

Seed Dressing and Foliar Spray of Green Bean (*Phaseolus vulgaris* L.) with Essential Oils and Disinfectants for Suppressing Root Rot and Wilt Incidence under Field Conditions

N. S. El-Mougy, M. M. Abdel-Kader, H. M. Abouelnasr

Abstract—The efficacy of two essential oils applied as a bean seed dressing followed by seedlings foliar spray with four commercial disinfectants against root rot and wilt incidence was evaluated under field conditions. The essential oils, grape seed and peppermint oils and the disinfectants, Gold, Revarest, Klenva, Malva were applied. Chitosan and the fungicide Topsin-M were used as comparison treatment. Essential oils individually or combined with disinfectants were applied as a bean seed dressing. Furthermore, emerged bean plants were sprayed with the same treatments. Under laboratory conditions, growth inhibition effect was observed for the isolated, tested fungi *R. solani* and *F. oxysporum* when exposed to essential oils individually or combined with disinfectants. A high inhibitor effect was recorded for peppermint followed by grape seed oils. Concentrations of 1% and 2% of chitosan as well as Topsin M at 400 ppm showed complete reduction (100%) in the two fungal growths. Under field conditions, the obtained results showed that the applied treatments of chitosan had a superior effect on root rot and wilt disease incidence compared with other tested treatments. It was found that seed coating treatment provides good protection of emerged green bean seeds against the root pathogens attack compared with the fungicide and control treatments. Also, the application of seed dressing with essential oils accompanied by seedling spray demonstrated similar results. It was observed that essential oils had an enhancing effect against disease incidence when combined with disinfectants compared with their application. The obvious yield increase was significantly higher in all applied treatments than in fungicide and control.

Keywords—Bean, disinfectants, essential oils, root rot, wilt

I. INTRODUCTION

LEGUMES are of main value in the Egyptian diet. Green bean (*Phaseolus vulgaris* L.) is one of the most paramount leguminous crops for local and exporting purposes. Certain soilborne fungi were reported to attack green beans inducing root rot and wilt diseases. The fungi *Rhizoctonia solani* (Kühn) and *Fusarium oxysporum* f. sp. *phaseoli* are considered as the main pathogens responsible for these diseases [1]-[3].

Bean plants affected by wilt disease, which is considered a widespread problem, results in a reduction in both yield and

quality especially in developing countries [1], [3], [4]. In developing countries, bean wilt disease resulted in more outstanding yield losses, mainly due to the addition of high favorable environmental stress. Recently, the application of pesticide alternatives for preventing the growth of plant pathogens through the discovery of plant-derived antimicrobial agents is receiving considerable interest for avoiding the environmental pollution [5], [6].

Volatile compounds and essential oils which have antimicrobial characters were found in plant extracts [7], [8]. Essential oils and volatile compounds are aromatic oily liquids extracted from plant materials such as flowers, seeds, leaves, herbs, wood, fruits and roots. Essential oils are known to have valuable sources of biologically active compounds and therefore, there has been an increased interest in investigating the antibacterial and antifungal, antiviral, insecticidal and an antioxidants character of aromatic plant extracts, especially essential oils [9], [10]. On the other hand, antioxidants were recorded by few investigators to have an important role in plant defense mechanism. Green bean wilt incidence was significantly decreased by using *T. harzianum* integrated with essential oils, growth regulator or antioxidants under field conditions [11]. Furthermore, [12] used *T. harzianum* and some essential oils as seed dressing for reducing the incidence of sunflower charcoal rot under field conditions.

A successful disease-control program requires the application of several control measures to be active for a long-term reduction of disease losses. Therefore, an integrated pest management (IPM) program is the best approach for diseases control [13].

The objective of the present work was to investigate a non-fungicidal control method against root rot and wilt diseases incidence. Therefore, evaluation of the effectiveness of some essential oils and disinfectants applied individually or in combination as seed dressing and seedlings spray against the incidence of root rot and wilt diseases of green bean was carried out under field conditions.

II. MATERIALS AND METHODS

Pathogenic Fungi

Samples of bean plants showing root rot or wilt symptoms were collected from the field study and subjected to isolation trials. This work was carried out in the laboratory of

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Department of Plant Pathology, National Research Centre, Giza, Egypt. The purified fungal cultures were identified with the aid of illustrated fungal genera [14], [15].

Essential Oils, Disinfectants and Fungicides

Grape seed and peppermint essential oils used in the present study were purchased from Chemical Industrial Development (CID) Company, Egypt. Chitosan was purchased from Al-Gamhoria Company Ltd. for chemicals and medicinal instruments, Cairo, Egypt. The tested disinfectants, Gold, Revarest, Klenva, Malva were purchased from AB Pharma Corporation for Pharmaceuticals Company, Egypt. Meanwhile, the fungicide, Topsin M 70 WP, Dimethyl 4,4-(o-phenylene)bis(3-thioallophanate), Agrichemicals group, Cerexagri, Inc. was obtained from local market of Agrochemicals shops, Egypt.

