Minufiya J. Agric. Res. Vol.35 No. 6: 2033-2047 (2010) "http://www.mujar.net"

AUGMENTATION OF ANTAGONISTIC EFFECT OF SOME BIOAGENTS AGAINST FUNGI CAUSING PEPPER FRUIT ROT BY GAMMA RADIATION

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(Received: Jul. 18, 2010)

ABSTRACT: *The effect of some biological control agents on the growth of seven isolates of the most frequent isolated pathogens from fruit rot of pepper, i.e. 3 isolates of Fusarium, 2 isolates of Alternaria and 2 isolates of Aspergillus were studied under laboratory conditions. Eight isolates of Trichoderma spp and one isolate of Bacillus subtilis were used for determination of the antagonistic activities against fungal pathogens in dual culture laboratory conditions.*

Three parameters were measured for antagonistic activities, i.e. linear growth and reduction of fungal growth under stress of bioagent, as well as over growth and/or inhibition zone. B. subtilis, Trichoderma hamatum, T. harzianum and T. viride were found to be the most potent biocontrol agents against most of the pathogens. The antagonistic effect was increased after irradiation of B. subtilis to radiation dose of 1 kGy and irradiation of Trichoderma sp. to a dose of 0.2 kGy.

Key Words: *Biocontrol Agents, Pepper, Fruit Rot, Radiation.*

INTRODUCTION

Bell pepper is an important commercial crop in the world. Sensory and nutritional characteristics make it a widely accepted vegetable by consumers. Peppers are grown in most countries of the world, being Asia the largest producer (Bosland and Votava, 2000).

Some fungi are plant pathogens and can start the spoilage from the field while others, although they could contaminate the fruits in the field, actually proliferate and cause substantial spoilage only after harvest when the main plant defenses are reduced or eliminated. Post-harvest fruit spoilage results in significant economic losses. Additionally, if the spoiling fungi are toxigenic or pathogenic, they could pose a health risk for the consumer. Toxigenic fungi have been isolated from spoiling fruits. Some of these moulds could produce mycotoxins while grown on fruits, even during refrigeration (Tournas and Stack, 2001). Moreover, pathogenic fungi could cause infections or allergies in susceptible individuals (Kurup, 2003; Monso, 2004).

Postharvest diseases cause considerable losses to harvested fruits and vegetables during transportation and storage. Synthetic fungicides are primarily used to control postharvest decay loss. However, the recent trend is shifting toward safer and more eco-friendly alternatives for the control of postharvest decays. Of various biological approaches, the use of antagonistic microorganisms is becoming popular throughout the world. Several postharvest diseases can now be controlled by microbial antagonists. Although the mechanism(s) by which microbial antagonists suppress the postharvest diseases is still unknown, competition for nutrients and space is most widely accepted mechanism of their action (Sharma *et al.*, 2009).

In addition, production of antibiotics, direct parasitism, and possibly induced resistance in the harvested commodity are other modes of their actions by which they suppress the activity of postharvest pathogens in fruits and vegetables. Microbial antagonists are applied either before or after harvest, but postharvest applications are more effective than preharvest applications. Mixed cultures of the microbial antagonists appear to provide better control of postharvest diseases over individual cultures or strains (Sharma *et al.*, 2009).

The use of biological agents to enhance the plant defense mechanisms against pathogens represents an ecologically friendly alternative to pesticides repeatedly used to control plant diseases. This strategy has more significance against soil borne pathogens

Postharvest decays of fruits and vegetables account for significant levels of postharvest losses. It is estimated that about 20-25% of the harvested fruits and vegetables are decayed by pathogens during postharvest handling even in developed countries (Zhu, 2006; Singh and Sharma, 2007). In developing countries, postharvest losses are often more severe due to inadequate storage and transportation facilities.

Synthetic fungicides are primarily used to control postharvest diseases of fruits and vegetables (Zhu, 2006; Singh and Sharma, 2007). Nevertheless, attempts to reduce chemical contamination of the environment as well as health hazards associated with consumption of pesticide residues dictate the reduction of the use of such chemicals. In addition, the recent trend is shifting toward safer and more eco-friendly alternatives for the control of postharvest decays.

There is a strong public and scientific desire to seek safer and eco-friendly alternatives for reducing the decay loss in the harvested commodities (Mari *et al.*, 2007). Among different biological approaches, use of the microbial antagonists like yeasts, fungi, and bacteria is quite promising and gaining popularity (Droby, 2006; Korsten, 2006). There are two basic approaches for using the microbial antagonists for controlling the postharvest diseases of fruits and vegetables: (1) use of microorganisms which already exist on the product itself, which can be promoted and

managed, or (2) those that can be artificially introduced against postharvest pathogens.

