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Effect of soil amendment with activated yeasts on controlling *Fusarium* and *Verticillium* wilt and growth characters of pepper

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Abstract

Wilt disease of pepper caused by *Fusarium oxysporum* f. sp. *capsici* (*FOC*) and *Verticillium dahliae* (*VD*) is serious worldwide disease and cause great problems in all pepper production areas of the world. Six isolates of *FOC* and three isolates of *VD* were obtained from naturally diseased pepper plants from different localities of Assiut Governorate, Egypt. These isolates had the ability to cause wilt symptoms either as a vascular browning or as a foliar yellowing with different degrees. Also, there was correlation between the degree of vascular browning and foliar yellowing which has occurred with the same tested isolate. The efficacy of activated yeasts of *Saccharomycess cerevisiae* (dry and soft) for controlling the wilt disease caused by *FOC* and *VD* was studied. In greenhouse experiments, application of dry and soft activated yeast at the concentrations of 4, 6 and 8 gL^{-1} into infested soil with *FOC* and *VD* one and three weeks after seedlings transplanting significantly reduced the disease severity of *Fusarium* and *Verticillium* wilts. Likewise, concentration of 6 gL^{-1} of yeasts was the most effective ones. Data indicated that the soft yeast treatment exhibited the highest reduction in the disease severity compared to other treatments in both tested seasons (2014 and 2015). Yeasts treatments significantly enhanced stem length and diameter of pepper plants compared to untreated plants.

Key words: Pepper, *Fusarium* wilt, *Verticillium* wilt, yeasts, biofertilizers.

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Introduction

Pepper (*Capsicum annuum* L.) is one of the most important vegetables crops in Egypt and the world, which has major pathological problems. Among the limiting factors to its production in the world are *Fusarium* and *Verticillium* wilt diseases. The *Fusarium* wilt disease, caused by the soil-borne fungus, *F. oxysporum* f. sp. *capsici* is the most important disease on pepper plants that reduces growth, fruit yield, and quality, threatening pepper production (Wongpia & Lomthaisong, 2010). *F. oxysporum* is difficult to control as it survives in field soil for several years. Commercial fungicides used to control outbreaks of the Fusarium wilt disease can be an environmental hazard (Dubey et al., 2007). Moreover, *F. oxysporum* species is able to detoxify fungicides by biological conversion causing fungicide resistance (Dekker, 1976). *Verticillium* wilt disease causes severe economic losses in many crops (Fradin & Thomma, 2006). Verticillium wilt of pepper is mainly caused by *V. dahliae* Klebahn. This pathogen alters physiology of infected plants, accelerates their senescence and reduces yield. The decrease in photosynthesis and consequently, in the supply of carbohydrates to forming fruits, together with a premature fall of flowers are related to the limited fruit yield observed in diseased plants (Goicoechea, 2006). It is well known that *V. dahliae* reduces growth, fruit yield and quality of pepper (Garcia-Mina et al., 1996). Control of *V. dahliae* is especially difficult due to its ability to survive in field soil for several years as various types of mycelia, clusters of hyaline cells and microsclerotia (Schnathorst, 1981). *S. cerevisiae* is considered a new promising plant growth promoting yeast for different crops (Agamy et al., 2013). It became in the last few decade a positive alternative to chemical fertilizers safely used for human, animal and environment (Omran, 2000). Application of yeasts as biocontrol agents acts as a new trend against different pathogens. Potential use of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters were recent investigated by El- Tarabily and Sivasithamparam (2006). The fungal activities of *Rhizoctonia solani* to infect sugar beet plants were well suppressed by using different yeasts (El-Tarabily, 2004). In this study, we aimed to control *Fusarium* and *Verticillium* wilt diseases of sweet pepper by activated yeasts and study also their effect on some growth characters parameters.

