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Antagonistic Yeast for Controlling Bean Root Rot Disease under Field Conditions

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Abstract*- The efficacy of the yeast Saccaromyces serivisae on the linear growth of Fusarium solani and Rhizoctonia solani the causal of bean root rot was evaluated in vitro. The results indicate that S. serivisae could inhibit the tested fungal growth in different degrees. The mycelium growth of F. solani and R. solani reduced by 18.6,39.3,60.7% and 28.5,51.8,67.6%, respectively when the population density of antagonistic S. serivisae increased as $1x10^3$, $1x10^6$ and $1x10^9$ in growth media. Under field conditions, all applied treatments were significantly reduced the incidence of root rot disease of bean comparing with untreated control at both pre-, and post emergence growth stages. Also, combined treatments showed superior effect against disease incidence comparing with single ones. At pre-emergence stage the lowest disease incidence was recorded as 12.0% at combined treatment of [Compost + yeast (seed and foliar spray)] followed by 18.0 and 22.0% at treatments of [Compost + Yeast (seed soaking)] and Compost + Yeast (seed soaking), respectively. Meanwhile, disease incidence was recorded as 24.0, 25.0 and 31.0% at treatments of [Compost (soil treatment)], [Yeast (seed soaking)] and [Yeast (foliar spray)], in respective order. Similar trend concerning root rot incidence was also observed at post-emergence growth stages. As for the obtained yield bean seeds treatment with S. serevisiae followed by foliar spray with the same components showed significant increase in the accumulated bean yield for plants grown in composted soil comparing with the other treatments.

***Index Terms*- Bean, compost, control, root rot, Saccaromyces serevisae, yeast.**

I. INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is one of the most important leguminous crops for local consumption and exportation purposes. Green pods are used for fresh meals and food industries, while plant wastes used as feeds. Bean is attacked by certain pathogenic fungi causing root-rot and leaf spot diseases which seriously affected both plant stand and yield production. Bean root-rot diseases caused by *Fusarium solani, Rhizoctonia solani, Macrophomina phaseolina* and *Sclerotium rolfsii* were reported by several investigators [1,2,3]. Root-rot disease appears during the growing season at both pre- and post-emergence stages of plant growth [4]. Plant diseases control mainly depends on fungicides, although their applications cause hazards to environmental pollution [5].

As control measures, some cultural practices, *i.e.* crop rotation, sowing date, fertilizers and irrigation were tried by many investigators but they failed to provide satisfactory control against plant pathogens [6]. Biological control using antagonistic microorganisms proved to be successful for controlling various plant diseases, however it still not easy and costly in application [7]. Thus, there is a growing need to develop alternative approaches for controlling plant diseases one approach that is being actively pursued involves the use of bioactive substances released from biocontrol agents. Biological control has been advanced as an alternative to synthetic fungicides and considerable success in laboratory and pilot scale tests has been realized utilizing antagonistic microorganisms to control postharvest diseases. Several antagonistic yeasts and bacteria have been isolated and shown to have a broad spectrum of activity against a number of postharvest pathogens on a variety of fruit [8].

Recently, a number of investigations have demonstrated the effectiveness of composts of various origins in suppressing soil-borne plant pathogens [9,10,11,12], and their application to soil has been proposed to control many different diseases. In this regards, several researchers have been recorded that bio compost application as soil amendment could suppress diseases caused by *R. solani* and Fusarium spp. on many economic crops [13,14].

Therefore, this study was undertaken to assess the effect of yeast *S. serisisae* on the growth of bean root-rot pathogens under laboratory conditions, to evaluate its efficacy for controlling such diseases when applied as seed dressing and/or foliar spray in soil amended with compost under field conditions.

II. MATERIALS AND METHODS

Laboratory studies

Bean root rot pathogenic fungi, *F. solani* and *R. solani* as well as antagonistic yeast *S. serevisiae* were kindly obtained from Plant Pathology Dept., National Research Centre, Egypt. These microorganisms proved their high pathogenic and antagonistic ability in previous work at the same Department.