Inhibition of Pathogenic Fungal Growth in vitro Tests

To obtain the proposed concentrations of 0.5%, 1.0% and 2.0%, certain volumes of the tested essential oils were added separately to flasks containing autoclaved PDA medium before its solidification. For combined treatments, another set of PDA media containing equal volumes of each of tested essential oils and disinfectants to obtain the same previous concentrations was also prepared. Different volumes of stock solution of the fungicide Topsin M 70 WP were added to other conical flasks containing PDA medium to obtain concentrations of 100 ppm, 200 ppm and 400 ppm. A separate PDA flask free of tested materials was used as check (control) treatment. Almost 20 ml of treated media were poured inside each sterilized Petri dishes (9 cm diameter). Vigor mycelial cultures of both isolated *Fusarium oxysporum* and *Rhizoctonia solani* grown for 10 days on PDA medium were used in this test. Disks (9 cm diameter) from the edge of abundant active fungal growth were obtained and placed on the surface of prepared media, as well as control, at the center of the Petri dishes. All inoculated Petri dishes were incubated for seven days at 25 ± 2 °C. Five Petri dishes were used as replicates for each particular treatment. The fungal growth diameter in each treatment was measured and compared relatively to control treatment, then the growth reduction in response to tested agent was calculated.

Field Experiment

The field experiments were conducted for two successive growing seasons (cultivation periods of March and September 2018) at Researches and Production Station, National Research Centre, Nubaria Region, Beheira Governorate, Egypt. This field is well known by the authors as naturally heavily infested with soilborne root rot and wilt pathogens.

This experiment was carried out to evaluate the efficacy of essential oils individually or in combination with disinfectants as seed coating and foliar spray against root rot and wilt diseases of bean. Chitosan individually as well as the fungicide Topsin-M were used as comparison treatment. The applied treatments were as the following design.

Concerning seed dressing, bean seeds were coated with the proposed treatment as follows: 1) Grape seed oil at the rate of

2 mL/100 gm seeds; 2) Peppermint oil at the rate of 2 mL/100 gm seeds; 3) Chitosan (2%) at the rate of 1 mL/100 gm seeds; 4) Grape seed oil (2 mL/100 gm seeds) + Gold (1 gm/100 gm seeds); 5) Grape seed oil (2 mL/100 gm seeds) + Revarest (1 gm/100 gm seeds); 6) Grape seed oil (2 mL/100 gm seeds) + Klenva (1 gm/100 gm seeds); 7) Grape seed oil (2 mL/100 gm seeds) + Malva (1 gm/100 gm seeds); 8) Peppermint oil (2 mL/100 gm seeds) + Gold (1 gm/100 gm seeds); 9) Peppermint oil (2 mL/100 gm seeds) + Revarest (1 gm/100 gm seeds); 10) Peppermint oil (2 mL/100 gm seeds) + Klenva (1 gm/100 gm seeds); 11) Peppermint oil (2 mL/100 gm seeds) + Malva (1 gm/100 gm seeds) and 12) Topsin-M (3 gm/Kg seeds). Bean seed dressing was applied before sowing; next, the emerged plants (at 2–3 true leaves age) were sprayed with the same above-mentioned treatment at the same previously sown plots three times with 15-day intervals starting at 15 days from plant emergence. Topsin M was applied as seed dressing at the recommended dose (3 g/kg seeds) and at the rate of 2 gm/L as foliar sprays. Meanwhile, chitosan (2%) was sprayed at the rate of 1 mL/100 L water.

The experimental field includes plots (7x6 m); each contains 12 rows with 30 bores per each for seed sowing. Five plots were used for each individual treatment and check control as well. All plots were managed in Complete Randomized Block Design (CRBD). In all experimental plots, seed sowing was carried out using bean seeds (Giza, 3 cv.) with three seeds on average per each bore. Then after, all conventional agricultural practices were followed as recommended.

At the pre-emergence stage, the average percent of root rot infection or wilt was recorded after 15 days after sowing. At post-emergence stage, bean plants showing root rot or wilt symptoms were recorded and the average accumulated disease incidence was calculated three times after 30 days, 45 days and 60 days after sowing throughout the growing season. At harvest time, the average accumulated yield was calculated for all applied treatments and the control as well.

The field experiments were carried out at the same field for two successive, spring (March) and winter (September) growing seasons 2018. Then, the average of root rot and wilt diseases incidence and accumulated yield for the two seasons was calculated.

Statistical Analysis

General Linear Model option of the Analysis System SAS [16] was used to perform the analysis of variance. Duncan's Multiple Range Test at $p \leq 0.05$ levels was used for means separation [17].

III. RESULTS AND DISCUSSION

According to [14] and [15], the isolated fungi from bean plants that showed diseases symptoms of root rot or wilt were identified as *Rhizoctonia solani* and *Fusarium oxysporum*. These fungi were recorded to have high pathogenicity on beans [1], [3].

The antifungal activity of grape seed and peppermint essential oils at different concentrations and/or disinfectants,

Gold, Revarest, Klenva, malva against *R. solani* and *F. oxysporum* bean root rot and wilt pathogens were determined *in vitro* by agar disc method.

Different concentrations of chitosan and the fungicide Topsin-M were also tested. Presented data in Table I reveal that the fungal growth decreased as the concentrations of tested agents were increased.

