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Comparative Study on the Activity of *Trichoderma asperellum* and *T. Album* and Their Role in Controlling Faba Bean Root Rot and Wilt Diseases

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Trichoderma spp are the most important soil fungi in Egypt. This study was initiated to evaluate *Trichoderma asperellum* and *T. album* used to produce Bio-Nagi and Bio-Zeid biocide products, respectively. The mycelial growth of Trichoderma isolates was tested in response to temperature effect. The most growth of *T. asperellum* was obtained at 25-30°C, while incubation at 35°C showed slow growth rate to fill up 9 cm Petri plate at 15days. No mycelial growth was observed after incubation at 4, 37 and 40°C for 5, 10 and 15 days, respectively. Meanwhile, when these Trichoderma plates were re-incubated in the optimum temperature at 25°C they showed fast growth rate in those exposed previously to 4 and 37°C to fill up the plates completely within 3 days, but for those exposed previously to 40°C, no growth was noticed. *In vitro*, the two Trichoderma isolates inhibited the mycelial growth of *Fusarium oxysporum* and *Rhizoctonia solani*. The highest growth reduction values (66.7 and 58.4%) and (54.2 and 53%), against *R. solani* and *F. oxysporum* were obtained with Bio-Nagi and Bio-Zeid, respectively. Also, Trichoderma isolates produced volatile compounds having significant effect in reducing the mycelial growth of the two pathogenic fungi. Four carriers' formulae (Imported and local soybean flours, Sawdust and Yeast extract) were separately mixed up, leach with the two isolates of Bio-Nagi and Bio-Zeid and were evaluated in vitro and in vivo. The most effective for preservation temperature of the two biocides in each formula, was calculated when stored at 4oC than at room temperature, which recorded the highest viability and spore germination, then decreased gradually by increasing storage period from 3 to 12 months. All tested Trichoderma carrier formulations added to soil infested with pathogen significantly decreased wilt and damping off diseases and increased plant survival of faba bean, in greenhouse compared with infected control. The two Bio-Nagi and Bio-Zeid biocide formulae were antagonistic against the two pathogenic fungi and recorded the highest efficacy in reducing wilt and damping-off caused by *F. oxysporum* and *R. solani*. Application of Bio-Nagi + Imported soybean flour was the most effective formulation, gave the highest reduction in the percentage of wilt and damping off followed by Bio-Nagi+ Local soyben flour and Bio-Zeid+ Imported soybean flour against the two pathogens.

Keywords: Optimum temperature, Mycelial growth, Volatile compounds, Carriers, Biocides, Wilt and damping-off, *Faba bean.*

Crop loss due to disease-causing fungi can be decreased using environment friendly means known as biological control to decrease the use of chemical fungicides. (Harman *et al.*, 2004). Many soil-borne fungi perform beneficial roles, such as biological control of plant pathogens or remediation of soil pollutants, but relatively little predictive information is available about their growth and activity in soil habitats. Application of biological control using antagonistic micro-organisms has proven to be successful method for controlling various plant diseases in Egypt (EL-Abbasi *et al.*, 2003 and Khalifa, 2016). Trichoderma group are well documented as effective biological control agents of plant diseases caused by soil borne fungi (McLean *et al.*, 2004; Asran *et al.*, 2005 and Mohamed and Haggag, 2006). Knowledge concerning the behavior of these fungi as antagonists is essential for their effective use since they can act against target organisms in several ways (Jeffries and Young, 1994). Much of the known biology and many uses of these fungi have been documented (Kubicek and Harman, 1998 and Mahmoud, 2016). There are several studies on growth and variability of the filamentous fungi of *Trichoderma* spp. based on mycelial growth, hyper-parasitism, biomass density and sporulation at different temperatures (Cross and Kenerley, 2004 and Singh *et al.*, 2014). Trichoderma spp. can grow at a wide range of temperature between 20-30ºC (Mishra and Khan, 2015). The optimum temperature at 25-30ºC was found to be best for growth and sporulation of the fungus (Singh and Kumar, 2009). Damping off and wilt diseases caused by soil-borne fungi are the most important diseases of many crops. Several fungi were recorded as causal pathogens of damping off and wilt diseases such as *Rhizoctonia solani* and *Fusarium* spp. (Abou-Zeid *et al.*, 2002 and 2003).

