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Biological Control of Postharvest Date Fruit Rots using Microbial Bioagents and Bio-Fungicides

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Abstract: Postharvest diseases cause considerable losses to harvested date fruits during transportation and storage. 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. In this study Trichoderma harzianum (1), T.asperellum, Trichoderma longibrachiatum, T.album, Trichoderma harzianum (2), and T.34 (T.asperellum) revealed different degrees of hyper parasitism to the growth of A.alternata, C.herbarum and Th.paradoxa on PDA medium. The greatest reduction occurring for C.herbarum, followed by A.alternata, while Th.paradoxa was less affected ones. Bacterial bioagents tested had inhibitory effect on the mycelial growth of the pathogenic fungi A.alternata, C.herbarum and Th.paradoxa compared with control. In this case, Brevandimonas vesicularis and Paenibacillus polymyxa gave the highest reduction of the mycelial growth of A.alternata, B.subtilis (1) and Ps.fiuorescens (1) came in the last. In addition, Different concentrations of four bio-fungicides and bacteriocides were evaluated for their effectiveness to control date fruit rots under cold storage during 2020 and 2021 seasons. Futhermore, using (bio-control) followed by plant guard and Bio-Arc at 3g/L.W were the most effective biocides which minimized date fruit rot caused with A.alternata to a large degree compared with untreated date fruits. Generally, all tested fungicides and bio-agents were effective against the causal fungus in vitro and in vivo trials, but with different degrees.

Keywords: Date palm, Biological control, Fruit rots, Microbial bioagents and Bio-fungicides

Introduction

The date palm (Phoenix dactylifera L.) is one of the oldest cultivated fruit trees in the world [1]. It has been cultivated in the Middle East and North Africa for over 5,000 years [2], belongs to the Arecaceae palm family, and can live for over 100 years [3]. Date is considered as one of the leading fruit crops. In Egypt, it covers a large area that extended from Aswan to the North Delta; Egypt is considered the leading country in producing dates. The total number of females reached 14865631 palms and their yield reached about 1710603 ton. Among all the Egyptian Governorates, Aswan Governorate is considered to be one of the leading Egyptian Governorates in dry dates production [4].

The most important dry date varieties in Egypt are Bartamoda, Barakawi, Abrimi, Sakouti, Gondaila and Shamia. A number of postharvest diseases attack date fruits during harvesting, packing, handling, marketing and storage. Date fruits are attacked by several diseases such as A.alternata, C.herbarum and Th.paradoxa, Aspergillus niger, Penicillium spp. and Rhizopus stolonifer [5]. Alternaria, Aspergillus, Fusarium and Penicillium, have been reported to cause fruit rots of date palm [6]. Penicillium, Cladosporium, Aspergillus and Alternaria fungi were the most genera in both part of date fruits [7]. Most postharvest rots of several fruits could be reduced considerally by

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spraying with spores of antagonistic fungi at different stages of fruit development or by dipping the harvested fruits in their suspensions [8].

Inhibitory effect of bio agents, *T. harzianum*, *T.hamatum, T.asperellum, B.subtilis, Penibacillus polymyxa, B.megaterium* and *Ps.fluorescens* against the causal pathogens of date palm root rot under Lab. Conditions. *Trichoderma* isolates were active more than *Bacillus* and *Pseudomonas* isolates inhibition of linear growth of the pathogenic fungi. It was found that the tested bio control agents significantly decreased rotted plants and increased survival plants compared with the control [9]. Biological control of plant pant pathogens by antagonistic microorganisms was a potential non-chemical means and was known to be a cheap and effective eco-friendly treatment for the management of crop diseases. The use of biological control agents as an alternative to fungicides was increased rapidly in the present day agriculture due to the deleterious effects of chemical pesticides [10]. The use of bioagents has become important as an alternative to fungicides to prevent postharvest losses. Under Lab. Conditions dual culture of nine bacterial bio-agents isolate *Bacillus megaterium, Bacillus subtilis, Bacillus cereus, paenibacillus polymxa, pantoea agglomerans, Ps.fluorescens* and 3 fungal bioagents *trichoderma harzianum* were tested for antagonistic properties against *Alternaria alternata.* They were found that all bio-agents have an inhibitory effect on the growth of pathogen fungus *in vitro pantoea agglomerans* was the most effective isolate (79.76%) of bio-agent bacteria isolates. All the bio-agent fungal isolates showed high hyperparasitic effect. It was important to carry out studies *in vivo* bioassays in order to control postharvest decay with bacterial and fungal bio-agents which were determined to be effective [11].