The objective of this study was to evaluate the potential of using *Trichoderma* species as well as *B. subtilis* as biological control agents to control the pepper fruit rot caused by some fungal pathogens under laboratory and green house conditions in artificially infected fruits. Also, to evaluate the effects of γ-irradiation on the control of Tricoderma and Bacillus *In vivo* and *in vitro* and investigate whether these agents can control the disease of fruit rot in pepper.

MATERIALS AND METHODS

Media:

Potato dextrose agar medium (PDA) containing (g/l): peeled and sliced potatoes 200, dextrose 20; and agar 20. Tryptone glucose yeast extract agar medium (TGY) containting the following in (g/l): tryptone 5; glucose 3; yeast extract 1; agar 15, pH 7.0 was used for culturing and maintenance of fungal and *Bacillus subtilis* strains, respectively.

Collection of infected plant materials:

This work was started by collecting naturally infected pepper fruits showing fruit rot symptoms, from different pepper growing areas in Minufiya governorate (Shebin El- Kom, El- Bagour, Quisna, Berkat Al- Sabie and El-Sadat Districts).

Isolation and purification of Pepper fruit rot causal organisms:

Isolation of the causal organisms and the associated fungi was done from the diseased samples that collected from the above mentioned locations. The infected fruits were cut into small pieces, washed thoroughly with running tap water to remove any adhering soil particles, surface sterilized by immersing it in 0.25% sodium hypochlorite solution for 4 min., followed by 70% ethanol for 2 min. The samples pieces were then washed several times in sterilized distilled water and blotted between two sterilized filter papers. The surface sterilized samples were than transferred onto PDA medium containing penicillin (50 units/ml), 20 ppm tetramycin and 40 ppm streptomycin sulphate to avoid the bacterial contamination. The inoculated plates were then incubated at 25°C for 3-7 days and examined daily for the occurrence of fungal growth. For purification, the developed fungal colonies were transferred to other Petri dishes contained the same medium and incubated at 25°C for 7 day.

Isolation and identification of biological control agents from soil and rhizosphere:

Soil and rhizosphere samples were taken from pepper fields by uprooting the infected plants with great care to obtain most of the intact root system.

The root system was shaken gently to get rid of most of the adhering soil particles. Root system with the remaining adherent soil particles was transferred to a wide mouth reagent bottle containing 99 ml sterile distilled water (soil weight was 1g). The bottles were shaken thoroughly or mechanically for 15 min, this gave an approximate dilution of less than 1/100, the root system was then discarded and serial dilutions were made to study the micro flora. Aliquots from these dilutions were inoculated on Trichoderma Selective Medium (TSM). The inoculated Petri dishes were incubated at 28°C and daily checked for the developing fungal colonies. The developed colonies were marked and transferred onto PDA slants. Moreover, *Bacillus subtilis* strain previously isolated from soil and identified in (Lab of Bacteria and Viruses, NCRRT) was used as the bacterial biological control agent.

Biological control:

Eight isolates of *Trichoderma* spp. [*T. harzianum* (5 isolates), *T. hamatum* (2 isolates) and *T. viride* (1 isolate] and one isolate of *Bacillus subtilis* were utilized as biological control agents, in dual cultures, against seven isolates of pepper fruit rot pathogenic fungi, namely, *Fusarium oxysporum* (2 isolates) and *F. semitectum*, *Alternaria alternata*, *Alternaria citri*, *Aspergillus niger* and *Aspergillus flavus* (one isolate of each), under laboratory conditions.

Petri dishes (90 mm in diameter) each contained 15 ml of PDA medium were used to detect the antagonistic effect between the above mentioned biological control agents and the pathogenic fungal isolates. Different plates were inoculated with 0.5 cm in diameter disks of each fruit rot pathogen isolate obtained from the periphery of 4 days old cultures. Each pathogenic fungus was inoculated at one side of the plate and the opposite side was inoculated with either disk of 0.5 cm in diameter, obtained from 3 days old culture of each biological agent (or with loop of bacterial growth cells in line form 3 days old bacterial culture). Four plates were used for each particular treatment as replicates. The plates inoculated with the pathogenic fungus only were served as a control treatment as replicates. The inoculated plates were incubated at 25°C, when the mycelial growth covered the entire medium in control treatment; all the plates were then examined for antagonism, hyper parasitism, growth reduction and/or inhibition zone and over growth. Growth reduction was determined by measuring the radial growth of the pathogens was recorded by estimating the mean of colony diameter (mm) as well as the percentage of growth reduction that pooled out using the following formula:

$$\text{Reduction (\%)} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

The width of the inhibition zone between the two colonies was recorded in all treatments. The percentage of inhibition of radial growth of the pathogenic fungal isolates was calculated by comparing radial growth of the colony directly opposite to the bioagent colony with the radial growth of the part of the colony not adjacent to the bioagent (Zhou and Reeleder, 1990).