TABLE I
THE INHIBITORY EFFECT OF DIFFERENT CONCENTRATIONS OF SOME ESSENTIAL OILS INDIVIDUALLY OR COMBINED WITH DISINFECTANTS ON THE LINEAR GROWTH OF *FUSARIUM OXYSPORUM* AND *R. SOLANI* IN VITRO

Treatment	Concentration	Average growth reduction	
		<i>F. oxysporum</i>	<i>R. solani</i>
Grape seed oil	0.5 %	45.7 j	51.2 g
	1.0%	62.4 h	63.3 f
	2.0%	80.7 d	78.7 c
Peppermint oil	0.5 %	72.3 ef	68.4 e
	1.0 %	88.8 b	78.3 c
	2.0%	100 a	100 a
Chitosan (2%)	0.5%	77.4 e	72.8 d
	1.0%	100 a	100 a
	2.0%	100 a	100 a
Grape seed oil + Gold	0.5 + 1%	55.3 i	57.7 g
	1.0 + 1%	68.7 g	71.2 d
	2.0 + 1%	86.4 b	84.6 b
Grape seed oil + Revarest	0.5 + 1%	51.3 ij	55.7 g
	1.0 + 1%	64.5 h	68.7 e
	2.0 + 1%	85.7 b	84.7 b
Grape seed oil + Klenva	0.5 + 1%	60.2 hi	58.8 g
	1.0 + 1%	78.3 e	77.8 c
	2.0 + 1%	88.2 a	86.4 b
Grape seed oil + Malva	0.5 + 1%	59.6 i	57.8 g
	1.0 + 1%	71.3 ef	70.3 de
	2.0 + 1%	87.4 b	80.7 c
Peppermint oil + Gold	0.5 + 1%	75.5 e	70.3 de
	1.0 + 1%	80.2 d	78.8 c
	2.0 + 1%	86.6 b	84.3 b
Peppermint oil + Revarest	0.5 + 1%	77.7 e	72.3 d
	1.0 + 1%	86.6 b	78.8 c
	2.0 + 1%	88.8 a	88.8 a
Peppermint oil + Klenva	0.5 + 1%	74.2 e	71.6 d
	1.0 + 1%	81.4 d	76.6 c
	2.0 + 1%	86.6 b	84.3 b
Peppermint oil + Malva	0.5 + 1%	74.4 e	70.2 de
	1.0 + 1%	76.7 e	75.4 c
	2.0 + 1%	80.3 c	81.1 bc
Topsin-M	100 ppm	83.3 c	76.6 c
	200 ppm	100 a	83.3 b
	400 ppm	100 a	100 a

Reduction of fungal growth in different treatment, calculated relatively to its growth in untreated control.

Concentrations of essential oils and disinfectants were calculated as (v:v) to the growth medium, while based on the active ingredient of the fungicide.

Mean values within columns followed by the same letter are not significantly different at $p \leq 0.05$.

Peppermint oil had more inhibitor effect against fungal growth than grape seed oil that it causes growth reduction at concentrations of 0.5%, 1.0% and 2.0% calculated relatively to the control as 72.3%, 88.8%, 100% for *F. oxysporum* and

68.4%, 78.4%, 78.3% for *R. solani* compared with 45.7%, 62.4%, 80.7% and 51.2%, 63.3%, 78.7% for grape seed oil, in respective order. Also, data presented in Table I showed an inhibitory effect of all tested agents against the growth of tested fungi. Concentrations of 1% and 2% of chitosan and 400 ppm of the fungicide Topsin M could completely (%) inhibit the growth of the two tested fungi. High inhibitor effect was recorded for peppermint followed by grape seed oils. It was observed that the addition of disinfectants enhances the inhibitory effect of essential oil. The tested concentrations of grape seed oil (0.5%, 1.0%, 2.0%) could reduce growth of *F. oxysporum* at a range of 45.7 up to 80.7%, meanwhile, higher reduction in fungal growth by (55.3%-86.4%); (51.3%-85.7%); (60.2%-88.2%) was recorded when the disinfectants, Gold, Revarest, Klenva, Malva were combined at 1% to grape seed oil concentrations, respectively. These results could explain the obtained efficacy of applied treatments under field conditions whereas the essential oils individually or combined with disinfectants applied as seed coating and/or seedling foliar spray reduced bean root rot and wilt diseases incidence.

TABLE II
AVERAGE ROOT ROT AND WILT DISEASES INCIDENCE (%) OF GREEN BEAN AFFECTED WITH THE APPLICATION OF SOME ESSENTIAL OILS AND DISINFECTANTS UNDER FIELD CONDITIONS THROUGHOUT TWO CULTIVATION SEASONS

Treatment	SEASONS			
	Average disease incidence and produced yield ^A			
	Seed dressing	Foliar spray		Yield ^b (Kg/plot)
	Pre-emergence ^B	Post-emergence ^C		
		Root rot	Wilt	
Grape seed oil	12.1 c	15.2 c	17.1 c	20.7 c
Peppermint oil	10.3 d	12.4 d	15.7 d	21.4 c
Chitosan	4.8 gh	5.3 i	5.7 j	35.2 a
Grape seed oil + Gold	10.3 d	11.4 e	13.5 f	24.1 d
Grape seed oil + Revarest	9.4 e	10.2 ef	12.1 fg	26.2 e
Grape seed oil + Klenva	8.8 e	9.4 f	11.4 h	27.8 ef
Grape seed oil + Malva	8.1 e	9.8 f	10.0 h	29.4 fg
Peppermint oil + Gold	5.6 g	6.2 h	7.8 i	32.6 h
Peppermint oil + Revarest	6.2 f	7.3 g	7.1 i	30.2 fg
Peppermint oil + Klenva	10.6 d	11.7 e	14.2 e	22.3 d
Peppermint oil + Malva	12.2 c	13.2 cd	12.8 fg	21.1 c
Topsin-M	16.7 b	18.7 b	20.0 b	19.6 b
Control	18.7 a	21.3 a	23.5 a	17.7 a

^A All figures calculated as average of the two growing seasons

^B Pre-emergence (%) based on the number of not emerged seeds in relation to the number of sown seeds

^C Post-emergence (%) based on the number of plants showing root rot or wilt disease symptoms in relation to the number of emerged seedlings

^D Yield rated as average of accumulated kg/plot green bean at different treatments

Mean values within each column followed by the same letter are not significantly different ($P \leq 0.05$).