Therefore, the main goal of this study is to find the effect of biological control of postharvest date fruit rots using microbial bioagents and bio-Fungicides on major "Bartamoda" date fruits were studied.

Materials and methods

1. Identification of mycoflora associated with diseased date fruits:

Diseased date fruits showing various types of rots were collected from date orchards, store houses, retail and wholesale markets at Aswan Governorate during 2019 season and used for mycoflora isolation. Small pieces of each collected date fruits were first washed with running water, dried between two filter papers, then cut and sterilized with 70% alcohol solution for two minutes, then dried using sterile filter papers. The sterilized pieces were directly transferred to petri dishes containing PDA medium, then incubated at 25±27°C for 5-7 days. Fungal hyphal tips or single spores were taken from growing colonies, transferred to PDA medium slant tubes, incubated for 7 days at 25°C, then kept in refrigerator at 5°C, as stock culture for further studies. Identification of mycoflora obtained was carried out in the Agric. Botany Dept., (Pathology branch) Fac. of Agric., Al-Azhar Univ. using the key of Imperfect Fungi according to [12].

2. Pathogenicity tests:

Apparently healthy mature date fruits of Bartamoda cv. were selected, washed by dipping in tap water for 5 minutes, rinsed by sterile water, then kept to dry at Lab. Temperature. Before, inoculation, date fruits were surface sterilized in 70% ethyl alcohol. Inoculation tests were used with the fungal growth of *A.alternata, C.herbarum, Th.paradoxa, Fusarium moniliforme, Aspergillus flavs, A.niger* and *Rhizopus stolonifer.* The fruits were scratched using sterile (0.5 mm deep and 1.0 mm long almost). Inoculation was made by spraying scratched fruits with spore suspension of the tested fungi. The spore suspension inoculum was adjusted directly before inoculation. Sterilized distilled water was

used for spraying Bartamoda date fruits that served as control. Fifteen date fruits were utilized for each replicate, each treatment contained three replicates. The inoculated fruits were packed in perforated boxes, and then stored at 25°C in refrigerator for 15 days. Data for diseases development were recorded after 15 days from starting time.

Severity of infection was determined as a percentage of the length of the external rotted area in proportion to the total length of the fruit according to [13]. While, percentage of infection was determined by counted of number infected fruits compared with total number of fruits and percent of infection was calculated as follows:

$$\text{Infection\%} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits}} \times 100$$

3. Biological control:

3.1. Evaluation of antagonistic activity of *Trichoderma* isolates against pathogenic fungi *in vitro*:

The used antagonistic fungi were *Trichoderma harzianum* (isolates No.1and7), *T.asperellum, T.34* (*asperellum), T.longibrachiatum* and *T.album* provided from biological control unit, ARC,Giza.

While, *T.34* (*asperellum)* was obtained on PDA medium from the commercial product (Bio control T.34) provided by Shoura chemical company. *Trichoderma* isolates and the tested pathogenic fungi *A.alternata,C.herbarum* and *Th.paradoxa* were grown on PDA medium for 7 days at (25°C±2°C) and used in this study. Petri dishes containing PDA medium were divided into two equal halves. The first half was inoculated with a disc (6 mm in diameter) of one *Trichoderma* isolates used in this study.

