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Effect of Some Botanical Powdered Plants against Root Rot Disease Incidence of Bean under Field Conditions

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Abstract- ***The antifungal effect of three botanical powdered plants and their extracts against root rot disease incidence of bean was evaluated under laboratory and field conditions. Powder of chilli pods (Capsicum annuum), Cabbage leaves (Brassica oleracea) and Eucalyptus leaves (Eucalyptus obliqua) were used in the present work. The botanicals powder or extract were added to the culture medium PDA to obtain the proposed concentrations of 2, 4 and 8%. High significant inhibitory effect on radial fungal growth was observed for different concentrations of botanicals tested. Extracts of botanical plants at the same concentrations indicate a superior inhibitory effect on radial fungal growth when compared with powdered ones. Under field conditions, the botanical plant powders were added individually or combined with compost to the cultivation soil before sowing. All applied treatments reduced root rot incidence comparing with untreated control. Higher significant reduction in disease incidence was observed for combined treatment than that of individuals. It is interesting to note that botanical plants powder gave a similar effect to the fungicide Rhizolex-T in reducing root rot incidence either at pre- or post-emergence stages of bean growth. Promising applicable technique could be suggested in the light of the results obtained. The use of soil drench treatment with botanical plants powder might be considered as safe, cheep and easily applied method for controlling such soil-borne plant pathogens considering the avoidance of environmental pollution and the side effect of pesticide application.***

Index Terms- **Botanicals powder, botanicals extracts, Root rot, control, Fusarium solani, Rhizoctonia solani.**

I. INTRODUCTION

Green Bean (*Phaseolus vulgarus* L.) is one of the most important leguminous crops in Egypt. Root rot disease caused by *Fusarium solani* and *Rhizoctonia solani* is a serious and persistent diseases problem of bean plants during growing season [1,2,3]. There is a growing need to develop alternative approaches for controlling plant diseases. Botanical plants made into powder were widely used by ancient civilizations to improve the palatability of foods and beverages as well as for their preserving characteristics. Many plants are used as insecticides, molluscicides and rodenticides [4,5,6,7].The use of plants or plant products as fungicides is of a great importance and needs more attention [8]. Various plant products like gum, oil, resins etc. are used as fungicidal agents [4,9]. The biological control of plant diseases may have minimum adverse effect on physiological processes of plant and less environmental hazards [10]. Biological fungicides, being plant products are easily convertible into a common organic material and may create fewer health problems compared synthetic alternatives. The garlic extract, oil [11] and juice [12] showed fungicidal properties against *Fusarium* of watermelon. Bio-control of fungal diseases is not common and investigations are required to find out suitable plants that can be used to control pathogenic fungi. Spices are recognized to prevent the microbial deterioration of food. Antimicrobial activity of spices depends on several factors, *i.e.* kind of spice, composition and concentration of spice and its occurrence level, substrate composition and processing conditions and storage [13,14]. Several scientific reports describe the inhibitory effect of spices on a variety of microorganisms, although considerable variation of resistance of different microorganisms to a given spice and of the same microorganisms to different spices has been observed [15]. Also, [16] emphasized a possible use of spices and their derivatives like alternatives for inclusion in a new perspective of food conservation called "natural antimicrobial system", which could use the synergistic effect of antimicrobial compounds from animal, plant and/or of microbial origin, in order to create an inhospitable environment for microbial survival in foods. Furthermore, the incidence of several soil-borne plant pathogens have also been reduced by using composts made of different raw materials [17,18,19,20,21]. Since [22] first suggested compost could be used as a peat substitute to control root pathogens, bio-control research has increasingly focused on developing the right combination of composts and antagonistic microbes.

The objective of the research work performed was to study the antifungal activity of some botanical plant powders alone or combined with compost against root rot disease incidence of green bean under field conditions.

II. MATERIALS AND METHODS

Source of plant materials, bean seeds, compost and pathogenic fungi

The botanical plant materials, *e.g*. chilli pods (*Capsicum annuum*), Cabbage leaves (*Brassica oleracea*) and Eucalyptus leaves (*Eucalyptus obliqua*) kindly obtained from Medicinal and Aromatic Plants Research Department, NRC, Egypt, were washed with distilled water and dried in shade. The dried plant materials were then finely grinded to powder.