A variety of formulations has been described as carriers for biocontrol fungi including *Trichoderma* spp., that may improve their storage or growth in soil. Biocontrol efficacy is likely increased with increasing growth of the biocontrol agent, suggesting that quantitative studies of non- biotic and biotic factors affecting growth and proliferation of biocontrol agents in soil are necessary, and that influence the ability to predict fungal growth in natural habitats and may improve the predictability efforts (Stack *et al.*, 1988 and Bin *et al.*, 1991). The development of formulations and delivery systems for biocontrol are well documented on suppress of incidence of the diseases (Çiğdem and Merih, 2005). There are several approaches described to optimize the formulation suitability of a biocontrol agent, as application of appropriate carrier and formulation additives which improve the biocontrol efficacy without any support the growth of the pathogen and/or cause any damage to the host plant (Sallam *et al.*, 2009 and Faruk *et al.*, 2014).

The objective of the present study was to evaluate each of *Trichoderma asperellum*, the major component of Bio-Nagi, a formula is still under registration compared with *T. album*, the major component of Bio-Zeid, commercial biocide to produce new biocide. Evaluation of different carrier formulations of *Trichoderma asperellum* and *T. album*, towards wilt and damping-of fungi of faba bean in greenhouse conditions was carried out. The effect of the storage period on activity of different formulations was also investigated.

Materials and Methods

1. Source of fungal pathogens:

Virulent pathogenic fungal isolates of *F. oxysporum* Schlecht and *R. solani* Kuhn were obtained from the Unit of Identification of Microorganisms, Plant Pathology Research Institute, A.R.C., Egypt. These isolates were tested and proved to be of high pathogenic ability during a previous work carried out by Khalifa (2016).

2. Source of antagonistic fungi and carriers:

Two plant fungal antagonistic isolates, *i.e. Trichoderma asperellum* (the major component of Bio-Nagi, a formula is still under registration) which was recorded as a first record in Egypt by Abou-Zeid and Mahmoud (2012) and *T. album*, the major component of Bio-Zeid, commercial biocide labeled on different crops in Egypt, and four carriers (Imported soybean flour, Local soybean flour, Sawdust and Yeast extract) obtained from Unit of Identification of Microorganisms.

3. Effect of temperatures on mycelial growth of Trichoderma isolates:

The mycelial growth rate of *Trichoderma asperellum* and *T. album* involved in Bio-Nagi and Bio-Zeid, respectively following the protocol of Samuels *et al.* (2002) was determined. Four PDA Petri dishes (9 cm diameter) were centrally inoculated with 5-mm agar disk from 5-15 days-old PDA cultures of *Trichoderma* spp. The Petri dishes were incubated in darkness at 4, 25, 30, 35, 37 and 40ºC with only intermitted exposure to light when they were examined. Colony radius was measured after 1, 2 and 3 days at 25 and 30ºC. Others Colony radius was also measured after 5, 10 and 15 days at 4, 35, 37 and 40ºC. Each growth rate was measured repeated twice and the results were averaged for each isolate.

4. Biological control:

The antagonistic activity of the obtained Trichoderma isolates was evaluated against two fungal pathogens in vitro.

4.1. Assay of antagonism in vitro:

Dual culture plates with PDA medium were used to chek the antagonistic effect of *Trichoderma asperellum* and *T. hamatum* against *R. solani* and *F. oxysporum* as described by Dennis and Webster (1971). Plate (9 cm diameter) each containing 15 ml of PDA medium was inoculated at one side with a disk (5 mm in diam.) obtained from the periphery of 4 days-old culture of each pathogen. The opposite side of the plate was inoculated with similar disc of each Trichoderma isolate, obtained from 3 days old culture. Three plates were used for each Trichoderma isolate. Plates inoculated only with the pathogenic fungus served as a control. All inoculated plates were incubated at 25°C for 5-7 days. When mycelial growth covered the surface in the control treatment, all plates were then examined and the linear growth of the pathogens was measured. Percentage of decrease in mycelial growth of fungal pathogens was calculated using the following formula:

$$X = 100- [G_2 / G_1 x100]$$

Where: X: % of reduction in growth.