Biological control under green house conditions:

The effect of *Trichodermia* spp. on artificial infection using two isolates of *F. oxysporum*, and one isolate of each of *F. semitectum, Alt. alternata, Alt. citri, Asp. niger* and *Asp. flavus* were tested by inoculating of 6 mm in diameter disks taken from the growing edges of one week-old cultures in the center of pepper fruits, of genotype Geadon. Infected pepper fruits (wounded, unwound and neck infected) were sprayed by Trichoderma spores and mycelial suspension. Then surface was sterilized and inoculated with agar discs (4 mm in diameter) from culture of each of the selected pathogenic fungi grown on PDA medium (7days old) at the base of the fruit individually. Four replicates, 14 fruits of each, were used for each treatment. Each replicate was covered with stretch film in foam plate 5x 15x 25 cm. The inoculated fruits were incubated at room temperature at about 22- 25°C. The control treatment was surface sprayed with distilled water and infected with the fungal pathogens. Percentages and disease index of rotted fruits were recorded, 5 days after inoculation.

Effect of Gamma irradiation of biological agents on their antagonistic activity:

The most potent bioagents were selected and irradiated to study the effect of radiation on the antagonistic activity against the pathogenic fungi causing pepper fruit rot under laboratory conditions as follows:

I- *T. hamatum* and *T. viride* were prepared by culturing on PDA medium at 28°C for 7 days then exposed to doses of γ-radiation of 0.2, 0.6 and 0.8 kGy.

II- *B. subtilis* strain was prepared by culturing in TGY medium at 32 °C for 24 h. and exposed to γ-radiation at doses of 0.5, 1.0, and 1.5 kGy.

Petri dishes (90 mm in diameter) each contained 15 ml of PDA medium were used to detect the antagonistic effect between the above mentioned biological agents and pathogenic fungal isolates. Different plates were inoculated with 0.5 cm in diameter disks from each of *F. oxysporum* (two isolates) and *F. semitectum*, *Alt. alternata*, *Alt. citri*, *Asp. niger* and *Asp. flavus* (one isolate from each) were obtained from the periphery of 7 days old cultures.

Each pathogenic fungus was inoculated at one side of the plate and the opposite side was inoculated with either disks of 0.5 cm in diameter, of each fungal biological agent, obtained from 7 days old culture, or with a loopful of

bacterial growth in line from 3 days old bacterial culture. Eight plates were used for each particular treatment (two plates for each of the three doses of γ- radiation and two plates for non irradiated bioagents). Non irradiated plates were served as the control treatment. The inoculated plates were incubated at 28°C. When mycelial growth covered the entire medium in the control treatment, all the plates were then examined for growth.

Application of irradiated bioagents on pepper fruits under laboratory conditions.

The effect of irradiated and non irradiated bioagents on artificial infection using two isolates of *F. oxysporum* and one isolate from each of *F. semitectum*, *Alt. alternata*, *Alt. citri*, *Asp. niger* and *Asp. flavus* were tested by placing 6 mm diameter disks taken from the growing edges of 2-week-old culture in the center of fruits.

T. hamatum, T.viride and *B. subtilis* exposed to selected dose of γ-radiation namely, 0.2 kGy for *Trichoderma* sp. and 1.0 kGy for *B. subtilis* were sprayed under aseptic conditions on fresh fruits of Geadon genotype. Three replicates, 14 fruits of each, were used for each treatment; each replicate was covered with stretch film in foam plate 5x 15x 25 cm. The sprayed fruits were classified into 3 groups, one was infected upon the surface, the second was wounded infected and the third was infected by neck infection, each of the infected fruits were left at room temperature at about 22- 25°C for 7 days, and examined for fruit rot disease symptoms. The control treatment (the same types of pepper fruits sprayed with non irradiated *Trichoderma* sp. and *B. subtilis*) were examined for fruit rot symptoms as shown above.