The second half were inoculated with an equal disc of *A.alternata, C.herbarum* and *Th.paradoxa* . Each treatment was replicated five times. The tested fungi were grown on PDA medium only and without Bio control agents served as control. All petri plates were incubated at 25c°±2c°until fungal growth of control grew to full petri plates. At the same time, the fungal growth was measured and recorded. The percentage of mycelial growth inhibition was calculated according to the following formula:

% of mycelial growth inhibition= (A-B /A) X 100

Where:

A= length of mycelial growth in control.

B= length of mycelial growth in treated Petri plates.

3.2. Evaluation of antagonistic bacteria against the pathogenic fungi *in vitro*:

The antagonistic bacteria *Pseudomona fluorescens* (isolates No.1 and 2), *Brevandimonas vesicularis, Bacillus Megaterium* (isolates No.1 and 2), *Bacillus subtilis* (1 and 2) and *Penibacillus Polymyxa* were obtained from Merin, Fac. of Agric. Ain Shams Univ. these isolates were tested against the tested pathogenic fungi *A.alternata ,C.herbarum* and *Th.paradoxa* under vitro conditions . The antagonistic bacteria were grown on each of nutrient sucrose agar and PDA media for 7days at 25° C ±2°C. Nutrient sucrose agar medium consisted of, peptone 5gm, beef extract 3gm, sucrose 5gm, yeast extract 2gm, agar 20gm and distilled water 1 Liter.

A 6mm in diameter mycelia disc of the pathogenic fungus taken, advancing zone of growing hypha was inoculated at the center of 9cm diameter petri plates containing nutrient and /or PDA media. On the other hand, the antagonistic

bacteria streaked at a distance of 2-3cm either in semicircular or in a circular pattern. Petri dishes were incubated at 25°C ±2°C and inhibition zone was examined after 24, 48, 72 and 96 hours. Each treatment was contained 5 petri dishes. Petri plates inoculated with the tested fungi only were used as control. Percentage of growth inhibition of pathogen was measured and calculated, when pathogen achieved full growth by the following formula:

Percentage inhibition of radial growth =

$$\frac{\text{Growth control (cm3)} - \text{Growth treatment (cm3)}}{\text{Growth control}} \text{ X } 100$$

3.3. Growing of bacterial isolates in liquid medium:

The bacterial isolates were grown in flasks of Gliotoxin fermentation medium (GFM) composed of [14].

GFM medium:

Dextrose	25gm
Ammonium tartarate	2gm
KH2PO4	2gm
MgSO4	1gm
FeSO4	0.01gm
Distilled water	1000ml

Each flask containing 200ml of the nutrient liquid medium was inoculated with bacterial cells obtained from (nutrient agar medium) growing for 4days old. These flasks were incubated under complete darkness at (25°C ±2°C) for 7, 14 and 21 days. The obtained cultures were used for further studies.

To study the effect of incubation period on filtrate production (Toxin), GFM medium was used against for growth of *A.alternata, C.herbarum* and *Th.paradoxa*. Fifteen flasks each contained 200 ml. Of GFM medium were used for growing each of *B. subtilis, B. Megaterium*, *P. fluorescens* and *B. vesicularis*. The filtrate of each bacterial isolate was obtained every week using sterilized filter. Culture filtrate was added to warm sterilized PDA medium at the rate of 5, 10 and 15% (v:v), then poured in petri plates. Five petri plates were inoculated with *A.alternata, C.herbarum* and *Th.paradoxa*. Petri plates containing PDA+ 5, 10 and 15% sterilized GFM medium (no bacterial isolates) were served as control. All treatments were incubated at Lab. Temperature (25°C ±2°C) when fungus mycelium covered one plate in control. Treatments, mycelial growth of the tested fungi in both treatments were measured and the average growth was calculated to find out the correlation between toxin concentration and mycelial growth.