At pre-emergence growth stage, data presented in Table II and illustrated in Fig. 1 reveal that the applied essential oils individually or combined with disinfectants could protect bean seeds against attack of soilborne pathogens during seed germination stage which reflected on the emerging seedlings above the soil surface at different degrees. In this concern, all applied essential oils in combination with disinfectants showed higher effect on root rot disease incidence than

applied treatment of essential oils individually. Chitosan proved high significant protective effect against pathogen seed invasion that its seed dressing resulted in 4.8% and 74.3% disease incidence and reduction, respectively. This treatment is followed by applied seed dressing with Peppermint oil + Gold (5.6% & 70.0%) and Peppermint oil + Revarest (6.2% & 66.8%), respectively for disease incidence and reduction. Moreover, moderate significant effect was observed for combined treatments of Grape seed oil + Malva; Grape seed oil + Klenva and Grape seed oil + Revarest resulted in the pre-emergence disease incidence of 8.1%, 8.8% and 9.4% with disease reduction calculated as 56.6%, 52.9% and 49.7%, in relevant respective order. Meanwhile, the other applied treatments showed lesser effect. The lowest significant effect was recorded at seed dressing with the fungicide Topsin-M treatment that disease incidence was 16.7% compared with the untreated control (18.7%) with a reduction calculated as 34.7%. Extended protective effect against invasion of soilborne pathogens to plant roots was observed at post-emergence growth stage of bean plants when essential oils individually or combined with disinfectants applied as foliar spray. Data in Table II and Fig. 1 show that chitosan had superior effect against root rot (5.3% & 75.1%) and wilt (5.7% & 75.7%) diseases incidence and reduction, respectively, compared with other applied treatments and control as well. Also, data in Fig. 1 reveal that treatments of Peppermint oil + Gold; Peppermint oil + Revarest followed by Grape seed oil + Malva; Grape seed oil + Klenva and Grape seed oil + Revarest could reduce disease incidence of root rot by 58.2%, 55.8%, 52.1% and wilt by 57.4%, 51.4%, 48.5% compared with Topsin-M which were 12.2% and 14.8%, in relevant respective order.

It is well known that the high density of healthy plants is reflected on yield increase. In the present study, reductions in root rot and wilt diseases reflected on the increase in plant stand and consequently, the produced yield. The obtained yield recorded for the chitosan treatment was 35.2 Kg/plot representing an increase 98.8%, followed by Peppermint oil + Gold treatment which revealed 32.6 Kg/plot with an increase in yield estimated as 84.1% as well as at Peppermint oil + Revarest treatment where the produced yield was 30.2 Kg/plot with an increase of 70.6%. Moderate produced yield as 29.4 Kg/plot, 27.8 Kg/plot, 27.8 Kg/plot with increase by 66.1%, 57.0%, 48.0% at combined treatments of grape oil with Malva, Klenva and Revarest, respectively. The other applied treatments showed the lowest yield increase which ranged between 16.9% - 36.1% over the control treatment; meanwhile, only 10.7% yield increase was recorded for the fungicide treatment. The obvious results in the present study show that essential oils, grape seed and Peppermint oils individually or combined with disinfectants, Gold, Revarest, Klenva, Malva had an inhibitor effect on the growth of isolated bean root pathogens *R. solani* and *F. oxysporum* under *in vitro* conditions. Furthermore, these treatments had efficient effect against root rot and wilt incidence of bean when applied as seed dressing followed by foliar spray under field conditions. These results revealed that the use of fungicide

alternatives against plant diseases, matching the need for active tools characterized by simplicity, fast acting and accurate for reducing pathogen populations, especially against the root pathogens. That is because the control of root rots and wilt diseases depends on conventional methods such as chemical, biological controls and resistant cultivars or cultural practices. Although the use of fungicides is considered one of the most effective methods to control these diseases, unfortunately, the pathogens could build resistance against these fungicides, in which case, higher doses are then demanded. Furthermore, the expected hazards of chemical application in the field to the environment and health are not desired. Therefore, researchers from all over the world paid more attention for developing fungicide alternative materials characterized as safe to human and environment as well as have rapid biodegradable ability. Botanicals are known to constitute a large origin of natural antimicrobial components which characterized as safe and potent alternatives to chemical fungicides. Such volatile components and essential oils could be used directly or served as origin for synthetic analogs if they formulated and applied. The herbal medicine, which is considered part of folk or traditional medicine commonly, used plants and their products for treating human diseases [18] and they were used for many centuries till nowadays to alleviate or prevent many diseases because of their resource of various ethnic groups [19].