RESULTS AND DISCUSSION

Biological control of soil borne pathogens comprises the decrease of inoculum or of the disease producing activity of a pathogen through one or more mechanisms. Interest in biological control of soil borne plant pathogens has increased considerably in the last few decades, because it may provide control of diseases that cannot or only partly be managed by other control strategies. Recent advances in microbial and molecular techniques have significantly contributed to new insights in underlying mechanisms by which introduced bacteria function (Choudhary and Johri, 2009).

In this study, seven isolates were selected as the most virulent from 370 fungal isolates isolated in pure cultures in 2005 and 2006 seasons from materials collected from different pepper growing areas in Minufiya governorate.

Data in Table (1) showed that with *F. oxysporum* isolate one, the highest linear growth (mm) was recorded by *T. viridi* while the least one (the best control) was recorded with *B. subtilis* (20 mm). The highest pathogen growth reduction was recorded by *B. subtilis* whereas the least one was recorded by *T. harzianum* (Th4). *T. harzianum* (Th5), *T. hamatum* (Tm1 and Tm2) and *B. subtilis* recorded inhibition zone with *F. oxysporum* isolate one. The rest fungal biocontrol agents were grown over pathogen mycelia growth. The most over growth was recorded with *T. harzianum* (Th4) followed by *T. viridi* and (Th2), while the least one was happened with (Th4).

Table (1): Effect of some bioagents on growth of *Fusarium* spp., the causal organism of fruit rot disease of pepper fruits under laboratory conditions.

Fungus Bioageants		*F. oxysporum-1*				*F. oxysporum-2*				*F. semitectum*			
		L. g	G. red	O. G	Inh. zone	L. g	G. red	O. G	Inh. zone	L.g	G. red	O. G	Inh. zone
T. harzianum	1	32.0	61.8	3.35	—	41.0	58.3	3.5	—	44.0	50.0	—	2.16
	2	35.0	56.6	4.25	—	35.0	69.4	—	2.17	39.0	61.0	—	1.63
	3	30.0	58.8	4.00	—	38.0	66.7	2.93	—	25.0	85.2	—	1.03
	4	45.0	47.8	4.50	—	43.0	55.6	3.63	—	27.0	80.7	—	2.13
	5	35.0	59.3	—	3.50	44.0	54.6	3.81	—	36.0	71.3	—	1.33
T. hamatum	1	42.0	55.9	—	3.80	45.0	52.4	—	2.31	46.0	52.7	—	1.87
	2	37.0	59.4	—	4.10	22.0	80.8	—	4.10	43.0	57.3	—	—
T. viride	1	45.0	47.00	4.20	—	25.0	75.0	—	2.8	34.0	60.2	—	2.53
B. subtilis		20.0	77.78	—	1.05	29.0	72.0	—	1.13	40.0	55.56	—	1.20
Control		90.0				90.0				90.0			

Fungi		*Alternaria alternata*				*Alternaria citri*			
		L. g	G. red	O. G	Inh. zone	L.g	G. red	O. G	Inh. zone
T. haraianum	1	29.0	60.0	1.54	—	40.0	62.5	—	1.97
	2	27.0	61.0	—	—	33.0	65.6	—	0.82
	3	29.0	60.0	2.05	—	50.0	56.4	—	0.93
	4	38.0	53.3	—	—	30.0	76.2	—	0.91
	5	40.0	50.0	2.18	—	40.0	62.5	—	1.55
T. hamatum	1	36.0	51.7	0.63	—	49.0	60.0	—	0.8
	2	26.0	62.0	2.17	—	20.0	82.5	—	—
T. viride	1	19.0	69.3	2.14	—	28.0	77.3	—	1.13
B. subtilis		10.0	88.9	—	1.9	53.0	52.2	—	1.4
Control		90.0				90.0			
Fungus		*Aspergillus niger*				*Aspergillus flavus*			
		L. g	G. red	O. G	Inh. zone	L. g	G. red	O. G	Inh. zone
T. haraianum	1	38.0	58.3	—	—	30.0	56.0	—	—
	2	42.0	55.6	—	0.6	43.0	50.8	—	—
	3	37.5	47.3	—	—	38.0	46.7	—	0.7
	4	43.0	58.0	—	—	48.0	40.7	—	0.5
	5	39.0	56.0	—	0.76	41.0	46.9	—	—
	1	38.0	58.2	—	0.77	37.5	50.0	—	—
T. hamatum	2	32.0	58.6	—	0.8	18.0	76.0	—	—
T. viride	1	27.5	62.8	—	—	22.5	66.7	—	0.8
B. subtilis		20.0	66.67	—	1.02	15.0	83.33	—	1.3
Control		90.0				90.0			