G_1: growth of pathogenic fungus in control plates.

G_2: growth of pathogenic fungus in dual plates with Trichoderma.

4.2. Confrontation to assess antibiosis mediated by volatile molecules in vitro:

Mycelial disk (5mm diam.) was taken from the margin of a 4-days old *Trichoderma* spp. culture, placed separately inverted in the centre of plates (9 cm diam.) containing 15 ml of PDA medium. Confrontation system consisted to place a plate containing the pathogenic fungus inverted on the top of a plate containing the Trichoderma and then hermetically closed with Para film. Petri dishes were then incubated in dark at 26°C. Controls consisted of each fungus growing separately in the middle of the plate without confrontation with the other fungus. Each treatment was replicated with 3 plates. Colony diameters were measured every 24h during incubation until the mycelium of the pathogenic fungus fully covered the control plates (Fravel, 1994).

4.3. Assay of carrier suitability in vitro:

The experiment was conducted in the laboratory of Unit of Identification of Microorganisms, with Bio-Nagi and Bio-Zeid to determine their shelf-life under different storage temperature (3, 6, 9 and 12 months) on different carriers (Imported soybean flour, Local soybean flour, Sawdust and Yeast extract), and kept into the polyethylene bags containing sterilized 150g from each carrier. Strains mass culturing were performed as spore powder. The mass production, 5g from pure spore of Trichoderma isolates were taken and mixed with 95g of each carrier. Trichoderma formula was stored at room temperature and in refrigerator at 4oC. Data were taken as tentative number of spores after 3, 6, 9 and 12 months of each Trichoderma formula (1g mixed with 99 ml sterilized water to make stock dilution). Number of spores was counted in 10-6 dilution on potato-dextrose agar (PDA) medium using four Petri plates for each one (Sallam *et al.*, 2009).

4.4. Assay of carrier suitability in vivo:

The experiment was conducted in greenhouse of Unit of Identification of Microorganisms, to evaluate the different carriers, imported soybean flour, Local soybean flour, Sawdust and Yeast extract with Bio-Nagi and Bio-Zied against damping-off and wilt caused by *R. solani* and *F. oxysporum* on faba bean susceptible cultivar (Giza-429 cv.), obtained from Legume Crops Dept, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Inocula of *R. solani* and *F. oxysporum* were grown on sorghum grain sand medium (25% clean sand and 75% sorghum grains and sufficient amount of distilled water to cover the mixture and autoclaved at 121 o C for 30 min) and incubated for 15 days at 26±2 °C. The previously prepared carriers were used herein. The mass production 5g from pure spore of Trichoderma isolates were taken and mixed with 95g of each formula. Pots (20 cm in diameter) were dipped in 5% formalin solution for 10 min and left to dry in

open air. Soil sterilization was carried out by the same formalin solution and left for 2 weeks, continuously mixed to ensure complete evaporation of formalin.

This experiment was carried out in sterilized pots containing 2 Kg sterilized clay soil in the greenhouse. Soil was infested individually with *R. solani* and *F. oxysporum* isolates, at the rate of 3 and 5% (w/w), respectively (Abou-Zeid et al., 2002), then irrigated and left 7 days to enhance fungal growth. Tested biocides were applied at the rate of 2.5 g/kg, as seed dressing. Five faba bean seeds (Giza-429 cv.) were sown per pot, and three replicates were used for each treatment. Untreated seeds were sown in infested soil as control treatment.

Disease incidence was recorded as the percentage of pre- and post- emergence damping-off as well as healthy survived plants at 15, 30 and 45 days after planting in case of *R. solani*, meanwhile disease incidence was recorded as the early and late wilt, 30 and 60 days respectively, after planting in case of *F. oxysporum* according to Abou-Zeid *et al.* (2002).