Results of *in vitro* tests showed that the mycelial growth of *R. solani* and *F. oxysporum* was highly inhibited when exposed to peppermint and grape seed oils as direct contact on growth medium. Moreover, this inhibitory effect increased in combination with disinfectants. Similarly, the essential oils from some plants, i.e. lemon, rose, geranium and mint were recorded to have inhibition ability against the mycelia growth of *R. solani* and *F. oxysporum* [4]. Moreover, field approaches of essential oils as seed dressing and/or foliar spray singly or integrated with disinfectants demonstrate their protection against germinated seeds or plant roots infection under field conditions which consequently leads to significant decrease in root rot and wilt diseases incidence of green bean under field conditions. Peppermint oil had a superior effect in this regard compared to grape seed oil either alone or combined with disinfectants. These results could be attributed to the stability, concentration and volatility of the active constitute in the essential oil used. It was reported that the major components of peppermint essential oil include menthol, menthone and menthyl acetate besides other active components, e.g. flavonoids, menthol, rutin and others; miscellaneous, rosmarinic acid, azulenes, choline, carotenes etc. [20], [21]. Also, [22] stated that grape seed oil composed of linoleic, oleic, palmitic, stearic, alpha-linolenic and palmitoleic fatty acids. Meanwhile, [23] recorded that grape seed oil is also rich in phenols and steroids. Similarly, the efficacy of several essential oils which had antifungal inhibitory effect against a wide range of soilborne pathogens were reported [24]-[26]. Moreover, [27] reported that wilt incidence was decreased at pre-emergence stage by 67% - 71%, and at post-emergence stage by 55% - 58% when clove and cinnamon oils were used

as soil improvers. Such compounds derived from plants provide disease control potential. In general, the mechanism system of natural products includes several tools for suppressing mold fungi. For example, they cause cytoplasm

granulation, detaching cytoplasmic membrane, inactivation or suppress both intracellular and extracellular enzymes of fungal cells.

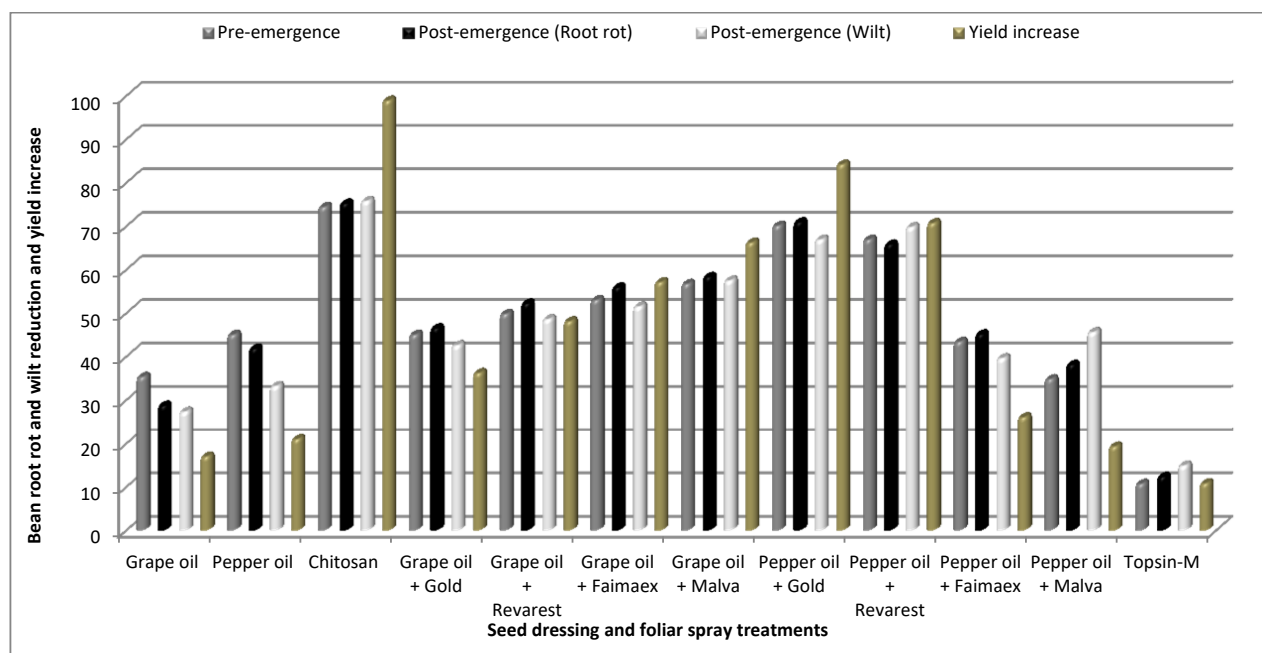


Fig. 1 Average root rot and wilt diseases reduction and yield increase in response to seed dressing followed by foliar spray with some essential oils and disinfectants at two successive seasons under field conditions

The mode of inhibitor action of essential oils and their chemical compounds on microorganisms seems to be involved in different mechanisms. The microbial inhibition supposed to be attributed to the phenolic compounds, which sensitize the phospholipid bilayer of the cytoplasmic membrane and lead to deficiency in the permeability and inaccessibility of vital intracellular components [28]. Many authors emphasized that the antimicrobial effect of essential oil constituents depends on their hydrophobicity and partition in the microbial plasmatic membrane that is the main or primary microbial target of antimicrobial compounds [6], and their higher volatility of active compounds increases their solubilization in the cell membranes, which are the first target of antimicrobial action [29].