The inhibitory effect of the antagonistic yeast, *Saccharomyces cerevisiae* against the linear growth of bean root rot pathogenic fungi was evaluated using the modified dual culture technique [15]. Abundant fungal and yeast growth was first prepared. Fifty mL of the yeast isolate was grown for 48 h on NYDB broth medium [8 g of nutrient medium (Difco Laboratories, Detroit, MI), 5 g of yeast extract, and 10 g of dextrose in 1 liter of water] and incubated in a rotary shaker at 200 rpm for 24 h at 28±2ºC. The yeast 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 yeast cells in the suspensions were adjusted to $1x\ 10^3$, $1x10^6$ and $1x10^9$ cells per milliliter with the aid of haemocetometer slide. For fungal growth, a 5-mm disk of each tested fungi was transferred to the centre of a PDA plate then incubated for seven days at $25±1^0$C.

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In vitro antagonistic studies between biocontrol yeast and pathogenic fungi were performed on PDA medium in 9-cm-diameter Petri dishes. A loop of each yeast concentration was streaked onto the PDA, 10mm from the edge of the Petri dish. A 5-mm disk of each tested pathogenic fungal growth culture was placed on the opposite side of the dish at the same distance. The control treatment was inoculated with a culture disk of either a pathogenic or antagonistic culture alone at the same conditions. Both experimental and control dishes were assigned to a completely randomized design, with five replicates per treatment. All inoculated Petri dishes were incubated at 25±1^{o}C and the fungal growth diameter away from and towards the antagonist agent was measured after the pathogenic fungal growth in the control treatment had reached the edge of the Petri dish. This test was repeated three times and the inhibition was calculated as linear growth in each treatment and the reduction percentage in colony diameter growth compared with the control treatment was calculated.

Field experiment

Field experiment was carried out in Research and Experimental Station of National Research Centre at El-Nubaria region El-Beheira Governorate during 2013 growing season in order to evaluate the efficacy of yeast, *S. serevisae* application individually or in combination with compost for controlling root-rot disease of bean. This field is well known as naturally heavily infested with bean root-rot pathogens. The fungicide Rizolex-T was used in this study as a reference for evaluating yeast treatments. The evaluated treatments were applied as follows:

(A) Single treatments

1. Yeast, *S. serevisae* as seed soaking.
2. Yeast, *S. serevisae* as foliar spray.
3. Compost as soil drench at the rate of 5Kg/m^2
4. Fungicide (Rizolex-T 50% at the rate of 3g/Kg seeds as seed dressing)

(B) Combined treatments

5. Compost +Yeast (seed soaking)
6. Compost +Yeast (foliar spray)
7. Compost + yeast (seed and foliar spray)
8. Control

Bean seeds were soaked in 1x10^9 concentration of yeast population. After 15 days of seedling emergence, certain plants (at 2-3 true leaves age) were sprayed with cell suspension at the same concentration. Rizolsx-T was applied as seed dressing at the recommended dose (3 g/kg seeds).

A field experiment consisted of plots (7x 6 m^2) each comprised of 12 rows and 30 holes / row which were arranged in a completely randomized block design with five plots as replicates for each particular treatment as well as untreated check treatment. Bean seeds cv. Giza 3 were sown in all treatments at the rate of 3 seeds/ hole. All plots received the traditional agricultural practices. Average percent of root rot infection at pre-emergence stage was recorded after 15 days of sowing. At post-emergence stage bean plants showing root rot symptoms were recorded and the disease incidence was calculated after 15 days of seedlings spray. Yield accumulated was determined as fresh pods (kg/m^2) for each particular treatment at the end of growing season. The increase of obtained yield in relative to comparison treatment was also calculated.

Statistical analysis

Tukey test for multiple comparisons among means was utilized **[**16**]**.

III. RESULTS AND DISCUSSION

Laboratory studies

The efficacy of yeast isolate was evaluated against the growth of bean root rot fungi *in vitro*. Results in Table (1) showed that *S. cerevisiae* has significantly the highest inhibitory effect on the growth of the pathogenic tested fungi when used at concentration of 1x10^9 followed by the population density of 1x10^6 and 1x10^3, respectively. The linear growth of *F. solani* and *R. solani* was recorded as 73.2, 64.3; 54.6, 43.3 and 35.3, 29.1 mm against the yeast, *S. cerevisiae* at population density of 1x10^6 and 1x10^3, respectively.