Statistical analysis:

Statistical analyses were subjected to Computer Statistical Package (CO-STATE) originated by Anonymous (1989).

Results and Discussion

1. Effect of temperature on growth of Trichoderma isolates:

Linear growth of *Trichoderma* spp. was measured as the diameter of colonies after incubation in the dark for 1-3 days at 25 and 30°C and after 15 days at the other temperature levels, *i.e.* 4, 35, 37 and 40ºC, was considered on PDA agar medium. Growth patterns at 25 and 30°C for 1-3 days showed significant differences in the mycelial growth. Trichoderma isolates showed fast growth and did not vary greatly, and needed 3 days to completely fill the plate at both 25 and 30°C (Table1). The growth patterns at 4, 37 and 40°C showed no significant differences in the mycelial growth. The two Trichoderma isolates incubated at 35°C showed slow growth at 15 days to completely fill the plate (9 cm). Meanwhile, no growth was observed when Trichoderma plates were incubated at 4, 37 and 40°C for 5 to 15 days (Table 2). In optimum temperature at 25°C after 15 days, fast growth rate at 4 and 37°C at 3 days incubation was noticed and filled up the plate (9 cm/3days) and showed no growth rate at 40°C (Table 3).

There are several studies on growth and variability of the filamentous fungi *Trichoderma virens* and *Trichoderma* spp. based on linear growth and properties of mycelia at different temperatures (Cross and Kenerley, 2004 and Singh *et al.*, 2014). All the species of Trichoderma could grow at different temperatures, viz. 20, 25, 30 and 35°C but they showed the best growth at a temperature range of 25-30°C (Srivastava *et al.*, 2014 and Maurya *et al.*, 2017). Also, Mishra and Khan (2015) indicated that *Trichoderma* spp. could grow at a wide range of temperature between 20-30ºC. The optimum temperature for the best growth and sporulation of the fungus was at 25-30ºC (Singh and Kumar, 2009).

Table 1. Growth of Trichoderma isolates on PDA incubated for 3 days at 25 and 30ºC.

Trichoderma isolate	Temp.	1 day /cm	2 days /cm	3 days /cm
T. asperellum	25°C	2.6	7.4	9.0
	30°C	3.0	7.1	9.0
T. album	25°C	2.5	6.6	9.0
	30°C	2.9	6.5	9.0
LSD $_{0.05}$	-	0.32	0.18	n.s.

Table 2. Growth of *Trichoderma* isolates on PDA incubated for 15 days at 4, 35, 37 and 40ºC.

Trichoderma isolate	Temp.	5days/cm	10days/cm	15days/cm
T. asperellum	4°C	0.0	0.0	0.0
	35°C	3.3	8.0	9.0
	37°C	0.0	0.0	0.0
	40°C	0.0	0.0	0.0
T. album	4°C	0.0	0.0	0.0
	35°C	3.0	7.8	9.0
	37°C	0.0	0.0	0.0
	40°C	0.0	0.0	0.0
LSD $_{0.05}$		0.22	0.22	n.s.

Table 3. Growth of *Trichoderma* isolates which showed no growth at 4, 37 and 40°C followed by reincubation again on PDA at 25ºC for 3 days.

Trichoderma isolate	Temp.	1 day /cm	2 days /cm	3 days /cm
T. asperellum	4°C	2.0	7.5	9.0
	37°C	2.5	9.0	9.0
	40°C	0.0	0.0	0.0
T. album	4°C	1.8	7.0	9.0
	37°C	2.0	8.0	9.0
	40°C	0.0	0.0	0.0
LSD $_{0.05}$		0.15	0.5	n.s.

2. Biological control:

The mycelial growth of the two phytopathogenic fungi was decreased and worked out by confronting the two Trichoderma isolates in vitro.