L. g: linear growth (mm) G. red: growth reduction (%), O. g: over growth (cm), Inh. zone: inhibition zone (cm).

F. oxysporum isolate two was affected by different biocontrol agents. The highest linear growth was recorded by (Th5) while the lowest linear growth (Tm1). The highest fungal growth reduction was recorded by (Tm2). Whereas the lowest fungal growth reduction was recoded by (Tm1). The highest inhibition zone diameter was recorded by Tm2 (4.1cm), while the lowest inhibition zone diameter was recorded by *B. subtilis* (1.13cm), the rest fungal biocontrol agents were grown over pathogen mycelia growth. The most over growth was recorded with (Th5), while the least one was happened with (Th3).

In the case of *F. semitectum*, the highest linear growth of pathogen noticed by Tm1. The lowest linear growth of the pathogen was recorded by Th3. The highest fungal growth reduction was recorded by Th3 (85%). The lowest growth reduction was recorded with (Th1) (50%). No one of biocontrol agents were grown over pathogen mycelia growth. *T. hamatum* (Tm2) was contacted with the pathogen mycelia growth only; over growth and/or inhibition zone absent, while the rest fungal biocontrol agents gave inhibition zone. The most inhibition zone recorded by *T. viride* (2.5cm), whereas the lowest inhibition zone recorded by (Tm3) (1.03cm).

Biocontrol agents achieve a better disease control via a microbial antagonism, which implies a direct interaction with the causal agent of disease and/or an indirect action involving the host (Benhamou *et al.*, 2002). The direct effect is usually a result of an antagonism between the biocontrol agent and the pathogen due to antibiosis or nutrient competition and/or parasitism, whereas the indirect interaction results from the enhancement of the plant defense mechanisms against the invading pathogen (Cherif *et al.*, 2002; Getha and Vikineswary, 2002). Association of both effects can be noticeable using some biocontrol agents (Benhamou *et al.*, 2002).

Trichoderma spp. have been developed into several commercial biological control products and are used in field crop and greenhouse systems (Harman, 2000). Rojo *et al.* (2007) found that *Trichoderma* species were effective in controlling *F. solani*, the causal pathogen of peanut brown rot root, in both naturally infested and artificially contaminated fields. Among the *Trichoderma* species evaluated *T. harzianum* was the most effective biocontrol agent.

It was also showed that linear growth of *Alt. alternata* was affected also by growing against biocontrol agents in dual culture. The highest linear growth was recorded by (Th5) (40mm). The lowest linear growth was recorded by *B. subtilis* (10.0 mm). The highest pathogen growth reduction was observed by *B. subtilis* (88.89%), whereas the lowest growth reduction was happened with Th5. All biocontrol agents gave inhibition zone except Th2 and Th4, *B. subtilis* was grown over pathogen mycelia growth (1.9cm). Th2 and Th4 were contacted with the pathogen mycelia growth only; over growth and/or inhibition zone absent. The most over growth was recorded with (Th5) (2.18cm), while the least one was happened with (Tm1) (0.63cm).

A. citri was affected by different biocontrol agents. The highest linear growth was recorded by *B. subtilis*, while the lowest linear growth Tm2. The highest fungal growth reduction was recorded by Tm2, whereas the lowest fungal growth reduction was recoded by *B. subtilis* (52.2%). (Tm1) was contacted with the pathogen mycelia growth only; over growth and/or inhibition zone absent. The most inhibition zone recorded by Th1 (1.97cm), while the lowest inhibition zone recorded by Tm1 and Th2 (0.82cm).

Asp. niger and *Asp. flavus* were reacted with biological agents with the same rate. The highest linear growth of pathogen noticed by Th4 against both isolates (43 and 48 mm, respectively). The lowest linear growth of the pathogen was recorded by *B. subtilis* (20 and 15 mm, respectively). The highest fungal growth reduction was recorded by *B. subtilis* (66.67 and 83.33% respectively). The lowest growth reduction was recorded with (Th3) (47.3%) with *Asp. niger* and Th4 (40.7%) with *Asp. flavus*.

Th1, Th3, Th4 and *T. viridi* were contacted with the pathogen fungal growth (*Asp. niger*). Th1, Th2, Th5, Tm1 and Tm2 were contacted with the pathogen fungal growth (*Asp. flavus*). No one of biocontrol agents were grown over pathogen mycelia growth with both isolates. The most inhibition zone recorded by *B. subtilis* (1.02 and 1.3mm respectively), whereas the lowest inhibition zone in isolate *Asp. niger* recorded by Th2 while in isolate *Asp. flavus* recorded by Th4.