4. *In vivo* experiments:

4.1. Effect of certain preharvest Bio-cides treatments on date fruit rots:

Six biofungicides were used to study their effect on controlling date fruit rot diseases in the field during 2019 season. Immature Bartamouda bunches grown at Al-Adowa province, Edfou, Aswan Governorate were sprayed with certain biofungicides. Bunches were sprayed with each of Plant guard (*T.harzianum*) 30× 10^6 spores/g, Bio-Arc (*Bacillus Megaterium*) 25×10^6 cells/g, Bio-Zeid (*T.album*) 10×10^6 spores/g, T-34 biocontrol (*T.asperellum*) 12×10^6 spores/g, Rizo-N (*B.subtilis*) 30×10^6 cells/g and Sernado (*B.subtilis*) 1.34 %Sc at the rates of 1, 2 and 3ml or gm/liter water three times, 21days between each of them. Six bunches of date fruits were used for each fungus. Each bunch was divided into three parts, each part was used for each concentration, as well as each part was separated about others with

polyethylene bags. Bartamouda bunches receiving no biofungicides were used as control. The bunches were kept under observation for 45 days. The natural infection on fruits were determined in the treated, as well as in the untreated bunches after harvesting and storing for 7days as mentioned before.

4.2. Effect of different biocides on date fruit rots:

Four biofungicides i.e. Plant guard, Bio-Arc, T34 (biocontrol) and Bio-Zeid at 1, 2 and 3 ml or gm were tested for control of date fruit rot diseases caused by *A.alternata ,C.herbarum* and *Th.paradoxa.* Bunches of mature date fruits Bartamoda variety obtained from Al-Adowa province were evaluated under Lab. Conditions. Any, bruised, wounded or unrepresentable fruits were discarded. Healthy date fruits were selected. Fruits were washed thoroughly with tap water, followed by sodium hypochlorite detergent for 5 minutes, and then left to air dry. Standard amount of inoculum of each fungus to be tested were introduced through small hole in the middle of fruit. Twenty four hours after inoculation, date fruits were immersed for 5 minutes in different concentrations of the tested biofungicides. The required concentrations of the biofungicides used freshly prepared lie for the experiment was performed. Similarly treated fruits immersed in sterile water were served as check. For each treatment, replicates were used and each replicate contained 30 fruits. Fruits were allowed to dry and packed in to perforated plastic boxes and stored for 15 days in refrigerator at 25c°±2c°. The amount of the disease severity was estimated by measuring the length of the rotted part of the fruit.

5. Statistical analysis:

Analysis of variance of the data was carried out on the mean values of the tested treatments according to the procedures described by [15]. The least significant difference (L.S.D) at 5% probability was used for testing the significance of the differences among the mean values of the tested treatments for each character.

Results and discussion

1. Biological control:

1.1. Effect of fungal bioagents against pathogenic fungi:

Trichoderma harzianum (1), *T.asperellum***,** *Trichoderma longibrachiatum***,** *T.album, Trichoderma harzianum* (2), and T.34 (*T.asperellum*) revealed different degrees of hyper parasitism to the growth of *A.alternata*, *C.herbarum* and *Th.paradoxa* on PDA medium. Table (1) exhibited hyper parasitism between *Trichoderma spp.* and the three tested fungi causing date fruit rot diseases. Different rates of growth inhibition were induced by the antagonists. It was clear from Fig (1) that the mycelium of *Trichoderma spp.* grew rapidly over the mycelium of *C.herbarum* and prevented its growth and developments, Meanwhile, *Trichoderma spp* gave the greatest inhibition of the mycelial growth of *C.herbarum*, as reached 100% growth inhibition, except *T.album,* which gave 82.88% growth inhibition *Alternaria alternata* was reduced by 72.2, 70.22, 68.88 and 67.77% respectively, in presence of *T. asperellum*, *Trichoderma harzianum* (2), T.34 and *T. longibrachiatum* while, *T.harzianum* (1) and *T.album* caused 53.68 and 48.44% reduction in mycelial growth of *A.alternata*. The same trend was observed in case of *Th.paradoxa,* wherever *T.harzianum* and