2.1. Assay of antagonism, in vitro:

Data presented in Table 4 indicate that the mycelial growth of *R. solani* and *F. oxysporum* was decreased over 5-7 days assessed due to the presence of Trichoderma isolates. This assay showed variations in the percentage decrease of the mycelial growth of these phytopathogen colonies by the different isolates of Trichoderma. The highest growth decrease values (66.7 and 58.4%) and (54.2 and 53%), were obtained against *R. solani* and *F. oxysporum* with Bio-Nagi and Bio-Zeid, respectively.

Table 4. Growth decrease (%) of *R. solani* and *F. oxysporum* isolates by Trichoderma isolates when grown in dual culture plates on PDA medium for 5-7 days at 26ºC.

Bioagent	*Rhizoctonia solani*			*Fusarium oxysporum*		
	Radial growth (cm)	Growth reduction %	* Over growth	Radial growth (cm)	Growth reduction %	* Over growth
T. asperellum	2.4	66.7	++	2.3	58.4	++
T. album	3.3	54.2	+	2.6	53.0	+
Control	7.2		-	5.53	-	-
$LSD_{0.05}$	0.43	4.98		0.32	2.92	

* Over growth: + Low over growth ++ high over growt

These results are in harmony with those obtained by Bell *et al.* (1982), Mohamed and Haggag (2006) who reported that biological control is known to be very effective against soil borne diseases. Trichoderma spp. showed an interesting control against various pathogens (EL-Abbasi *et al.*, 2003 and Asran *et al.* 2005). Trichoderma spp. and Gliocladium spp. caused the highest growth decrease of *F. oxysporum*, *R. solani* and *F. solani in vitro* (Eisa *et al*., 2006 and Khalifa, 2016). Antimicrobial metabolites produced by different fungal bioagents (*T. hamatum, T. harzianum* and *T. viride*) inhibited the growth of various soil-borne pathogens *in vitro* (Kubicek and Harman, 1998; McLean et al., 2004 and Mahmoud, 2016).

2.2. Confrontation to assess antibiosis mediated by volatile molecules in vitro:

In this respect, the bioagents used in the two products of Bio-Nagi and Bio-Zeid were tested for their ability to produce volatile metabolites against *R. solani* and *F. oxysporum* (Table 5). Trichoderma isolates produced volatile compounds having significant effect in reducing the mycelial growth of the two pathogenic fungi. Volatile metabolites produced by *T. asperellum* recovered isolate was most effective

in decreasing the mycelial growth of *R. solani* and *F. oxysporum*, being 6.8 and 4.8 cm after 5 days, respectively followed by *T. album* isolate, being 7.6 and 5.2 cm against the two pathogenic fungi, respectively.

Table 5. Growth of *R. solani* and *F. oxysporum* isolates due to volatile products of Trichoderma isolates grown on PDA medium for 7 days at 25ºC.

Treatment	Linear growth (cm)							
	R. solani				*F. oxysporum*			
	1 day	3 days	5 days	7 days	1 day	3 days	5 days	7 days
T. asperellum	1.8	5.2	6.8	9.0	0.6	2.0	4.8	7.1
T. album	2.0	5.9	7.6	9.0	1.5	3.2	5.2	8.8
Control	2.3	6.5	8.2	9.0	1.8	4.25	6.8	9.0
$LSD_{0.05}$	0.32	0.32	0.36	n.s.	0.33	0.31	0.74	0.73

These results are in agreement with those obtained by Dennis and Webester (1971) and Baracat *et al.* (2014) who stated that *Trichoderma* spp. released toxic metabolite and volatile compound caused inhibition of mycelial growth of the pathogenic fungi. The large members of volatile secondary metabolites produced by *Trichoderma* spp. such as ethylene, carbon dioxide, aldehydes and ketones are playing an important role in controlling the plant pathogens (Vey *et al.*, 2001 and Faheem *et al.*, 2010).

2.3. Assay of carrier in vitro:

Obtained results indicated that spore germination and growth of the two Trichoderma isolates from four carriers formula (Local soybean flour, Sawdust, Yeast extract and imported Soybean flour) the components of Bio-Nagi and Bio-Zeid formulations were still active, different effects were found on the viability of spores, at different storage temperatures, from 3 to 12 months under 4ºC and at room temperature as well (Tables 6 and 7).