Table (1) Effect of yeast *Saccharomyces cerevisiae* on the growth of bean root rot pathogenic fungi *in vitro*

Tested yeast	Concentration (cfu)	Fungal growth (mm)	
		F. solani	R. solani
S. cerevisiae	1x10^3	73.2 b	64.3 c
	1x10^6	54.6 d	43.3 e
	1x10^9	35.3 f	29.1 g
Control		90 a	90 a

Figures with the same letter are not significantly different (P≤ 0.05)

Furthermore, the high reduction in the growth (Fig. 1) of *F. solani* and *R. solani* was recorded as 60.7 and 67.6%, respectively, when the yeast *S. cerevisiae* was inoculated at concentration of 1x10^9 in the growth medium. Meanwhile, *S. cerevisiae* at concentration of 1x10^3 showed the lowest inhibitory effect which recorded as 18.6 and 28.5%, respectively. Similar results are also reported by [17]. They found that mixture containing isolates of the backing yeast *S. cerevisiae mixture* (CBY) proved itself to have the highest inhibitory effect on the growth of the pathogenic tested fungi followed by the two other yeast isolates *S. cerevisiae* and *C. tenuis*. They added that under storage conditions, application of carnauba wax formula containing either *S. cerevisiae* or *S. cerevisiae* (CBY) combined with peppermint oil (1%) had more superior effect for reducing gray mould, soft rot and black rot incidence as well as disease development of tomato fruits, reaching up to 100% under artificial inoculation of decay pathogenic fungi, *Botrytis cinerea*, *Rhizopus stolonifer* and *Alternaria alternate.*

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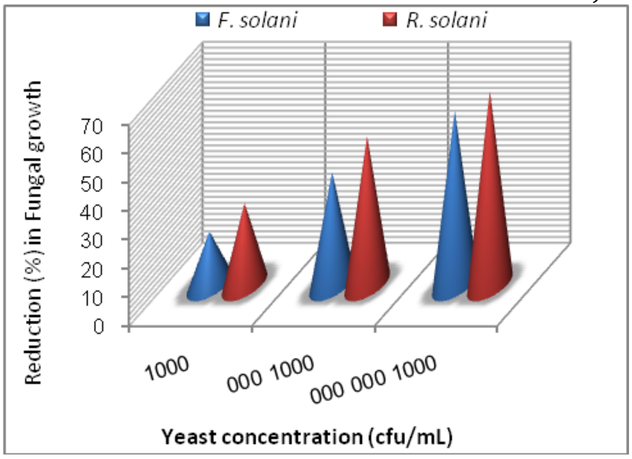

Fig. (1) Reduction in the growth of bean root rot pathogenic fungi in response to the antagonistic yeast Saccharomyces cerevisiae

Also, it was reported that Yeasts such as *Pichia guilliermondii* [18] and *Cryptoccocus laurentii*, a yeast that occurs naturally on apple leaves, buds, and fruit [19] were the first to be applied for control of postharvest decay on fruit. Although there is no doubt that biocontrols are effective, they do not always give consistent results. This could be because biocontrol efficacy is so directly affected by the amount of pathogen inoculums present or antagonistic ability of the bio agent itself [20]. Biological control has been advanced as an alternative to synthetic fungicides and considerable success in laboratory and pilot scale tests has been realized utilizing antagonistic microorganisms to control postharvest diseases. Several antagonistic yeasts and bacteria have been isolated and shown to have a broad spectrum of activity against a number of postharvest pathogens on a variety of fruit [8].

Field experiment

Different field approaches of the yeast S. serevisae as seed soaking, foliar spray either individually or in combination with compost for controlling root-rot disease of bean were carried out. Data in Table (2) showed that all applied treatments were significantly reduced the incidence of root rot disease of bean comparing with untreated control at both pre-, and post emergence growth stages. Also, combined treatments showed superior effect against disease incidence comparing with single ones. At pre-emergence stage the lowest disease incidence was recorded as 12.0% at combined treatment of [Compost + yeast (seed and foliar spray)] followed by 18.0 and 22.0% at treatments of [Compost + Yeast (seed soaking)] and Compost + Yeast (seed soaking), respectively. Meanwhile, disease incidence was recorded as 24.0, 25.0 and 31.0% at treatments of [Compost (soil treatment)], [Yeast (seed soaking)] and [Yeast (foliar spray)], in respective order. Similar trend concerning root rot incidence was also observed at post-emergence growth stages. On the other hand, disease incidence was recorded as 28.0 and 30.0% at the fungicide Rizolex-T treatment comparing with 45.0 and 53.0% in control treatment.