T.34 gave the best effect in reducing the linear growth 73.11 and 70.22%, followed by *T.asperellum* and *T.harzianum* (1), as reached 66.88 and 54.66% inhibition percentage. In this respect, *T. longibrachiatum* and *T.album* were less effective in reducing the mycelial growth of *Th.paradoxa,* as reached 47.33 and 35.77%, respectively. Generally, the greatest reduction occurring for *C.herbarum,* followed by *A.alternata,* while *Th.paradoxa* was less affected ones.

Table (1): Efficacy of fungal bioagents against postharvest diseases of date *in vitro*.

Fungal bioagents	Colony diameter of pathogens (cm)			Mycelial growth inhibition %		
	A.alternata	*C.herbarum*	*Th.paradoxa*	*A.alternata*	*C.herbarum*	*Th.paradoxa*
***Trichoderma harzianum* (1)**	4.16	0.0	4.08	53.7	100.0	54.66
Trichoderma asperellum	2.5	0.0	2.98	72.2	100.0	66.88
Trichoderma longibrachiatum	2.9	0.0	4.74	67.77	100.0	47.33
Trichoderma album	4.64	1.54	5.78	48.44	82.88	35.77
***Trichoderma harzianum* (2)**	2.68	0.0	2.42	70.22	100.0	73.11
***Trichoderma asperellum* (T.34)**	2.80	0.0	2.68	68.88	100.0	70.22
Control	9.0	9.0	9.0	0.0	0.0	0.0

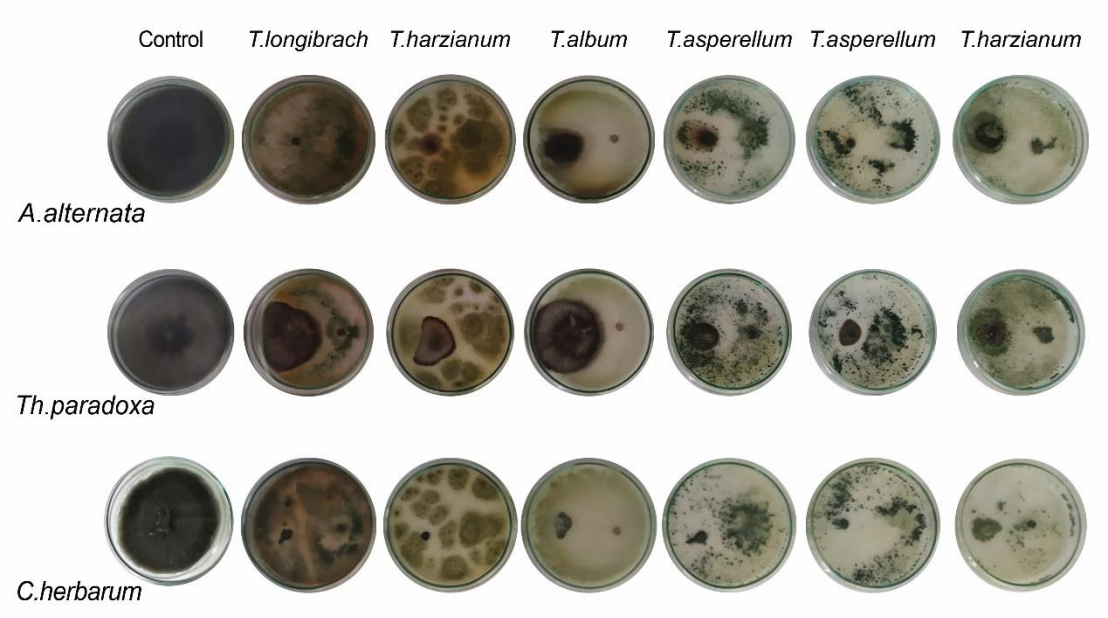

Figure (1): Antagonistic effect of *T. longibrachiatum*,*T.harzianum* (1), *T.album*, *T.asperellum* (1), *T.asperellum* (2) and *T.harzianum* (2) aginst the pathogenic fungi *A.alternata*, *C.herbarum* and *Th.paradoxa* under lab. conditions.