In the present study, commercial disinfectants, i.e. Gold, Revarest, Klenva, Malva individually or in combination with essential oils had a suppression effect against root rot and wilt pathogens when used as an amending growth medium (*in vitro*) and seed coating or foliar spray (*in vivo*). In this regard, disinfectants consisting of chemical active agents are able to destroy or inhibit non-spore or vegetative growth state of pathogenic microorganisms. Disinfectants are applied to the surfaces of instruments and health care objects to control and prevent infection [30], [31]. As a mechanism of action against microorganisms, they work by destroying the cell wall of microbes or interfering with the metabolism. Also, it can be seen that they interact with the cell surface penetrating into the

cell and act on the target site(s) [32], [33]. Disinfectants are not able to kill all microorganisms in certain mediums, but they could reduce their population to a minimum harmful scale, for example, to a level that does not injure the health or the quality of perishable goods. These disinfectants vary in their effectiveness depending on their constituents. The active ingredients of the disinfectants used in the present study reveal that they share in their constituents with that of aloe vera extract, menthol, thymol, chamomile extract, tea tree oil and other antioxidants as well. These ingredients could be considered the main active components that affected the target pathogens under study. In this regard, it was reported that Gold contains aloe vera extract, menthol, thymol, chamomile extract, tea tree oil and Borax [34] which kills or helps to prevent the growth of pathogens, and a wide spectrum of bacteria and yeasts. Also, Revarest contains aloe extract, menthol, thymol, tea tree oil, borax and propylene glycol [35]. Klenva contains alum root, thymol, chamomile extract, menthol, tea tree oil and pro vitamin B5 [36]. It was reported that Malva liquid commercial product contains Malva extract (Mallow), TEGO-Betain, citric acid, chlorhexidine gluconate, chlorocresol, thymol, menthol, chamazulene, natural rose extract [37]. Malva (*Malva sylvestris* L.), known as the common mallow, is a plant with a long history of use as food, and due to its therapeutic relevance, some parts of this plant, particularly the leaves, have been utilized in conventional medicines because of their potent anti-inflammatory and

antioxidant properties. On the other hand, Chitosan is a polysaccharide produced from chitin, and had no solubility unless through alkalic or enzymatic deacetylation. Chitosan can produce some substances which influence the reaction of the plant's defense mechanism, such as phytoalexins [38] and chitinases [39]. Under field trails, [40] suggested the commercial use of chitosan for controlling tomato root rot diseases. Foliar application of chitosan of tomatoes improved significantly the powdery mildew disease control and increased the produced yield by about 20% under field conditions [41]. The self-defense mechanisms in plant tissues were activated in parallel with chitosan treatments which inhibited the growth of different pathogens [42]. Therefore, chitosan has been reported as an alternative to synthetic fungicides [43]-[45].

In the present study, results showed that chitosan had a growth inhibitor effect against *F. oxysporum* and *R. solani* under *in vitro* and also acts as elicitor of plant defense mechanisms against the two pathogens for inducing root rot and wilt diseases under field conditions (Tables I and II). In this consideration, many investigators recorded similar results with various crops. Induction of various plants self-resistance was achieved against potato late and early blight diseases, bean and lupin root rot diseases by the use of chitosan treatment under field conditions [46], [47]. Furthermore, chitosan has different characters, for instance its inhibitory effect against pathogenic fungal growth [48] and its ability to be active elicitors of self-plant defense response [49], [50]. Also, [51] reported that the use of chitosan at 6 g/L could inhibit completely the growth of all tomato root rot fungi and the total count of pathogenic fungi was also reduced. According to the antimicrobial activity of essential oils and disinfectants, in the present investigation, it could be suggested that all of the tested materials were active against *R. solani* and *F. oxysporum* the incidents of root rot and wilt diseases. These findings can be exploited further with a view to generate new potent antimicrobial agents able to protect cultivated crops against such diseases; of particular note, is that they are cost effective, environmentally safe and easily applied.