Table (2) Effect of yeast application on root rot disease incidence of bean plants under field conditions

Treatment	Root rot disease %	
	Pre-emergence	Post-emergence
Single treatments		
Yeast (seed soaking)	25.0 c	31.0 c
Yeast (foliar spray)	31.0 b	36.0 b
Compost (soil treatment)	24.0 c	24.0 d
Fungicide (Rizolex-T 50%, 3g/Kg seeds)	28.0 e	30.0 f
Combined treatments		
Compost +Yeast (seed soaking)	18.0 d	20.0 e
Compost +Yeast (foliar spray)	22.0 cd	25.0 d
Compost + yeast (seed and foliar spray)	12.0 e	15.0 f
Control	45.0 a	53.0 a

Figures with the same letter are not significantly different (P≤ 0.05)

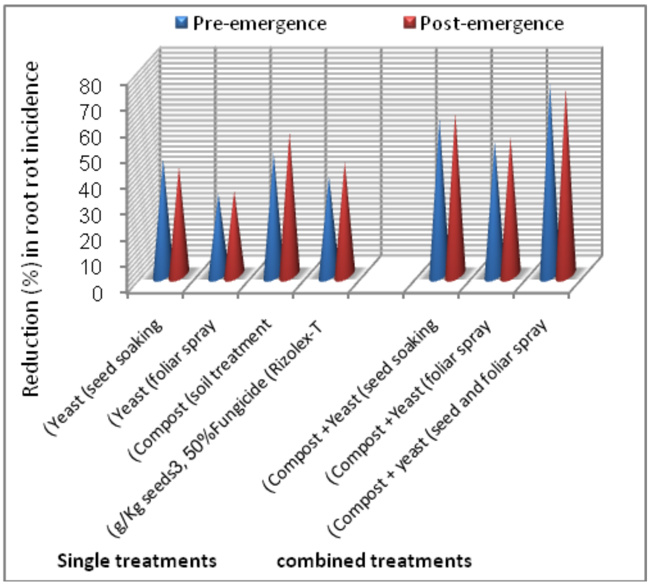

Fig. (2) Reduction in root rot incidence of bean in response to different application of yeast

Illustrated data (Fig. 2) showed that the highest reduction in root rot incidence was recorded as 73.3, 71.7%; 51.1, 52.8% and 60.0, 52.3% for pre-, and post-emergence stages at combined treatments of [Compost + yeast (seed and foliar spray)], [Compost +Yeast (foliar spray)] and [Compost +Yeast (seed soaking)], respectively. Also, at single treatments of [Compost (soil treatment)], [Yeast (seed soaking)] and [Yeast (foliar spray)] reduced the disease incidence by 46.7, 54.7%; 44.4, 41.5% and 31.1, 32.1%, in respective order. The obtained results indicate that compost could enhance the efficacy of the yeast for suppressing the invasion of bean roots by pathogenic fungi. In this concern, it was suggested that to enhance biocontrol activity of antagonists against fungal pathogens, certain strategies, such as adding calcium salts, carbohydrates, amino acids and other nitrogen compounds to biocontrol treatments, were suggested [21,22]. Biocontrol involves harnessing disease-suppressive microorganisms to improve plant health. Disease

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suppression by biocontrol agents is the sustained manifestation of interactions among the plant, the pathogen, the biocontrol agent, the microbial community on and around the plant, and the physical environment. One of the main procedures used in the research of biologically active substances is using compost for controlling soil borne plant pathogens and increasing the soil fertility as well. The suppressive activity of different compost types toward several plant pathogens is well documented in the recent review by [23]. Further, the investigation on the biological activity of these materials on antagonistic soil-borne fungi is of great interest, in that their contribution to biological control should be safeguarded. Very few information is reported in the literature on the suppressive effects of soil native humic substances (HS) and HS-like fractions on phytopathogenic fungi and also no information is reported on antagonistic soil-borne fungi. Although some information is available on the mechanisms responsible for the suppressive action of compost and compost extracts on plant pathogenic fungi [23] very limited information exists on the relationship between chemical properties of HS and HS-like fractions and fungal suppressive capacity. A number of investigations have demonstrated the effectiveness of composts of various origins in suppressing soil-borne plant pathogens [9,10,11,12], and their application to soil has been proposed to control many different diseases. The use of organic agricultural wastes in this respect can be an advantageous both in soil fertility, recycling of agricultural residues and could provide a powerful tool for management of plant diseases. It has been reported that several composts and/or composts fortified with bio control agent used as soil amendments reduced pathogens propagules density and protected plants from soil borne plant pathogens [24,25,26].