Commercial compost (a mixture of different aromatic plants, sugar beet and sugarcane) was purchased from

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Egyptian Company for Solid Waste Utilization (ECARU), Giza, Egypt.

Bean seeds cv. Giza 3 were obtained from Vegetable Crops Research Dept., Agricultural Research Centre, Giza, Egypt.

Fusarium solani and Rhizoctonia solani were isolated from bean plants. These plants showed typical root rot disease symptoms and collected at the previous growing season from grown at the Experimental Research Station of National Research Centre at El-Noubaria region, Behera Governorate, Egypt.

Laboratory experiment

Fifty grams of each of above mentioned botanical plant materials in powder form was homogenized by laboratory blender in 200 ml of ethanol (96%) and distilled water (20:80, v:v) for 10 min, then left in dark glass bottles for 72 h for tissue maceration. The extracts were filtered through thin cheesecloth sheets. The final extracts were collected separately in other dark glass bottles and exposed to 60°C in water bath for 30 min for ethanol evaporation. The collected extracts were then stored in a refrigerator at 5°C until needed. Furthermore, the powdered botanical plant materials were added to sterilized PDA flasks before solidifying to obtain the proposed concentrations of 2, 4 and 8% (w/v), while the botanical plant extracts were added to other PDA flasks to obtain the same concentrations of 2, 4 and 8% (v:v). A bactericide (chloramphenicol, 0.10 mg/l) was added to the media to avoid bacterial contamination. Amended media were poured into 9 cm diameter Petri dishes, and another set of untreated PDA medium was used as control. All plates were inoculated individually with 0.5 cm diameter discs of the tested Fusarium solani or Rhizoctonia solani cultures. After seven days of incubation, at 25 ± 2°C, orthogonal measurements of colonies were taken using the control plates as a reference. The reduction in fungal growth was calculated in relation to control treatment.

Field experiment

The field experiment carried out at the Experimental Research Station of National Research Centre at El-Noubaria region, Behera Governorate, Egypt.

Under field conditions, the efficacy of some botanical plant powders alone or combined with compost against root rot disease incidence of green bean under field conditions. The field trail conducted in 40 plots, each 12m², established in naturally heavily infested soil with root rot pathogens. The evaluated treatments were applied as follows:

(A) Single treatments
1-Compost
2-Powder of chilli pods (*Capsicum annuum*)
3-Powder of Cabbage leaves (*Brassica oleracea*)
4-Powder of Eucalyptus leaves (*Eucalyptus obliqua*)
5-Fungicide (Rizolex-T 50% at the rate of 3g/Kg seeds as seed dressing)

(B) Combined treatments
6-Compost + Powder of chilli pods
7-Compost + Powder of Cabbage leaves
8-Compost + Powder of Eucalyptus leaves
9- Untreated control

The botanical plants powder was added to compost at rate of 1.0 % (w/w) meanwhile, Compost treatments were applied at rate of 20 m^3/feddan (4200 m^2). Compost and/or botanical plants powder were incorporated in the top of 20 cm of the soil surface at planting row sites considering relevant treatments (Abdel-Kader 1997). All treatments were applied in Complete Randomized Block Design with five replicates (plots) for each particular treatment. The Bean seeds cv. Giza 3 were sown as 3 seeds per hill and 30cm distance between hills.

Disease assessment

Percentage of root rot incidence at the pre-emergence stage was calculated as the number of absent emerged seedlings in relative to the total number of sown seeds. Meanwhile, percentage of post- emergence root rot was calculated as the number of bean plants showing disease symptoms in relative to the total number of emerged seedlings. Undeveloped, germinated seeds as well as diseased plants were picked up from the soil, washed and sterilized with 3% sodium hypochlorite, then subjected to re-isolation trail in order to identify the causal pathogens. Plant roots showing rot lesions in addition to the visual root rot symptoms on the shoot system were considered diseased plants.