*Trichoderma spp***.** have been to be effective in suppressing plant diseases caused by fungi. Modes of action of beneficial microorganisms in suppressing plant diseases include direct parasitism of pathogens, competition for space or nutrients or the production of antibiotics and enzymes [16]. [17] who reported that *Trichoderma spp.* exhibited the appreciable

inhibition of mycelial growth of the tested fungi. Also, the obtained results are in accordance with those reported by [18 -19] who mentioned that all tested antagonistic *Trichoderma spp*. inhibited growth of the tested pathogenic fungi.

1.2. Effect of certain bacterial bioagents on the mycelial growth inhibition of the tested fungi:

The efficacies of different bacterial bioagents were investigated on the mycelial growth of the tested fungi to study their antagonistic effects under vitro conditions. Data in Table (2) and Figure (2) revealed that bacterial bioagents tested had inhibitory effect on the mycelial growth of the pathogenic fungi *A.alternata*, *C.herbarum* and *Th.paradoxa* compared with control. In this case, *Brevandimonas vesicularis* and *Paenibacillus polymyxa* gave the highest reduction of the mycelial growth of *A.alternata*, as reached 56.33 and 53.33%, respectively. At the same time, *Ps.fiuorescens* (2), *B.megaterium* (1), followed by *B.subtilis* (2) exhibited the moderate effect in reducing mycelial growth of the same fungus, whereas, mycelial growth inhibition reached it 45.55, 41.88 and 40.44%, respectively. As mean,

B.megaterium (2), *B.subtilis* (1) and *Ps.fiuorescens* (1) gave the lowest effect in decreasing mycelial growth inhibition of the same fungus. On the other hand, *B. vesicularis* exhibited the most effect in suppressing mycelial growth of *C.herbarum,* which entirely suppressed mycelial growth of this fungus. Meanwhile, *Ps.fiuorescens* (2), *B.megaterium* (2), followed by *Paenibacillus polymyxa* revealed the best effect in reducing mycelial growth inhibition of *C.herbarum* to a large degrees, as recorded 93.77, 84.88 and 81.11%, respectively. Whatever, *B.megaterium* (1) and *B.subtilis* (2) gave moderate effect in reducing mycelial growth inhibition of the same fungus, as recorded 77.77 and 76.0%, respectively. On the converse, *B.subtilis* (1) and *Ps.fiuorescens* (1) came in the last, reached it 68.55 and 56.33%, respectively.