Table 6. Spore germination of the two Trichoderma isolates grown on PDA after storage for 12 months on different formulations at 4ºC.

Treatment	Storage period/ month	Local soyabean 10^6	Sawdust 10^6	Yeast extract 10^6	Imported soybean 10^6
T. asperellum	3	215	203	233	223
	6	190	127	168	196
	9	130	72	140	166
	12	123	45	105	144
T. album	3	105	90	99	165
	6	90	68	83	131
	9	77	57	70	119
	12	66	47	69	104
$LSD_{0.05}$		19.16	14.33	12.69	11.40

Table 7. Spore germination of Trichoderma isolates grown on PDA after storage for 12 months on different formulations at room temperature.

Treatment	Period/ month	Local soya bean 10^6	Sawdust 10^6	Yeast extract 10^6	Imported soybean 10^6
T. asperellum	3	203	186	214	209
	6	180	168	151	174
	9	123	100	82	130
	12	86	70	46	93
T. album	3	96	80	87	152
	6	81	63	69	145
	9	72	44	54	93
	12	47	29	41	71
$LSD_{0.05}$		11.88	16.74	13.23	15.24

Storage of the two Trichoderma isolates at 4oC in each formula was the most effective, in most cases, for viable preservation. The highest spore germination was recorded after 3 months storage period and decreased gradually by increasing storage period from 3 to 12 months. Imported Soybean flour was the best formula for the two Trichoderma isolates, in most cases, followed by Local soya bean. Meanwhile the lowest spore germination was recorded due to using Sawdust for the two isolates when stored at 4ºC. Meanwhile at room temperature, the highest spore germination viability was recorded by imported soybean and local soybean formulae with the two isolates, and the lowest viability was recorded for Yeast extract with Bio-Nagi and Sawdust with Bio-Zeid.

These results are in agreement with those obtained by Küçük and Kivanç (2005) and Sallam *et al.* (2009). They assessed the efficacy of various carriers in sustaining

the population of bioagents isolates during storage. Different formulations have been used in control soil borne pathogens, these are fungal spores (Harman *et al.*, 1980) and powdery preparations of fungal mycelia (Latunde-Dada, 1993) have been used to increase the survival rate of the organism. A biocontrol formulation should possess several characteristics such as: easy preparation and application, stability, adequate shelf life and low cost (Churchill, 1982).

More recently commercial formulations of biological control have been developed which have consistently given good control of some plant diseases (Stewart *et al.*, 2001). The viability of *Trichoderma* spp. was more than 40% in the prepared formulations (wood flour and talc-based powder) at room temperature storage after 4 months (Sallam *et al.*, 2009). The carriers of Wheat bran, Soybean, Maize bran Sawdust and Sawdust + CMC were the most suitable tested carries for keeping the viability of *B. subtilis* and *T. harzianum* with no significant reduction all over the storage period up to ten months (Abdel-Kader *et al.*, 2012 and Faruk *et al.*, 2014).

Assay of carrier in vivo:

In this regard, data in Tables 8 and 9 indicate that all tested applications of antagonistic Trichoderma carrier formulations to pathogen infested soil significantly decreased wilt and damping-off diseases and increased survived plants of faba bean in greenhouse experiments compared with infected control. The antagonistic ability of Bio-Nagi and Bio-Zeid was the highest, against the tested pathogenic fungi which recorded the highest efficacy in reducing wilt and damping-off caused by F. oxysporum and *R. solani* with the range of (58.38 - 91.63% and 50.00- 75.00%) and (53.86 - 84.66% and 46.14 - 76.93%), respectively.