The percentages of pre-emergence root rot incidence was calculated after 20 days from sowing, meanwhile post-emergence bean root rot incidence was recorded periodically every 10 days starting from 20 up to 60 days of plant growth. Determination of accumulated bean yield per m2 was calculated. The average percentage of disease incidence was calculated and presented.

Statistical analysis

Tukey test for multiple comparisons among means was utilized **[23]**.

III. RESULTS AND DISCUSSION

Laboratory experiment

Results presented in Table (1) show the response of Bean root rot fungi to the inhibitory effect of tested extracted and botanical plants. Radial growth of tested fungi decreased significantly with increasing concentrations of added powder of botanicals. High significant inhibitory effect on radial fungal growth was observed at different concentrations of Eucalyptus leaves followed by Cabbage leaves in powder form. Meanwhile, Chili pods showed a low significant inhibitory effect on tested fungi. On the other hand, a similar pattern of

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reduction in radial fungal growth at increasing concentrations of botanical extracts was observed. Extracts of botanical plants at the same concentrations of 2, 4 and 8% indicate a superior inhibitory effect on radial fungal growth when compared with powdered ones.

Concentration of 6% of spice extracts caused a complete growth inhibition of the tested fungi. Concentration of 8% was chosen to show the differences between the activity of tested botanical extracts and powder against various pathogenic fungi, as illustrated in Fig. (1).

It is also observed that extracted Eucalyptus leaves have a highest effect on the radial growth of *F. solani* and *R. solani* that caused reduction in their growth recorded as 44.0 and 46.0%, respectively. Meanwhile, 39.6 and 36.3% reduction in the fungal growth was recorded when the growth media was amended with Eucalyptus leaves powder at concentration of 8%.

Table (1) Effect of different amounts of extracted and powdered botanicals on linear fungal growth *in vitro*

Treatment	Con. (%)	Fungal growth (mm)	
		F. solani	*R. solani*
Botanical plant extract			
Chili pods	2	83.4 b	86.3 b
Cabbage leaves	4	76.8 c	79.5 c
Eucalyptus leaves	8	50.4 e	48.6 e
Botanical plant powder			
Chili pods	2	77.8 c	78.9 c
Cabbage leaves	4	68.7 d	67.3 d
Eucalyptus leaves	8	54.3 e	57.3 e
Control		90.0 a	90.0 a

Figures with the same letters are not significant ($F \leq 5\%$)

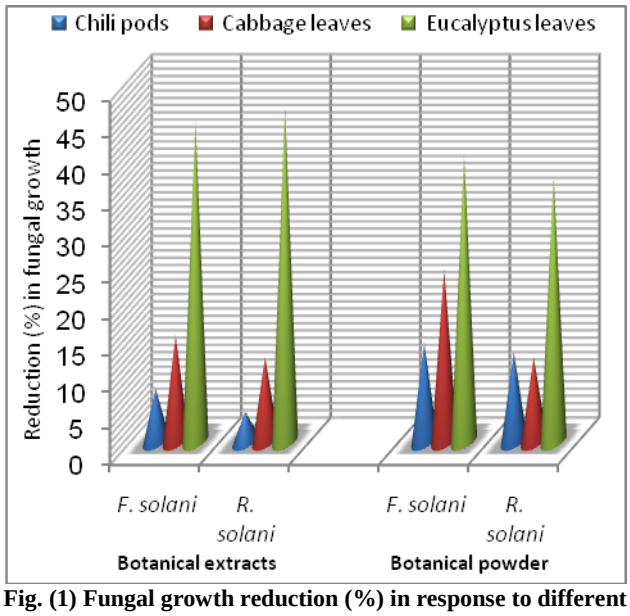

Fig. (1) Fungal growth reduction (%) in response to different amounts of extracted and powdered botanicals *in vitro*

Field experiment

Powdered chilli pods, powdered cabbage leaves and powdered eucalyptus leaves alone or in combination with compost were applied under field conditions. Results in Table (2) indicate that all treatments significantly reduced the root rot disease of bean plants comparing with untreated check control treatment. The obtained results in the present study indicate that higher significant reduction in disease incidence was observed for combined treatment between botanicals powder with compost than that of their individual application. Data in Table (2) showed that the highest effective treatment for reducing root rot incidence was observed in eucalyptus leaves combined with compost followed by Eucalyptus leaves treatment alone, they recorded disease incidence as 12.0, 10.0% and 17.0, 15.0% at pre-, and post-emergence growth stages of bean plants, in respective order. These treatments are followed by cabbage leaves and chili pods with or without compost, respectively. Root rot incidence in control treatment recorded as 42.0 and 51.0%, meanwhile the fungicide treatment recorded 14.0 and 11.0% at pre-, and post-emergence growth stages of bean plants, in respective order.