Materials and methods

Isolation and identification of the causal pathogens: Diseased sweet pepper plants, showing wilt symptoms were collected from different localities of Assiut Governorate, Egypt, during the 2013 growing season. They were cut in small pieces, thoroughly washed with tap water, surface sterilized for two minutes with 2% sodium hypochlorite solution, then rinsed several times in sterilized distilled water and dried between folds of sterilized filter papers. Then the pieces were plated onto Potato Dextrose Agar (PDA) medium and incubated at 25°C. After 4-5 days incubation period, developed fungal colonies were purified

by hyphal tip and single spore isolation techniques. Identification of the fungal isolates of *Fusarium* and *Verticillium* was carried out by using the morphological characteristics of mycelia and spores as described by Nelson et al., (1983); Isaac (1955). Pure cultures of all identified fungi were maintained at 5°C PDA slants until use.

Pathogenicity tests: Pathogenic capability of pathogenic fungi [six isolates of *F. oxysporum* f. sp. *capsici* (*FOC*) and three isolates of *V. dahlia*e (*VD*)] was carried out on sweet pepper plants (cv. Norhan) under greenhouse conditions, at the farm of Faculty of Agriculture, Al-Azhar University (Assiut Branch), during the 2013 growing season. Inocula of isolated fungi were grown on barley medium (150g barley + 50 g clean sand + 4 g glucose + 0.2 g yeast extract + 200 mL water) (Sallam Nashwa et al., 2014) in 500-mL flasks and incubated at 25±2°C for 15 days. Each bottle was inoculated with two disks (5 mm diameter) taken from the margin of a one-week-old culture of the isolates grown on PDA medium in Petri dishes. The bottles were then incubated at 25±2ºC in the dark for two weeks. The test was carried out using sterilized plastic bags (30 cm in diam) containing sterilized clay-sand soil, sterilization of plastic bags and soil was carried out by using 5% formaldehyde solution. The content of bottles was thoroughly mixed in plastic container and used as a source of inoculum. Inoculum of each isolate of the tested fungi was added at a rate of 1 and 2% to the plastic bags soil (w/w), one week before sowing, mixed well whit the soil and then thoroughly irrigated. Three pepper seedlings were planted into each infested plastic bag, and the culture practice were carried out as usual, after 45 days from planting the degree of disease was estimated to vascular browning and foliar yellowing. Disease index of vascular browning was determined by estimating the internal discoloration (browning) area in vascular bundle by making longitudinal sector of root according to the scale described by Gothoskar et al., (1953)**.** Where, 0= no brown discoloration in vascular bundle of root, 1= 0- < 25 of vascular root bundles are brown, 2= 25- < 50 of vascular root bundles are brown, 3= 50- < 75 of vascular root bundles are brown and 4= 75-100% of vascular root bundles are brown. For calculating the percentage of foliar yellowing indices the following formula was used.

$$\%\ Vascular\ browning = \frac{\text{Sum of vascular browning values}}{4 \times \text{total number of plants}} \times 100$$

$$\%\ Foliar\ yellowing = \frac{\text{Sum of foliar yellowing values}}{4 \times \text{total number of leaflets}} \times 100$$

Disease severity index (DSI) of foliar yellowing was scored at 45 days after inoculation based on the modified disease severity scale of Silva & Bettiol (2005). They were as follows: 1= no symptom; 2= plant showed yellowing leaves 1<20%, 3= plant showed yellowing leaves 20<40%, 4= plant showed yellowing leaves 40<60%, 5= plant showed yellowing leaves 60<80% and 6= plant showed yellowing leaves 80-100% or died. For calculating the percentage of foliar yellowing indices the following formula was used.

Effect of soil treatment with activated bread yeasts on controlling pepper wilt disease under greenhouse conditions: Sterilized plastic bags (30 cm) that containing sterilized clay-sand soil (2:1w/w), were infested separately by *FOC* isolate No. 5 and *VD* No. 1 at the rate 1% (w/w), inocula were prepared by growing fungi on barely grain medium as described before, mixed whit the soil and irrigated well. After one week, three disinfected pepper seedlings (Norhan cv.) were transferred into each plastic bag. Three replicates were used for each treatment. Irrigation was done twice (one and three weeks after transplanting) with yeast suspensions at concentrations of 4, 6, and 8 gL^{-1}. Activated yeasts were obtained from Nobaryia yeast factory, El-Behera, Egypt and used in this study. Yeasts were well dissolved firstly in slight sugar solutions and cultivated for 12 h. Before application, the solutions were diluted to the required concentrations using sterile distilled water as described by Shalaby & El-Nady (2008). The fungicide (Moncut 25%) at concentrations 0.25, 0.50 and 1 gL^{-1} was used as comparison (positive control), inoculated with the pathogens and irrigated plastic bags with water only used as control. After 45 days from planting the disease index was recorded as a vascular browning and foliar yellowing percent as mentioned before. Some growth parameters including plant height (cm) and stem diameter (mm) were also estimated.