REFERENCES

- [1] M.M. Abdel-Kader, Field application of *Trichoderma harzianum* as biocide for control bean root rot disease. Egypt. J. Phytopathol., vol. 25, pp. 19–25, 1997.
- [2] R.A. Burnchara, L. Camacho, Common bean reaction to *Fusarium oxysporum* f. sp. *phaseoli*, the cause of severe vascular wilt in central Africa. J. Phytopathol., vol. 148 pp. 39-45, 2000.
- [3] N.S. El-Mougy, Field application of certain biological and chemical approaches for controlling bean wilt disease. Egypt. J. Phytopathol., vol. 29 pp. 69–78, 2001.
- [4] N.S. El-Mougy, N.G. El-Gamal, M.M. Abdel-Kader, Control of wilt and root rot incidence in *Phaseolus vulgaris* L. by some plant volatile compounds. J. Plant Protec. Res., vol. 47 pp. 255-265, 2007.
- [5] O. Sagdiç, A.G. Karahan, M. Ozcan, G. Ozcan, Effect of some spices extracts on bacterial inhibition. Intern., J. Food Sci. Technol., vol. 9 pp. 353–359, 2003.
- [6] R. Lanciotti, A. Gianotti, N. Patrignani, N. Belletti, M.E. Guerzoni, F. Gardini, Use of natural aroma compounds to improve shelf-life of minimally processed fruits. Trends Food Sci. Technol., Vol. 15: pp. 201–208, 2004.
- [7] J. Jenny, Essential oils: A new idea for postharvest disease control. Good Fruit and Vegetables magazine. Vol. 11 (3) pp. 50, 2000.
- [8] M. Krauze-Baranowska, M. Mardarowicz, M. Wiwart, L. Poblocka, M. Dynowska, Antifungal activity of the essential oils from some species of the genus *Pinus*. Z Naturforsch., vol. 57 pp. 478–482, 2002.
- [9] G. Milhau, A. Valentin, F. Benoit, M. Mallie, J. Bastide, Y. Pelissier, In vitro antimalarial activity of eight essential oils. J. Essential Oil Res., vol. 9 pp. 329-333, 1997.
- [10] S. Kordali, R. Kotan, A. Mavi, A. Cakir, A. Ala, A. Yildirim, Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracuncululus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A. dracuncululus*, *A. santonicum* and *A. spicigera* essential oils. J. Agric. Food Chem., Vol. 53 pp. 9452-9458, 2005.
- [11] M.M. Abdel-Kader, N.S. El-Mougy, Potential control of beans (*Phaseolus vulgaris* L.) wilt disease using growth regulators, bioagent, antioxidants and essential oils as foliar application under field conditions. African J. Microbio. Res., vol. 10(46), pp. 1952-1960, 2016.
- [12] N.S. El-Mougy, M.M. Abdel-Kader, Application of *Trichoderma harzianum* and essential oils as seed dressing against charcoal rot disease incidence of sunflower under field conditions. BioScience Research., vol. 15(3) pp. 2711-2720, 2018.
- [13] A. Dik, J. Wubben, Y. Elad, J. Kohi, D. Shtienberg, Combination of control methods against powdery mildew diseases in glasshouse grown vegetables and ornamentals. Bull. OIL B-SROP., vol. 25 (10) pp. 5-8, 2002.
- [14] J.C. Gilman, A manual of soil fungi. Iowa State University Press, Ames, Iowa, U.S.A. 450 pp., 1957.
- [15] H.L. Barnett, B.B. Hunter, Illustrated Genera of Imperfect fungi. Burgen Publ. Co., Minnesota, 4 pp., 1972.
- [16] SAS Institute Inc., SAS/STAT User's Guide. Version 6, 12th ed. Vol. 2, SAS Institute, Inc. Cary, North Carolina, USA, 846 pp., 1996.
- [17] B.J. Winer, Statistical Principles in Experimental Design. 2nd ed. McGraw-Hill Kogakusha, LTD, 596 pp., 1971.
- [18] W.B. El-Ghaoui, E.B. Ghanem, L.A. Chedid, A.M. Abdelnoor, The effects of *Alcea rosea* L., *Malva sylvestris* L. and *Salvia libanotica* L. water extracts on the production of anti-egg albumin antibodies, interleukin-4, gamma interferon and interleukin-12 in BALB/c mice. Phytother Res., vol. 22 (12) pp.1599-604, 2008.
- [19] M. Kaileh, W. Vanden Berghe, E. Boone, T. Essawi, G. Haegeman, Screening of indigenous Palestinian medicinal plants for potential anti-inflammatory and cytotoxic activity. J. Ethnopharmacol., vol. 113(3) pp. 510-6, 2007.
- [20] E.E. Aziz, L.E. Craker, Essential Oil Constituents of Peppermint, Pennyroyal, and Apple Mint Grown in a Desert Agrosystem. J. Herbs, Spices & Medi. Plants, vol. 15(4): 361-367, 2010. <https://doi.org/10.1080/10496470903507940>
- [21] M.L. Tsai, C.T. Wu, T.F. Lin, W.C. Lin, Y.C. Huang, C.H. Yang, Chemical Composition and Biological Properties of Essential Oils of Two Mint Species. Trop. J. Pharma. Res., vol. 12(4): 577-582, 2013. <https://www.ajol.info/index.php/tjpr/article/viewFile/93279/82692>
- [22] B.S. Kamel, H. Dawson, Y. Kakuda, "Characteristics and composition of melon and grape seed oils and cakes". Journal of the American Oil Chemists' Society., vol. 62 (5) pp. 881–883, 1985.
- [23] B.D. Oomah, J. Liang, D. Godfrey, G. Mazza, "Microwave Heating of Grape seed seed: Effect on Oil Quality. J. Agric. Food Chem., vol. 46 (10) pp. 4017–4021, 1998.
- [24] A.K. Singh, A. Dickshit, M.L. Sharma, S.N. Dixit, Fungitoxic activity of some essential oils. Econ. Bot., vol. 34 pp. 186–190, 1980.
- [25] A. Kumar, S.C. Tripathi, Evaluation of the leaf juice of some higher plants for their toxicity against soilborne pathogens. Plant and Soil, vol. 132 pp. 297–301, 1991.
- [26] U.P. Singh, K.P. Singh, V.K. Tripathi, B.B. Pandey, Antifungal activity of some naturally occurring plant alkaloids. Intern. J. Trop. Plant Dis., vol. 12 pp. 209–212, 1994.
- [27] S. Brull, P. Coote, Preservative agents in foods: mode of action and microbial resistance mechanisms. Int. J. Food Microbiol., vol. 50 pp. 1–17, 1999.
- [28] B.J. Juven, J. Kanner, F. Sched, H. Weisslowicz, Factors that interact with the antibacterial of thyme essential oil and its active constituents. J. Appl. Microbiol., vol. 76 pp. 626–631, 1994.
- [29] S. Inouye, K. Uchida, N. Maruyama, H. Yamaguchi, S. Abe, A novel method to estimate the contribution of the vapor activity of essential oils in agar diffusion assay. Japan J. Med. Mycol., vol. 47 pp. 91–98, 2006.
- [30] S.S. Block, Historical review, p. 3–17. In S. S. Block (ed.), Disinfection,