Furthermore, reduction in root rot disease incidence in response to botanicals applied as single treatments or combined with compost was illustrated in Fig. (2). The most effective treatments were combined treatments between compost and Eucalyptus or cabbage which reduced the disease incidence more than 71.4 and 79.4% followed by 57.1% at chili pods powder for pre-, and post-emergence root rot stages, respectively. Similar effect in a lesser extend was observed concerning single treatments. This effect was recorded as reduction in disease incidence at pre-, and post-emergence stages recorded as (42.9, 60.1%), (57.1, 70.5%) and (59.6, 70.5%) in ascending order relevant to Chili pods, Cabbage leaves and Eucalyptus leaves treatments. Individual compost treatment could reduce disease incidence as (40.5, 58.8%) comparing with (66.7, 78.4%) at fungicide treatment.

Table (2) Effect of different botanical plants powder on root rot diseases of bean plants under field conditions

Treatment	Root rot disease %	
	Pre-emergence	Post-emergence
Single treatments		
Chili pods	24.0 b	20.0 b
Cabbage leaves	18.0 c	15.0 c
Eucalyptus leaves	17.0 c	15.0 c
Compost	25.0 b	21.0 b
Combined treatments		
Compost + chili pods	18.0 c	16.0 c
Compost + cabbage leaves	12.0 d	10.5 d
Compost + Eucalyptus leaves	12.0 d	10.0 d

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Fungicide (Rizolex-T)	14.0 d	11.0 d
Control	42.0 a	51.0 a

Figures with the same letters are not significant ($F \leq 5\%$)

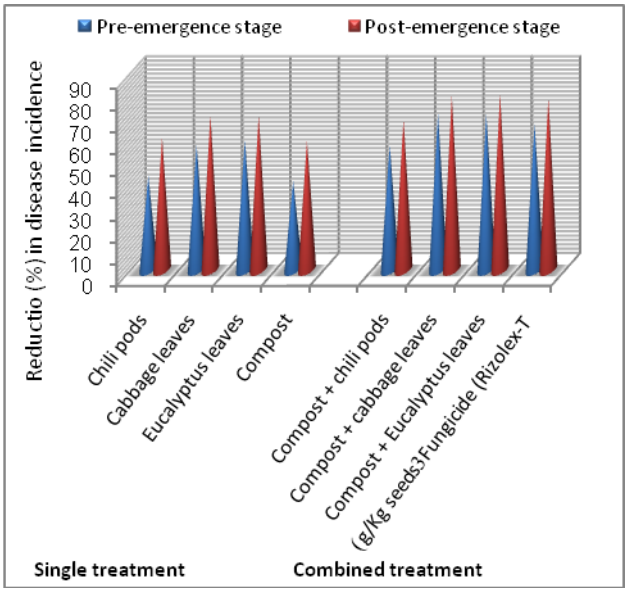

Fig. (2) Reduction (%) in root rot diseases of bean plants in response to single or combined botanical plants powder and compost under field conditions