Statistical analysis: The obtained data were subjected to statistical analysis using MSTATC computer program (Michigan Statistical Program Version C). Least significant difference (LSD., $p = 0.05$) for comparison between means of treatments was used as mentioned by Gomez and Gomez (1984).

Results

Isolation and identification of the causal organism: Nine fungal isolates were isolated from naturally diseased sweet pepper plants showing wilt symptoms and identified as *Fusarium oxysporum* f.sp. *capcici* (six isolates) and of *Verticillium dahliae* (three isolates), based on the morphological characteristics (Nelson et al., 1983; Isaac, 1955).

Pathogenicity tests: Results presented in Table (1) indicated that all the tested isolates were pathogenic and caused vascular browning and foliar yellowing symptoms on sweet pepper plants (Norhan cv.). All the tested isolates causing typical wilt symptoms with different degrees of disease severity. *FOC* isolates were the most destructive and caused the highest percentage of infected plants. *FOC* (isolate No. 5) gave the highest percentage of disease severity at tested both rates (1 and 2%) of the inoculum density followed by isolate No. 1. Isolates No. 2 of *FOC* showed a moderate effect of disease severity. While isolates No. 6 and No. 4 of *FOC* gave the lowest disease incidence followed by isolates No. 1, 2 and isolate No. 3 of *VD* then the isolate No. 3 of *FOC*. Also, our data showed that, 2% of inoculum density of tested fungal isolates was the most effective on disease incidence.

Table 1: Pathogenic variations among *FOC* and *VD* isolates on Norhan cultivar of sweet pepper cultivar under greenhouse conditions.

Isolates	Inoculum Density %	Disease index (severity) %		
		Vascular browning (%)	Foliar yellowing (%)	Mean
FOC No. 1	1	37.37	39.24	38.31
	2	44.44	46.60	45.52
FOC No. 2	1	33.33	37.19	35.26
	2	40.47	45.18	42.83
FOC No. 3	1	11.11	14.82	12.97
	2	22.22	24.71	48.93
FOC No. 4	1	7.40	10.15	8.78
	2	14.81	16.67	15.74
FOC No. 5	1	62.97	65.96	64.47
	2	66.67	74.08	70.38
FOC No. 6	1	3.70	7.41	5.56
	2	7.41	12.50	9.96
VD No. 1	1	18.52	21.25	19.89
	2	22.22	24.71	23.47
VD No. 2	1	11.11	14.82	12.79
	2	18.52	21.00	19.76
VD No. 3	1	11.11	15.03	13.07
	2	14.81	16.67	15.74
Mean		23.59	26.74	
LSD at 5% :				
Isolates (A)		13.37	13.45	
Inoculum density % (B)		ns	ns	
Interaction (A×B)		18.90	19.02	

Effect of soil amendment with activated yeasts (dry and soft) on controlling *Fusarium* and *Verticillium* wilt of pepper under greenhouse conditions: Data in Tables (2 and 3) indicated that application of these yeasts to infested soil with pathogenic fungi reduced disease severity of *Fusarium* and *Verticillium* wilt of pepper. Application of activated yeasts into infested soil with *VD* was the more effective in reduction of disease severity than infested soil with *FOC* in both seasons (2014 and 2015). Soft yeast was the more effective in controlling the disease than dry yeast compared to the control. Data also indicated that all concentrations of yeasts were effective for controlling the disease. Concentration 6 gL^{-1} was the more effective than the other tested concentrations. In general, all treatments containing activated yeasts at rate 6 gL^{-1} led to the effective control of vascular browning and reduced disease incidence. Addition of soft yeast into infested soil with pathogens led to the effective control of wilt disease and reduced disease incidence to 11.11 and 14.81% of vascular browning in the seasons 2014 and 2015, respectively and 16.67 and 17.42 of foliar yellowing in both seasons 2014 and 2015, respectively. Moreover, fungicide (Moncut 25%) was effective in reducing the disease at the tested concentration 1 gL^{-1}compared to the control.