- sterilization, and preservation, 4th ed. Lea & Febiger, Philadelphia, Pa., 1991.
- [31] W.A. Rutala, APIC guidelines for selection and use of disinfectants. Am. J. Infect. Control, vol. 23 pp. 313–342, 1995.
- [32] W. Gottardi, The influence of the chemical behavior of iodine on the germicidal action of disinfectant solutions containing iodine. J. Hosp. Infect., vol. 6 (Suppl. A) pp. 1–11, 1985.
- [33] A.D. Russell, I. Chopra, Understanding antibacterial action and resistance, 2nd ed. Ellis, Horwood, Chichester, England, 1996.
- [34] Anonymous 2016a. Gold Plus Cleanser. Macro Pharmaceutical. <http://www.macro.com.eg/product/gold-plus-cleanser/>
- [35] Anonymous 2016b. Revarest plus Liquid Cleanser. Macro Pharmaceutical. <http://www.macro.com.eg/product/revarest-plus-liquid-cleanser/>
- [36] Anonymous 2016c. Klenva Cleanser. Macro Pharmaceutical. <https://www.macro-egy.com/portfolio/klenva-cleanser/>
- [37] Anonymous 2016d. MALVA Intimate hygiene cleanser. Medicine.com <https://www.medicine.com/malva-intimate-hygiene-cleanser-for-female-use-only-427.html>
- [38] M. Walker-Simmons, L. Hadwiger, C.A. Ryan, Chitosans and pectic polysaccharides both induce the accumulation of the antifungal phytoalexin pisatin in pea pods and antinutrient proteinase inhibitors in tomato leaves. Bioch. Biophys. Res. Communicat., vol. 110 pp. 194–199, 1983.
- [39] H. Dornenburg, D. Knoor D., Elicitation of chitinases and anthraquinones in *Morinda citrifolia* cell cultures. Food Biotechnol., vol. 8 pp. 57–59, 1994.
- [40] N.S. El-Mougy, N.G. El-Gamal, Y.O. Fotouh, F. Abd-El-Kareem, Evaluation of different application methods of chitin and chitosan for controlling tomato root rot disease under greenhouse and field conditions. Res. J. Agric. Biol. Sci., vol. 2 pp. 190–195, 2006.
- [41] R. Walker, S. Morris, P. Brown, A. Gracie, Evaluation of potential for chitosan to enhance plant defense. A Report for the Rural Industries Research and Development Corporation, Australia, RIR DC Publication No. 04., 2004.
- [42] N.I. Vasyukova, S.V. Zinoveva, L.I. Ilinskaya, E.A. Perekhod, G.I. Chalenko, N.G. Gerasimova, , Modulation of plant resistance to diseases by water-soluble chitosan. Appl. Biochem. Microbiol., vol. 37 pp. 103–109, 2001.
- [43] N. Benhamou, P. Lafontaine, M. Nicole, Induction of systemic resistance to *Fusarium* crown and root rot in tomato plants by seed treatment with chitosan. Phytopathology, vol. 84 pp. 1432–1444, 1994.
- [44] El-Ghaouth, Effect of chitosan on cucumber plants: suppression of *Pythium aphanidermatum* and induction of defense reactions. Phytopathology, vol. 84 pp. 313–320, 1994.
- [45] S. Tiuterev, Chitosan. Mechanism of action and ways of using chitosan as ecologically safe means in enhancement of plant disease resistance. Arch. Phytopathol. Pl. Prot., vol. 30 pp. 323–332, 1996.
- [46] F. Abd-El-Kareem, Integrated treatments between bioagents and chitosan on root rot diseases of pea plants under field conditions. Egypt J. Appl. Sci., vol.17, pp. 257-279, 2002.
- [47] F. Abd-El-Kareem, M.A. Abd-Alla, R.S.R. El-Mohamedy, Induced resistance in potato plants for controlling early blight disease under field condition. Egypt J. Appl. Sci., vol. 17, pp. 51-66, 2002.
- [48] J.L. Leuba, P. Stossel, Chitosan and other polyamines: Antifungal activity and interaction with biological membranes. In Muzzarelli, R. and Goody, G.W. (eds.), Chitin in nature and technology. Plenum Press, New York, pp: 215-222, 1986.
- [49] N. Benhamou, Elicitor-induced plant defence pathways. Trends Plant Sci. vol.1pp. 233-240, 1996.
- [50] J. Kuc, Concepts and direction of induced systemic resistance in plants and its application. Eur. J. Plant Pathol., 107: 7-12, 2005.
- [51] F. Abd-El-Kareem, N.S. El-Mougy, N.G. El-Gamal, Y.O. Fotouh, Use of chitin and chitosan against tomato root rot disease under greenhouse conditions. Res. J. Agric. Biol. Sci., vol. 2(4) pp. 147-152, 2006.