Application of Bio-Nagi + Imported soy flour was the most effective formulation, gave the highest reduction in the percentage of wilt and damping-off diseases by (91.63%) and (84.66%), respectively. Follwed by Bio-Nagi+ Local soy flour and Bio-Zeid + Imported flour (83.38 and 75.00) and (76.93 and 76.93%) against the two pathogens, respectively. Meanwhile formulation of Bio-Zeid + Sawdust showed the lowest reduction in the percentage of plants infected by *F. oxysporum* and *R. solani* (50.00- 46.14%), respectively compared to control.

These results are in harmony with those obtained by Chet *et al.* (1979) who reported that application of biological control using antagonistic microorganisms proved to be successful for controlling various soil-borne pathogens. *Trichoderma* spp. has been reported more frequently as effective biocontrol agents against various pathogens (Ngo *et al.*, 2006 and Mahmoud, 2016). Soil amendment with formulated Trichoderma proved to be effective against fungal pathogens, such as *R. solani*, *F. oxysporum*, and *Sclerotium rolfsii* (Tran, 1998; and Khalifa, 2016). The formulated *T. harzianum* grown on peat soil based black bran and rice bran was found to be effective in controlling damping off and seedling blight of eggplant and also promoted seed germination (Sangeetha *et al.*, 1993). Also, Faruk *et al.* (2014) found that the maximum reduction in total seedling mortality was recorded when the carrier material was wheat bran, followed by grass pea bran, rice bran, and maize bran. Formulations

(wood flour and talc-based powder) of biocontrol agents were more effective when added at the time of planting (Rose *et al.*, 2004).

Carriers could improve product stability, shelf life, and also protect the fungi against environmental extremes in soil. A successful antagonist should colonize rhizosphere during seed germination and inducing defense reactions immediately after treatment or by enhancing a capacity for rapid and effective activation of cellular defense responses (El-Katatny *et al.*, 2006 and Haikal, 2007).

Table 8. Effect of different biocides and carriers on faba bean (Giza 429) wilt caused by *F. oxysporum*.

Treatment	Wilt			Efficacy %	Survived plants %
	Early %	Late %	Total		
Bio-Nagi+ Imported soy flour	0.0	6.7	6.7	91.63	93.3
Bio-Nagi+ Local soy flour	6.7	6.7	13.4	83.38	86.7
Bio-Nagi+ Yeast	6.7	13.3	20.0	75.00	80.0
Bio-Nagi + Sowdust (wood)	13.3	20.0	33.3	58.38	66.7
Bio-Zeid + Imported flour	6.7	13.3	20.0	75.00	80.0
Bio-Zeid + Local soy flour	0.0	26.7	26.7	66.63	73.3
Bio-Zeid + Yeast	13.3	20.0	33.3	58.38	66.7
Bio-Zeid + Sowdust (wood)	20.0	20.0	40.0	50.00	60.0
Control (infected)	20.0	60.0	80.0	-	20.0
Control (uninfected)	0.0	100.0	100	-	100.0
$LSD_{0.05}$	12.7	14.11	14.92	-	15.09

Table.9 Effect of different biocides and carriers on faba bean (Giza 429) damping off caused by *R. solani*.

Treatment	Damping-off			Efficacy %	Survived plants %
	Pre*	Post**	Total		
Bio-Nagi+ Imported soy flour	13.3	0.0	13.3	84.66	86.7
Bio-Nagi+ Local soy flour	13.3	6.7	20.0	76.93	80.0
Bio-Nagi+ Yeast	20.0	13.3	33.3	61.59	66.7
Bio-Nagi + Sowdust (wood)	33.3	6.7	40.0	53.86	60.0
Bio-Zeid + Imported flour	13.3	6.7	20.0	76.93	80.0
Bio-Zeid + Local soy flour	20.0	6.7	26.7	69.20	73.3
Bio-Zeid + Yeast	26.7	13.3	40.0	53.86	60
Bio-Zeid + Sowdust (wood)	40.0	6.7	46.7	46.14	53.3
Control (infected)	60.0	26.7	86.7	-	13.3
Control (uninfected)	0.0	0.0	0.0	-	100
$LSD_{0.05}$	14.97	12.1	17.21		17.25

*Pre= pre-emergence, **Post= Post-emergence.