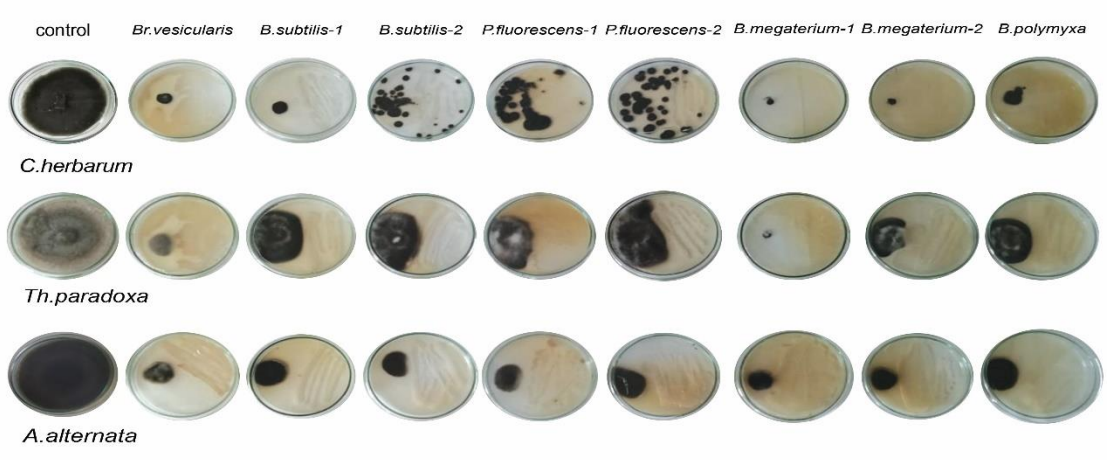

Figure (2): Antagonistic effect of certain bacterial isolates. i.e. *B.vesicularis , B. subtilis* (1)*, B. subtilis* (2)*, P. fluorescens* (1), *P. fluorescens* (2), *B. Megaterium* (1)*, B. Megaterium* (2) and *Paenibacillus Polymyxa* aginst the pathogenic fungi *.A.alternata, C.herbarum and Th.paradoxa i* under lab. Conditions.

Table (2): Antagonism of bacterial bioagents against postharvest pathogens of dates on PDA medium.

Bacterial Bioagents	Colony diameter of pathogens (cm)			Mycelial growth inhibition %		
	A.alternata	*C.herbarum*	*Th.paradoxa*	*A.alternata*	*C.herbarum*	*Th.paradoxa*
***Pseudomonas fluorescens* (1)**	5.70	3.93	4.76	36.66	56.33	47.11
Pseudomonas fluorescens (2)	4.90	0.56	4.33	45.55	93.77	51.88
Brevandimonas vesicularis	3.93	0.0	2.80	56.33	100.0	68.88
B. megaterium (1)	5.23	2.0	5.40	41.88	77.77	40.0
***B. megaterium* (2)**	5.43	1.36	5.20	39.66	84.88	42.22
***Bacillus subtilis* (1)**	5.43	2.83	5.30	39.66	68.55	41.11
Bacillus subtilis (2)	5.36	2.16	5.06	40.44	76.0	43.77
Paenibacillus polymyxa	4.20	1.70	4.40	53.33	81.11	51.11
Control	9.0	9.0	9.0	0.0	0.0	0.0

It was clear from the same Table that *B. vesicularis* recorded high efficacy in reduction of mycelial growth of *Th.paradoxa*. As regard, *Ps.fiuorescens*(2), followed by *Paenibacillus polymyxa* recorded moderate effect in reducing mycelial growth of the same fungus, reached it 51.88 and 51.11% mycelial growth inhibition, While, *Ps.fiuorescens*(1), *B.subtilis* (2), *B.megaterium* (2), *B.subtilis* (1), followed by *B.megaterium* (1), exhibited the lowest effect in decreasing mycelial growth inhibition, whearas the reduction reached it 47.11, 43.77, 41.11 and 40.0%, respectively. Among different biological approaches, the use of microbial antagonists such as bacteria offers an effective, safely and ecofriendly strategy to control many fungal disease [20]. The obtained results are in accordance with those obtained by [21 -22]. Results from bioassays suggest that production of antifungal substances by these bacteria may be responsible for the inhibition of fungal growth, where no direct contact between bacteria colonies and mycelial growth of the pathogenic fungi so that the fungal growth inhibition was caused by diffusion of substances into the agar medium. On the other hand, most of bacteria that used as biocontrol agents produce antibiotics responsible for their antifungal activities [23].

