidence of faba bean under field conditions.

II. MATERIALS AND METHODS

The activity of integrated treatment of bio-agents, T. harzianum and/or B. subtilis soaked faba bean seeds took place with 2% of any of the lemongrass and thyme oils (v:w) against root rot incidence. Activity of these treatments was evaluated under field conditions. The antagonistic microorganisms, i.e.T. harzianum and Bacillus subtilis, were obtained from the Plant Pathology Department of the National Research Centre, Giza, Egypt. 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 high pathogenic or antagonistic ability during previous work at the same department. Fungal and bacterial cultures were maintained on potato dextrose agar (PDA) and nutrient agar slant media at 5±1oC as stock cultures until use. All isolates were refreshed by growing at the optimum growth conditions at the beginning of the present experiments. 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 0Cuntil required. The bio-agents primed faba bean seeds were coated with a different essential oil tested at the rate of 4 ml/kg seeds. Seed dressing was carried out by applying the tested essential oil 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. 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 [19] was used to perform the ANOVA. Duncan's multiple range test at $P \leq 0.05$ level was used for means separation [20].

III. RESULTS AND DISCUSSION

The essential oils evaluated in this work have a great variety of phytochemicals (Table 1) that could be consid-ered as responsible for a larger or smaller antifungal ac-tivity. Those phytochemicals are as follows: Citrol or citral in lemongrass oil; Thymol, carvacrol, geraniol, thymol methyl ether, α -pinene in thyme essential oil and menthol, menthone, menthyl acetate, viridiflorol, ledol in peppermint oil [21]. The inhibitory effect of thyme, peppermint and lemongrass essential oils against the plant pathogenic fungi was reported earlier. Aantifungal activity of selected Turkish spices (black cumin, coriander, cumin, dill, laurel, oregano, parslely, spearmint, white mustard) was studied [22] on some foodborne fungi. They found that ground (1.0, 1.5, 2.0% w/v) plus essential oil (0.05%, 0.025%) showed an inhibitory effect on Aspergillusflavus, A. niger, Geotricumcandidum, Mucorspp., Penicilliumroqueforti. Also, [23] reported that 4% of the tested essential oils geranium, rosa, lemon and

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mint have an inhibitory effect against the mycelial growth of R. solani and F. oxysporumf. sp. Phaseoli under in vitro, causing complete inhibition in fungal growth. Furthermore, [24] studied the biochemical reaction of onion, garlic, eucalyptus, caraway, fennel, black cumin, mustard, carnation, neemix and trilogy essential oils against mycelial growth of R. solani and Pythiumdebaryanum in vitro. They found that complete inhibition of both fungi was obtained by only carnation oil at 4%, however, considerable inhibition (more than 90%) was obtained with neemix and trilogy oils.

Table 1. Botanical plant classification and main active principles of their essential oil*

Common name	Scientific name	Family	Major active component
Lemongrass	Cymbopogoncitratus	poaceae	Citrol or citral, which occurs in it in quantities varying from about 70 % up to 85 % or more.
Thyme	Thymus vulgaris	Labiatae	Thymol, carvacrol, geraniol, thymol methyl ether, α -pinene
Peppermint	Geranium viscosissimum	Geraniaceae	menthol, menthone, menthyl acetate, viridiflorol, ledol

***herb information (www.holisticonline.com/Herbal-Med/_Herbs/h280.htm)**

Data in Table (2) show that at pre-emergence growth stage essential oils could highly significantly reduced disease incidence of faba beans which have been artificially infested with root rot pathogens, compared to fungicide and untreated control treatments. Seed coating with any of the tested essential oils gave significant protection to emerged bean seeds against invasion of pathogenic fungi at the pre-emergence stage. Seed coating with essential oils recorded 31.7 to 39.4% protection increased in range 60.5 to 74.0% when combined with the bio-agents compared with 21.1% protection when Rhizolex-T treatment was used, over the untreated control (Fig. 1). Moreover, individual seed coating with T. harzianum and B. subtilis recorded root rot incidence at pre-emergence stage as 10.4 and 10.2% (Table 1) comparing with 20.8 at untreated treatment with reduction of 50.0 and 50.9%, respectively (Fig. 1).