In this study *B. subtilis* was found to be more effective as a biocontrol agent and this may be due to its ability to form endospores, and because of the broad- spectrum activity of its antibiotics.

Bacillus species are well known for their ability to control plant diseases through various mechanisms, including the production of secondary metabolites. *B. subtilis* an antagonist of *Fusarium graminearum*, and other *Bacillus* spp. that are antagonists of common fungal pathogens of canola were screened for peptide synthetase biosynthetic genes of fengycin and bacillomycin D (Ramarathnam *et al.*, 2007).

Chung *et al.*, (2008) demonstrated that *B. subtilis* suppressed the growth of 39 out of 42 plant pathogens tested. The strain also suppressed the disease caused by *F. oxysporum* on cucumber and *Phytophthora capsici* on pepper in pot assays.

Cazorla *et al.*, (2007) observed that *B. subtilis* strains isolated from healthy avocado rhizoplantes have shown diverse antagonistic activities and promising biocontrol abilities, which are closely linked with the production of antifungal lipopeptides and good colonization aptitudes.

Pepper fruits genotype i.e., Geadon (the highest susceptible) wounded, unwounded and neck infection were sprayed with the biological control agents; five species of *T. harzianum*, two species of *T. hamatum*, one strain of all from *T. viride* and *B. subtilis*. Discs of infected species i.e., two isolates of *F. oxysporum*, one isolate of each of *F. semitectum*, *Alt. alternata*, *A. citri*,

Asp.niger **and** ***Asp. flavus*** **were spoted on the sprayed pepper fruits and left for fifteen days.**

The infected area was measured in mm. The results represented in Table (2) showed that the most smelter infected area was found when using ***B. subtilis*** **strain as a biological control agent followed by** *T.* **viride and** ***T. hamatum*** **isolate (2) while the five species of** ***T. harzianum*** **and** ***T. hamatum*** **isolate(1) have no apparent effect compared with** ***B. subtilis,*** *T.* **viride and** ***T. hamatum*** **isolate (2).**

It was noticed from data in Table (2) that the infected area ranged from 10.8 to 18.5 mm with ***B. subtilis,*** *T.* **viride and** ***T. hamatum*** **isolate (2), while the infected area measured 20.1 up to 76.8 mm with other the bioagents.**

Table (2): Biological control of some bioagents on pepper fruit rot infection under laboratory conditions using seven causal organisms.

Isolate	Treatment	*T. harzianum*					*T. hamatum*		*T. viride*	*B. subtilis*
		1	2	3	4	5	1	2		
F. oxysporum 1	W	40.4	52.1	36.5	53.1	54.7	64.3	17.3	14.9	13.8
	Un	32.8	34.2	42.0	42.5	66.3	62.5	13.9	15.1	11.9
	NI	20.1	42.1	32.5	46.2	57.6	43.5	13.4	12.5	11.4
F. oxysporum 2	W	32.4	32.6	62.1	51.3	42.5	38.4	15.9	13.5	14.6
	Un	35.4	42.5	58.3	42.3	53.6	25.7	12.3	11.8	11.6
	NI	41.1	23.4	42.6	38.6	59.3	29.4	13.3	12.2	19.0
F. semitectum	W	37.0	53.4	47.3	32.5	39.8	46.5	12.6	11.7	11.4
	Un	45.8	51.2	53.2	36.5	37.5	38.5	17.0	11.3	11.6
	NI	37.1	46.2	43.1	41.2	40.1	35.1	16.7	13.5	12.6
A. alternata	W	45.2	56.2	43.5	70.2	59.4	45.3	15.1	10.9	12.5
	Un	32.5	56.4	64.2	52.6	57.3	37.6	11.9	12.1	10.8
	NI	31.8	46.7	32.5	51.5	42.3	38.3	12.1	11.9	12.5
A. citri	W	29.1	53.4	43.5	42.5	61.7	29.5	12.8	11.2	11.9
	Un	37.1	38.7	72.0	57.3	43.3	37.6	12.6	13.2	11.0
	NI	40.1	46.5	43.2	41.2	41.2	35.4	12.3	15.4	11.9
Asp. niger	W	42.5	76.8	53.2	70.6	52.1	41.2	[illegible]	12.1	12.2
	Un	75.4	46.7	49.8	73.1	43.6	51.6	11.2	11.8	12.0
	NI	32.4	46.7	37.9	43.3	35.9	42.3	15.4	13.1	11.6
Asp. flavus	W	24.6	36.9	54.2	53.2	40.3	38.2	16.3	18.5	17.1
	Un	38.2	53.4	51.3	43.4	39.5	31.5	12.7	11.8	12.1
	NI	34.8	35.6	51.6	46.1	36.9	28.6	17.1	11.1	11.6