These results are confirmed with other investigators. They reported that incidence of several soil-borne plant pathogens have also been reduced by using composts made of different raw materials (Borrero *et al.* 2004; Cotxarrera *et al.* 2002; Hoitink and Boehm 1999; Hoitink and Fahy 1986; Lumsden *et al.* 1983). Under field conditions, results indicated that the pronounced treatments were combined treatments between compost and Eucalyptus or cabbage which reduced the disease incidence more than 70% at pre-, and post-emergence growth stages. Furthermore, many soil-borne pathogens can be reduced by application of composts made of different raw materials [17,18,19,20,21] and matured composts can sustain biological control agents [24]. In this respect [25] reported that significant biocontrol of rhizoctonia disease of cucumber could be obtained by an application of a mixture of agricultural compost and *Trichoderma* spp. There has been constant increasing of alternative search on efficient compounds for plant disease control, aiming at partial or total replacement of antimicrobial chemicals. Systematic investigation of biological interactions between microorganisms and plant products has been a valuable source of new and effective antimicrobial substances, which could have act differently on/in the microbial cell compared to other conventional antimicrobials. Plants synthesize secondary metabolites and some of them as well as their derivatives have antimicrobial. Among these secondary metabolites are found alkaloids, flavonoids, isoflavonoids, tanins, cumarins, glycosides, terpens and phenolic compounds [26].

In agricultural studies, these compounds have broad-spectrum activities against fungi, nematodes, and insects [27,28,29]. Botanical and spices powders offer a promising alternative for food safety and plant protection. Inhibitory activity of botanical and spices and their derivatives on the growth of bacteria, yeasts, fungi and microbial toxin synthesis has been reported [30,31]. Regarding inhibitory effect of botanical and spice plants, there were many investigators conducted. It was reported that various powder concentrations of mint, sage, bay, anise and red pepper significantly inhibited the growth of *Aspergillus parasiticus* [32]. Chilli, coriander, pepper, cumin and asafoetida were found to inhibit food spoilage moulds [33]. Several scientific reports describe the inhibitory effect of spices on a variety of microorganisms, although a considerable variation in resistance of different microorganisms to different spices has been observed [15]. The present study revealed that chilli, Eucalyptus and cabbage used for soil drench had significant efficacy against root rot incidence. They could protect bean against both pre- and post-emergence root rot up to 60 days when applied as soil drench alone or combined with compost. This might be attributed to a slow release of active components of plant powder when introduced into the soil. There are a number of plant-based antimicrobial constituents, including many essential oils, tannins, glycosides, and resins, that can be found in certain botanical plants. Specific examples include eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allyl isothiocyanate in mustard, eugenol and thymol in sage, and carvacrol (isothymol) and thymol in oregano [34]. It was reported that [35] peanut seed treatment with the test compounds (citral, eugenol, geraniol, limonene, and linalool) had no effect on the incidence of crown rot in peanut in *Aspergillus niger* infested soil. However, soil amendment with 0.25% (v/w) clove oil and cinnamon oil reduced pre-emergence rotting by 71 and 67% and post-emergence wilting by 58 and 55%, respectively. It has been demonstrated that some compounds derived from plants, such as thymol and palmarosa oil, provide disease control potential. In general, inhibitory action of natural products on moulds involves cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of interacellular and extracellular enzymes. These biological events could take place separately or concomitantly culminating with mycelia growth inhibition [36]. Also, it was reported that plant lytic enzymes act in the fungal cell wall causing breakage of b-1,3 glycan, b-1,6 glycan and chitin polymers [37]. Moreover, the mode by which microorganisms are inhibited by botanicals and their chemical compounds seem to involve different mechanisms. Also, it has been

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hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability and unavailability of vital intracellular constituents [38]. Reports have indicated that spices containing carvacrol, eugenol and thymol (phenolic compounds) had highest antibacterial performances [39]. Many authors emphasized that the antimicrobial effect of botanicals constituents are dependent on their hydrophobicity and partition in the microbial plasmatic membrane. The effect of specific ions due to their addition in/on plasmatic membrane had a great effect on the proton motive force, intracellular ATP content and overall activity of microbial cells, including turgor pressure control, solutes transport and metabolism regulation [40]. Hence, the objective of this study was to determine if botanical plants powder could provide effective control of some soil borne plant pathogens. Considering their attribute and broad-spectrum activities, successful development of such compounds as antifungal would not only provide a potent tool for control of bean root rot, but also could promise success in multipurpose biorational alternatives to conventional fungicides for the management of other plant diseases.

Promising applicable technique could be suggested in the light of the results obtained in the present study. The usage of botanical plants powder as soil drench combined with compost might be considered as safe, cheep and easily applied method for controlling soil borne plant pathogens taking in consideration the avoidance of environmental pollution and side effects of pesticide application.

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