Table 2. Root rot incidence of faba bean in response to seed dressing with essential oils and/or bio-agentsunder field conditions

Seed treatment	Root rot disease incidence %	
	Growth stage	
	Pre-emergence	Post-emergence
Thyme oil	14.2 c	16.4 c
Peppermint	14.4 c	16.6 c
Lemongrass oil	12.6 d	14.8 d
T. harzianum	10.4 e	12.2 e
B. subtilis	10.2 e	12.4 e
Thyme oil + T. harzianum	8.2 f	11.4 f
Thyme oil + B. subtilis	6.6 g	11.2 f
Peppermint + T. harzianum	8.6 f	10.4 g
Peppermint + B. subtilis	6.8 g	10.6 g
Lemongrass oil + T. harzianum	5.4 h	8.6 h
Lemongrass oil + B. subtilis	5.6 h	8.4 h
Fungicide (Rixolex-T)	16.4 b	18.6 b
Untreated	20.8 a	28.6 a

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

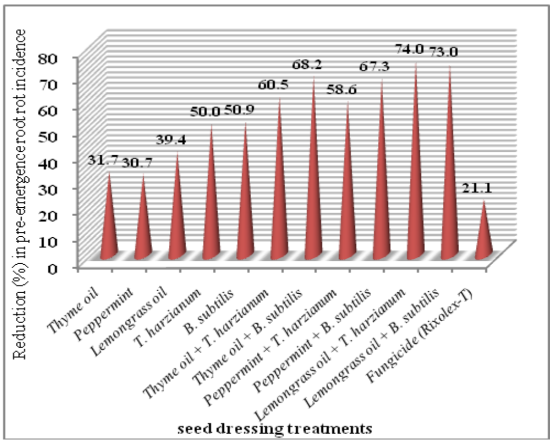

Fig. 1. Reduction in pre-emergence root rot incidence of faba bean in response to seed dressing with essential oils and/or bio-agentsunder field conditions

At the post-emergence stage, data (Table 2) also showed that all applied treatments could significantly reduce the percentage of root-rot incidence whereas it ranged between 8.4-16.4% compared with 28.6% in the untreated control treatment. Seed coating with fungicide showed a lowest effect on disease incidence that it record 18.6%. Treatment with essential oils caused reduction in the percentage of root-rot incidence recorded as 16.4, 16.6 and 14.8% in seed coated with thyme, peppermint and lemongrass oils, respectively. Seed treatment with essential oils combined with bio-agents had a superior effect on disease incidence whereas it caused root rot reduction recorded between 60.1–70.5% over control treatment (Fig. 2). Similar results, that essential oils inhibit large number of soilborne pathogens were reported by many researchers [25,26,27,28].

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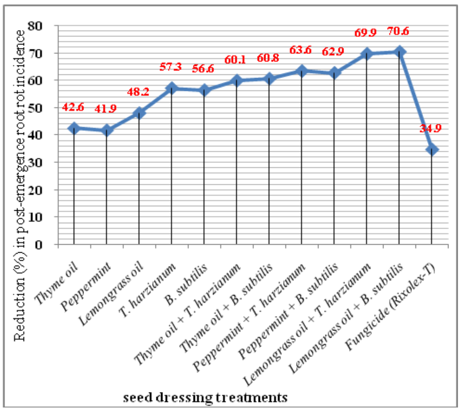

Fig. 2. Reduction in post-emergence root rot incidence of faba bean in response to seed dressing with essential oils and/or bio-agentsunder field conditions

The present investigation has demonstrated the anti-fungal activity of all treatments tested. This work proves that some essential oils have potential and could be useful when integrated with bioagents against faba bean fungal pathogens. From the earlier reports [29,30] it is evident that some of the plant products have antifungal compounds which do have the capacity to inhibit the fungal pathogens. Therefore, there is increasing interest in obtaining alternative antimicrobial agents for use in plant disease control systems. One of the main procedures used in the research of biologically active substances is a systematic screening for the interaction between microorganisms and plant products. This procedure has been a source of useful agents to control the microbial survival [31]. Plant products of a recognized antimicrobial spectrum could appear in food conservation systems as main antimicrobial compounds or as adjuvant to improve the action of other antimicrobial compounds [32]. Among other chemical products, aromatic plants possess essential oils resulting from secondary metabolism. These substances have a great economic potential, especially in the food, pharmaceutical and perfumery sectors. Thus, the number of studies on the chemical composition and biological properties of these oils, as well as the taxonomic, environmental and cultivation factors that lead to variation in their quantity and quality, has been increasing [33]. In the present study the combination of the bioagents, T. harzianum and B. subtilis with essential oils as seed coating increased the efficacy of essential oils against root rot incidence under field conditions. Similar results were reported by [1]. He stated that T. harzianum introduced to the soil was able to reduce root rot incidence of faba bean plants significantly more than the fungicide Rizolex-T. Moreover, the application of biological controls using antagonistic microorganisms, has proved to be successful for controlling various plant diseases in many countries [5,34,35,36,37].

IV. CONCLUSION

The present work demonstrated that thyme, peppermint and lemongrass essential oils as a seed coating, under field trails, resulted in a significant reduction in root rot incidence of faba bean. It may be concluded that application of essential oils combined with bioagentT. harzianumor B. subtilis is considered an applicable, safe and cost-effective method for controlling such soilborne diseases.

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