Table 2: Effect of activated yeasts as soil treatment on disease severity percentages of *Fusarium* wilt disease under greenhouse conditions during 2014 and 2015 growing seasons.

Treatments	Conc. gL-1	Season 2014			Season 2015		
		Vascular browning %	Foliar yellowing %	Mean	Vascular browning %	Foliar yellowing %	Mean
Soft yeast	4	22.22	27.31	24.77	25.92	29.35	27.64
	6	18.52	22.34	20.43	14.81	17.42	16.12
	8	29.63	35.75	32.69	29.63	37.36	33.50
	Mean	23.46	28.47		23.45	28.04	
Dry yeast	4	37.03	41.00	39.02	40.74	50.03	45.39
	6	22.22	27.31	24.77	22.22	27.00	24.61
	8	51.85	55.45	53.65	55.56	60.00	57.78
	Mean	37.03	41.25		39.51	45.68	
Fungicide (Moncut)	0.25	44.44	50.33	47.39	44.44	52.91	48.68
	0.50	33.33	30.18	31.76	37.04	43.06	40.05
	1.00	25.92	27.95	26.94	33.33	37.50	35.42
	Mean	34.56	36.15		38.27	44.49	
Control		74.08	88.89	81.49	62.97	69.52	66.25
Mean		35.92	40.65		36.67	42.42	
LSD at 5% :							
Treatments (A)		16.06	1.61		10.06	1.16	
Concentrations (B)		13.91	1.39		8.71	1.00	
Interaction (A×B)		27.82	2.78		17.43	2.01	

Table 3: Effect of activated yeast as soil treatment on disease severity percentages Verticillium wilt disease under greenhouse conditions during 2014 and 2015 growing seasons.

Treatments	Conc. gL-1	Season 2014			Season 2015		
		Vascular browning %	Foliar yellowing %	Mean	Vascular browning %	Foliar yellowing %	Mean
Soft yeast	4	14.81	22.24	18.53	14.81	20.07	17.44
	6	11.11	16.67	13.89	14.81	17.42	16.12
	8	22.22	26.75	24.49	44.44	33.33	38.86
	Mean	16.05	21.89		24.69	23.61	
Dry yeast	4	33.33	38.06	35.70	33.33	33.33	33.33
	6	25.92	30.71	28.14	25.92	28.14	27.03
	8	44.44	47.05	45.75	33.33	37.00	35.17
	Mean	34.56	38.61		30.86	32.82	
Fungicide (Moncut)	0.25	44.44	48.85	46.65	29.63	38.06	33.85
	0.50	22.22	31.14	26.68	29.63	36.36	33.00
	1.00	18.52	21.00	19.76	22.22	28.59	25.41
	Mean	28.39	33.66		27.16	34.34	
Control		66.67	83.33	75.00	66.67	71.13	68.90
Mean		30.37	36.58		31.48	34.34	
LSD at 5% :							
Treatments (A)		13.86	2.22		9.42	1.11	
Concentrations (B)		12.00	1.92		8.16	0.96	
Interaction (A×B)		24.00	3.84		16.32	1.93	

Effect of soil treatment with activated yeasts on some plant growth characters of sweet pepper plants under greenhouse conditions: Data presented in Tables (4 and 5) and Figure (1) indicated that soil amended with activated yeasts improved plant growth characters of sweet pepper plants (Norhan cv.) in both seasons (2014 and 2015) compared to untreated plants. The highest plant length was achieved in soil amended with dry yeast at the rate of 6 gL^{-1} (43 and 44 cm.) in both seasons 2014 and 2015, respectively, followed by 4 then 8 gL^{-1}. The application of yeasts to infested soil with *FOC* caused the best increase of plant length and stem diameter. Treated infested soil with fungicide (Moncut 25%) at the concentration of 0.25 gL^{-1} showed the lowest plant length and stem diameter compared to the treatment with yeast. Data also showed that all tested yeasts were effective in reducing dead plants and increased survival plants as well as improved plant growth characters compared to untreated plants.