W: Wounded, Un: Unwounded, NI: Nick infection

To augment the antagonistic activity of biocontrol agents against the pathogenic fungi causing fruit rot of pepper, the biocontrol agents were exposed to low dose of g- radiation. Two isolates of *Trichoderma* **spp. i.e.** ***Trichoderma hamatum*** **(Tm2) and** ***Trichoderma viridi*** **were irradiated at doses of 0.2, 0.4 and 0.6 kGy and one isolate from** ***Bacillus subtilis*** **was irradiated at doses of 0.5, 1.0 and 1.5 kGy, the irradiated bioagents were used for determination the antagonistic activities against fungal pathogens in dual culture laboratory conditions, non irradiated bioagents were used as control.**

Compared to the control (non irradiated) biocontrol agents namely, *T. hamatum* (Tm2) and *T. viridi*, the irradiated strains at 0.2 kGy (Table 3) showed a significant inhibition to all the fungi causing pepper rot, while the biocontrol agents exposed to does higher than 0.2 kGy (0.4 or 0.6 kGy) have no effect on the growth of causal agents. In the same time, the irradiated *B. subtilis*, has significant inhibition of the causal agents when exposed to doses of 0.5 and 1kGy, which shown the higher inhibition of growth causal agents compared to the control (non irradiated) while *B. subtilis* exposed to 1.5 KGy has no effect on the growth of causal agents.

Pepper fruits genotype i.e. Geadon (the highest susceptible) wounded, unwounded and neck infected were sprayed with the biological control agents i.e. *B. subtilis, T. viride* and *T. hamatum* (non irradiated and irradiated). Discs of infected species i.e. two isolates of *F. oxysporum*, one isolate of each of *F. semitectum, Alt.alternata, A. citri, Asp. niger* and *Asp. flavus* were spotted on the sprayed pepper fruits and left for 15 days. The infected area measured in cm.

B. subtilis species was irradiated at 1 kGy while *T. viride* and *T. hamatum* were irradiated at 0.2 kGy and examined as biological control for the infection caused by seven species, non irradiated species were used as control.

Table (3): Effect of biocontrol agents exposed to different doses (in kGy) of γ-radiation on the growth of fungi causing pepper fruit rot under laboratory conditions.

Isolate	*T. hamatum* 2				*T. viride*				*B. subtilis*			
		0.2	0.4	0.6	C	0.2	0.4	0.6	C	0.5	1.0	1.5
F. oxysporum 1	3.0	0.6	3.0	3.0	3.5	2.0	3.0	3.5	5	3.0	1.5	3.0
F. oxysporum 2	4.5	1.5	3.0	4.5	1.5	0.5	1.5	1.5	4.6	2.5	1.3	3.5
F. semitectum	3.3	0.5	2.5	2.5	1.5	0.7	2.0	2.0	4.0	3.5	1.0	4.5
A. alternata	2.0	0.5	2.0	2.5	2.0	0.5	1.5	1.5	5.0	1.0	0.5	4.5
A. citri	1.0	0.2	1.3	1.3	1.3	0.5	1.5	1.5	4.9	4.0	3.0	5.0
Asp. niger	2.5	1.5	3.0	2.5	1.3	0.5	1.5	1.5	3.8	2.0	0.5	1.7
Asp. flavus	1.5	0.5	1.5	1.5	2.0	1.0	2.0	2.5	4.7	1.4	0.6	1.5

C: control , 0.2, 0.4, 0.6, 0.5, 1.0&1.5: doses of γ-radiation.

Data in Table (4) showed that *B. subtilis* irradiated at 1 kGy has the highest effect compared to the non irradiated species especially with the unwounded fruit. The infected area measured between 10.87-19.0 cm when the non irradiated *B. subtilis* was used, while the infected area ranged from 0.13 to 1.17cm when irradiated *B. subtilis* was used. Also it was noticed that there was no detected infection of any of the infectious agents.

In case of using *Trichoderma* spp., it was noticed that the irradiated *T. viride* and *T. hamatum* have the highest effect on the growth of infections species compared to the non irradiated species. *T. viride* the infected area ranged from 10.9 to 18.5 cm with the non irradiated species, while the infected area measured between 0.03- 1.47cm with irradiated species. On using *T. hamatum* the infected area measured between 11.2-17.3 with non irradiated species and 0.1-1.4 cm with the irradiated species.