Reduction in bean infection with root-rot disease, indicate increase in the numbers of healthy plants, resulted in high quantity of produced bean yield (Table, 3 and Fig. 3). Bean seeds treatment with *S. serevisiae* followed by foliar spray with the same components showed significant increase in the accumulated bean yield for plants grown in composted soil comparing with the other treatments. Data also showed that all combined treatments produced significantly higher accumulated yield than individual treatment. The highest obtained yield was recorded as 38.7 Kg/plot at treatment of [Compost + yeast (seed and foliar spray)] followed by 35.3 Kg/plot at treatment of [Compost +Yeast (foliar spray)]. Meanwhile the lowest increase in bean yield was recorded as 21.2 and 20.7% at treatments of [Compost +Yeast (seed soaking)] and [Yeast (seed soaking)], respectively comparing with 2.4% in fungicide treatment. In this concern, some investigators indicated that addition of organic manures as opposed chemical fertilizers increased vegetative growth characters, yield and fruit quality of vegetable crops [27, 28]. On squash, [29] showed that plants had increased yields when planted in municipal solid waste compost amended soil in spite of application of NPK fertilizers at recommended rates. On pepper, [30] stated that early and total yields of all organic sources were significantly higher than that of chemical fertilizer. In the same line on cucumber, [31] found that organic treatment (compost) produced significantly greater early yield (1.85 kg/m2) and total yield (4.49 kg/m2) than chemical treatment which produced 1.38 kg/m^2 and 3.51 kg/m^2 for early and total yields, respectively.

Table (3) Bean yield in response to treatment with different yeast application and compost under field conditions

Treatment	Accumulated bean yield (Kg/plot)
Single treatments	
Yeast (seed soaking)	27.3 ab
Yeast (foliar spray)	28.3 ab
Compost (soil treatment)	30.3 b
Fungicide (Rizolex-T 50%, 3g/Kg seeds)	25.2 a
Combined treatments	
Compost +Yeast (seed soaking)	29.8 ab
Compost +Yeast (foliar spray)	35.3 bc
Compost + yeast (seed and foliar spray)	38.7 c
Control	22.6 a

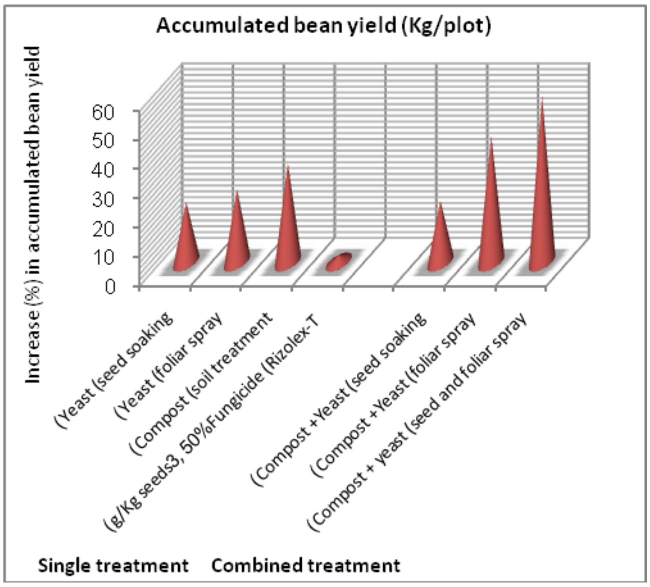

Fig. (3) Increase in bean yield in response to treatment with different yeast application and compost under field conditions

In the light of the present results, it could be suggested that combined treatments between yeast *S. serevisiae* as seed and foliar spraying for plant grown in soil amended with compost might be used commercially for controlling such soil borne diseases replacing fungicidal treatments.

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