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دراسات مقارنة على نشاط الفطرين *Trichoderma asperellum* و *T. album* ودورها فى مكافحة أمراض أعفان الجذور والذبـــول فى الفـــول البلـــدي

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تُعتبر الفطريات التابعة لجنس الترايكوديرما من أهم فطريات التربة في مصر. أجريت هذه الدراسة لتقييم المركب الحيوى البيوناجى والمحتوى على *Trichoderma asperellum*) والبيوزيد المحتوى على (*T. album*)، والتي تم الحصول عليهما من وحدة تعريف الكائنات الحية الدقيقة بمعهد بحوث أمراض النباتات. أختبر النمو الميسليومى لعزلات الترايكودرما كأستجابة لتأثير درجة الحرارة. سجل معظم النمو بين درجتى ٢٥-٣٠ °م، أظهر التحضين عند ٣٥° م معدل نمو بطيء ليكتمل النمو فى طبق بترى ٩ سم بعد ١٥ يوم، في حين أنه لم يلاحظ أي نمو ميسليومى بعد ٥، ١٠، ١٥ يوما عند درجات ٤، ٣٧ ، ٤٠° م. أظهر إعادة تحضين عزلات التريكودرما التى لم تنمو عند ٤ ، ٣٧ ، ٤٠° م على درجة الحرارة المثلى عند ٢٥° م معدل نمو سريع عند ٤ و ٣٧° م حيث تم إكتمال الطبق في ٣ أيام بينما لم تظهر أي نمو عند ٤٠° م في المعمل. بينما الأطباق التى سبق تحضينها على ٤٠° م ولم يظهر عليها أى نمو، فعند إعادة تحضينها على الدرجة المثلى (٢٥° م) لم يلاحظ أيضا أى نمو فطرى فيها. ثبطت عزلات الترايكودرما النمو الميسليومى لفطرين *F. oxysporum* و *R. solani* . تم الحصول على أعلى قيم لإختزال النمو (٦٦,٧ و ٥٨,٤ ٪) و (٥٤,٢ و ٥٣ ٪) ، ضد فطرى *R. solani* و *F. oxysporum* مع البيوناجى والبيوزيد على التوالي. تنتج عزلات الترايكودراما أثناء نموها مركبات طيارة لها تأثير كبير في الحد من النمو الميسليومى لأثنين من الفطريات المسببة للأمراض. تم تقييم ٤ أشكال/ بدائل للمادة الحاملة (دقيق فول الصويا المستوردة، دقيق فول الصويا المحلي، نشارة الخشب ومستخلص الخميرة) مع عزلتى البيوناجى والبيوزيد تحت ظروف المعمل والصوبة. كانت عزلتى المبيدين الحيوين في كل تركيبة والمخزنة على ٤° م هى الأكثر فعالية للحفظ عن التخزين في درجة حرارة الغرفة ، والتي سجلت أعلى معدل للحيوية وإنبات الجراثيم، ثم انخفضت تدريجيا بزيادة فترة التخزين من ٣ إلى ١٢ شهرا. أنخفضت بشكل معنوى جميع التطبيقات المختبرة لتركيبات المادة الحاملة والترايكودرما المضادة للكائنات المسببة لأمراض الذبول وموت البادرات كما زادت من نباتات الفول الحية، تحت ظروف الصوبة مقارنة بالكنترول المعدى. وقد سجلت أعلى فعالية للقدرة التضادية لعزلتى البيوناجى والبيوزيد ضد الفطرين الممرضين في الحد من أمراض الذبول وأعفان الجذور الناتجة عن فطرى *F. oxysporum* و *R. solani* . وكان تطبيق البيوناجى مع دقيق فول الصويا المستورد هو التركيبة الأكثر فاعلية والتى أعطت أعلى انخفاض في نسبة الذبول وأعفان الجذور متبوعة بالمعاملة بالبيوناجى مع دقيق فول الصويا المحلي و البيوزيد مع دقيق فول الصويا المستورد ضد مسببات الأمراض.