Table 4: Effect of yeasts on some plant growth characters of sweet pepper plants (Norhan cv.) infected by *F. oxysporum* f.sp. *capsici* under greenhouse conditions.

Treatments	Conc. gL-1	Season 2014		Season 2015	
		Plant length (cm)	Stem diameter (mm)	Plant length (cm)	Stem diameter (mm)
Soft yeast	4	36.67	6.33	40.00	6.33
	6	41.67	6.67	40.33	7.00
	8	36.00	6.00	38.00	6.00
	Mean	38.11	6.33	39.44	6.44
Dry yeast	4	41.67	7.00	43.00	7.00
	6	43.00	7.33	44.33	7.33
	8	37.67	6.00	42.00	6.00
	Mean	40.78	6.78	43.11	6.78
Fungicide (Moncut)	0.25	32.33	6.00	35.33	5.67
	0.50	35.00	6.00	36.33	6.00
	1.00	37.67	6.00	38.33	6.33
	Mean	35.00	6.00	36.66	6.00
Control		29.67	5.67	30.67	6.00
Mean		37.14	6.30	38.83	6.37
LSD at 5% :					
Treatments (A)		3.77	3.13	1.37	1.14
Concentrations (B)		3.27	2.71	1.19	0.99
Interaction (A×B)		6.53	5.42	2.38	1.97

Discussion

Pepper wilt diseases, incited by *FOC* and *VD* are major problems which attack pepper plants and causing severe damage to pepper production worldwide (Goicoechea, 2006; Wongpia & Lomthaisong, 2010). Nine isolates of pathogenic fungi were isolated from naturally diseased sweet pepper plants showing wilt symptoms and identified as *FOC* (six isolates) and of *VD* (three isolates). Pathogenicity tests of isolated fungi indicted that all tested isolates were

able to infect sweet pepper plants (Norhan cv.) causing wilt disease with different degrees of disease severity. Such results are in agreement with those reported by Garcia-Mina et al., (1996); Mushtaq & Hashmi (1997).

Table 5: Effect of yeasts on some plant growth characters of sweet pepper plants (Norhan cv.) infected by *Verticillium dahlia* under greenhouse conditions.

Treatments	Conc. gL-1	Season 2014		Season 2015	
		Plant length (cm)	Stem diameter (mm)	Plant length (cm)	Stem diameter (mm)
Soft yeast	4	37.33	6.33	34.33	6.33
	6	40.67	8.00	38.33	6.67
	8	35.67	6.00	35.00	5.67
	Mean	37.89	6.78	35.89	6.22
Dry yeast	4	39.00	6.33	40.67	6.00
	6	43.00	8.00	41.33	6.00
	8	37.67	7.00	37.67	5.67
	Mean	39.89	7.11	39.89	5.89
Fungicide (Moncut)	0.25	33.67	5.33	3400.	5.67
	0.50	35.67	5.67	36.00	5.67
	1.00	37.00	5.67	36.67	5.67
	Mean	35.47	5.56	35.56	5.67
Control		29.33	6.00	28.67	5.33
Mean		36.90	6.43	36.27	5.87
LSD at 5% :					
Treatments (A)		4.52	1.17	3.99	1.10
Concentrations (B)		3.91	1.02	3.46	0.96
Interaction (A×B)		7.83	2.03	6.91	1.91