These results indicated that the increase in the antagnestic activity of irradiated *B. subtilis* or *Trichoderma* species may be due to the increase in the production of secondary metabolites responsible for the antifungal activity

Table (4): Effects of γ-irradiation on three bioagents in relation to pepper fruit rot infection causing by seven causal organisms.

Isolate	Isolate	*T. hamatum*			*T. viride*			*B. subtilis*		
		W	Un	N I	W	Un	NI	W	Un	NI
F. oxysporum 1	Non	17.3	13.9	13.43	14.9	15.17	12.57	13.8	11.9	11.4
	Irr	0.2	0.13	0.2	0.37	0.0	0.57	0.93	0.0	0.33
F. oxysporum 2	Non	13.1	11.2	13.33	12.1	11.87	12.23	12.23	12.0	19.0
	Irr	0.1	0.1	0.17	0.33	0.0	0.43	0.3	0.0	1.06
F. semitectum	Non	12.8	12.6	16.77	11.2	13.2	13.5	11.9	11.03	12.53
	Irr	1.4	0.3	0.27	1.47	0.2	0.41	0.23	0.03	0.37
A. alternata	Non	15.1	11.9	12.1	10.9	12.17	11.97	12.53	10.87	12.67
	Irr	0.2	0.17	0.33	0.3	0.3	0.77	0.2	0.0	0.17
A. citri	Non	12.6	17	12.3	11.7	11.3	15.43	11.43	11.6	11.93
	Irr	0.9	0.87	0.27	0.13	0.13	0.23	0.33	0.2	0.13
Asp. niger	Non	15.9	12.3	15.47	13.5	11.8	13.1	14.6	11.6	11.63
	Irr	0.1	0.37	0.2	0.37	0.23	0.13	1.17	0.17	0.37
Asp. flavus	Non	16.3	12.7	17.17	18.5	11.8	11.17	17.13	12.17	11.6
	Irr	0.1	0.3	0.17	0.33	0.33	0.03	0.77	0.3	0.7

W: Wounded, Un: Unwounded, NI: Nick infection, Non: non irradiated, irr: irradiated

In conclusion, this study has investigated the selection of a set of microorganisms that can act as antagonists against pepper fruit rot pathogens based on their ability to inhibit more or less strongly their mycelial growth and/or sporulation. Among the in vitro selected antagonists, one isolate of *B. subtilis*, and 5 isolates of *Trichoderma* have also the ability to induce defense reactions of pepper without causing any seedlings mortality.

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زيادة المقاومة البيولوجية لبعض الميكروبات ضد الفطريات المسببة لعفن ثمار الفلفل باستخدام أشعة جاما

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الملخص العربي

تم فى هذا البحث دراسة تاثير المقاومة البيولوجية على نمو سبعة عزلات ممرضة كمسببات لعفن ثمار الفلفل وكانت كالتالى, ٣ عزلات من *الفيوزاريوم*, عزلتان من *الألترناريا* وعزلتان من *الأسبراجيلس*. تم استخدام ٨ عزلات من جنس *التريكوديرما* وعزلة من جنس *الباسيلس ساتلس* لتحديد المقاومة البيولوجية لهذه العزلات المضاد لنمو الفطريات الممرضة المسببة لعفن ثمار الفلفل فى الظروف المعملية والحقلية.

وقد استخدمت كمقاييس للمقاومة البيولوجية للفطريات الممرضة والمعزولة من ثمار فلفل مصابة بأعفان الثمار فى محافظة المنوفية ثلاثة مقاييس هى قطر النمو ومعدل اختزال النمو% ثم مقاييس المقاومة الحيوية من نمو زائد على الفطر الممرض ومقاييس التضاد ومنطقة التثبيط حيث وجد ان *الباسيلس ساتلس والتريكوديرما هاماتم والتريكوديرما فيردى* هى اكثر القوى الميكروبية نشاطا ضد نمو الفطريات الممرضة. وجد كذلك أن تعريض سلالة *لباسيلس* لجرعة ١ كيلو جراى وسلالتى *التريكوديرما* لجرعة ٠,٢ كيلو جراى من اشعة جاما قد ادى الى زيادة النشاط المضاد لهذه السلالات ضد نمو الميكروبات الممرضة بصورة عالية جدا مقارنة بالكنترول وذلك بباقى كائنات التضاد الحيوى المختبرة.