Application of activated yeasts for controlling *Fusarium* and *Verticillium* wilt under greenhouse conditions affected significantly the disease severity compared to the control. Addition of these yeasts at the concentration of 6 gL^{-1} to infested soil with pathogenic fungi gave higher reduction in disease severity. Application of activated yeasts was more effective in controlling *Verticillium* wilt than *Fusarium* wilt disease. Data indicated that the treatment with soft yeast was more effective on pathogenic fungi than dry yeast. The obtained results are in agreement with those obtained by many other researchers (Urquhart & Punja 1997; Zheng et al., 2003). A similar behavior was observed by Hassan & Abd El-Rehim (2002) for controlling onion neck rot disease. They observed that, the increasing of yeast concentration (0.05 to 0.1%) led to an increasing reduction of the disease incidence. Several mechanisms have been reported to play a significant role in the biocontrol activity of antagonistic yeasts. Among them, interaction between yeast and postharvest pathogens is involved. It has been suggested that attachment of the yeast to fungal hyphae and extensive production of an extracellular matrix by yeasts may play a key role by either enhancing nutrient competition or by some other undetermined mechanisms (Wisniewski et al., 1991; Jijakli & Lepoivre, 1998; Wan & Tian, 2002). Wisniewski et al., (1991) indicated that in the presence of

the pathogen, the yeast cells produced lytic enzymes that could enhance the attaching ability of yeast to hyphae of pathogens. Nutrient competition into wounds appeared to be the principal mode of action of yeasts (Vero et al., 2001). El-Tarabily (2004); Madi et al., (1997) reported that *R. solani* and *Sclerotium rolfsii* were effectively suppressed by some plant growth-promoting yeasts, respectively.

Figure 1. Effect of activated yeasts as infested soil treatment with pathogenic fungi (*F. oxysporum* and *V. dahliae*) on some plant growth characters under greenhouse conditions.

Under greenhouse conditions in the two growing seasons, soil amended with activated yeasts improved plant growth characters of sweet pepper plants when compared to untreated plants. The best plant length and stem diameter of pepper plants were achieved in soil amended with dry yeast at the rate 6 gL^{-1}. Application of yeasts into infested soil with *FOC* caused the highest increase of plant length and stem diameter. Data also showed that all tested yeasts were effective for increasing survival plants as well as improved plant growth characters compared to untreated plants. At the same time, the treated plants were very strong when compared to the control and fungicide. These results are in agreement with the finding of Bakry (2007) who found that the maximum yield of Jafa orange was noticed when these trees were sprayed with active dry yeast. In addition, Mohamed (2008) found that spraying Balady mandarin trees with active dry yeast and ZnSO4 reflected high yield values. Yeast as a natural stimulator is also characterized by its richness in protein (47%), carbohydrates 33%, nucleic acid 8%, lipids 4%, and different minerals 8% such as Na, Fe, Mg, K, P, S, Zn, Mn, Cu, Si, Cr, Ni, Va and Li in addition to thiamin, riboflavin, pyridoxine, hormones (Nagodawithana, 1991). Many investigators reported that, spraying plants with yeast extract improved plant growth, yield and quality of many vegetable crops *i.e.* pea, tomato, potato, pepper and cucumber (EL-Ghamriny et al., 1999; Yeo et al., 2000; Abd-El-Hafez & Shihata, 2001; Mahmoud, 2004; Mahmoud et al., 2013; Shalaby & El-Ramady, 2014). Moreover, El-Desuki & EL-Gereadly (2006) reported that, the vegetative growth of pea plant, leaves content of photosynthesis pigments, free amino acids, carbohydrates and cytokininis, pod yield and quality as well as nutritive value were increased by increasing the concentration of yeast extract in spraying solution from 1% up to 3%. Earlier reports explained the effect of yeast application on vegetative and fruit growth due to its richness in tryptophan which is consider as a precursor of IAA (indole acetic acid) and on flower ignition due to its effect on carbohydrate accumulation (Warring & Philips, 1973). Due to its cytokinin content, yeast treatments were suggested to play a beneficial role in cell division and cell enlargement (Natio et al., 1981). Ahmed et al., (1995); Glick (1995) recorded that the yeast is capable to increase the stimulative growth compounds like gibberellins, auxins and cytokinins that act in improving plant cell division and growth. Finally, the obtained data indicated that the application of yeasts to the soil not only resulted in reducing the disease severity but also increased plant height and stem diameter of the plant.

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