1.2. Evaluation of antagonistic activity of *Trichoderma* isolates against pathogenic fungi *in vitro*:

This study was carried out using four isolates of *Trichoderma longibrachiatum, T.harzianum* and *T.asperellum.* The cultures were left to grow on GFM medium for a period of 3 weeks at 27°C. A bioassay technique for toxin detection was applied weekly through the incubation periods. Data in Table (3) demonstrated that growth of the three tested fungi was retarded and inhibited to varying degrees. The toxic effect was produced from one week old culture of *Trichoderma spp.* The fungal growth of *C.herbarum* was decreased to a large degree with the three tested

concentrations (5, 10 and 15%), followed by *Th.paradoxa* and *A.alternata.* The culture filtrate of *T.album* and *T. asperellum* with all tested concentrations caused complete inhibition to growth of *C.herbarum.* However, *Th.paradoxa* and *A.alternata* growth was decreased to varying degrees compared with the control treatment. On the other hand, the toxin produced by *T. longibrachiatum* after one week was less effective on *Th.paradoxa* and *A.alternata*. The same trend was observed after two weeks, whereas, *A.alternata*, *C.herbarum* and *Th.paradoxa* growth was completely suppressed with filtrate of *T.asperellum* at 10 and 15%. Also, growth of, *C.herbarum* was entirely inhibited with filtrate of *T.harzianum* at 10 and 15 %. Meanwhile, growth of *C.herbarum* and *Th.paradoxa* was inhibited by filtrate of *T.album* at 15%. In this respect, *T. longibrachiatum* with all tested concentrations was effective in inhibiting growth of *A.alternata* and *Th.paradoxa* .It was clear from the same data that all tested *Trichoderma spp.* with all concentrations 5, 10, and 15% completely inhibited *A.alternata*, *C.herbarum* and *Th.paradoxa*. At the same time, *T.asperellum* entirley suppressed growth of *Th.paradoxa*. Also, *T. longibrachiatum* caused complete inhibition when used at 10 and 15%, while *T. longibrachiatum* and *T.harzianum* each at 15% caused the same effect.

Table (3): Bioassay of the toxins produced by *Trichoderma longibrachiatum, T.harzianum, T.album* and *T.asperellum* later incubated for different periods on growth of the tested fungi.

Incubation periods fungi	Filtrate concentration (%)	Control	Linear growth of the tested fungi											
			A.alternata				*C.herbarum*				*Theilav.paradoxa*			
			T.long	T.album	T.h	T.asp	T.long	T.album	T.h	T.asp	T.long	T.album	T.h	T.asp
One week	**5**	**9.0**	7.00	2.15	4.80	2.25	2.35	0.00	2.80	0.00	5.85	2.95	5.50	2.25
	10	**9.0**	6.15	1.45	3.80	1.75	1.70	0.00	2.25	0.00	4.30	1.95	3.25	1.80
	15	**9.0**	4.40	1.10	2.25	1.25	1.10	0.00	2.10	0.00	2.90	1.55	2.75	1.20
Two week	**5**	**9.0**	7.95	2.95	6.60	3.50	3.00	1.80	1.95	2.35	5.00	2.20	5.80	2.45
	10	**9.0**	6.80	2.65	5.45	0.00	2.40	1.60	0.00	0.00	4.15	1.75	4.75	0.00
	15	**9.0**	5.95	1.85	3.55	0.00	1.80	0.00	0.00	0.00	1.50	0.00	3.70	0.00
Three week	**5**	**9.0**	3.10	1.45	2.95	2.10	0.00	0.00	0.00	0.00	2.45	1.85	3.60	0.00
	10	**9.0**	0.75	2.15	2.10	1.85	0.00	0.00	0.00	0.00	0.00	1.05	2.25	0.00
	15	**9.0**	0.00	2.00	0.00	0.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

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Generally, fungal growth inhibition was obvious at the beginning of the incubation period and then inhibition activities were lowered according to the tested *Trichoderma sp.* and its age where isolates of *Trichoderma* were varied in toxin production. Biological control is defined as the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state by one or more organisms. *Trichoderma* is among the most common saprophytic fungi. Many *Trichoderma* strains have been identified as having potential applications in biological control, being effective against a wide range of plant pathogenic fungi [24]. Many recent studies have revealed the effect of various *Trichoderma spp.* on postharvest rots caused by many fungal pathogens [25]. *Trichoderma harzianum* is used to control the fungal diseases caused by *A.alternata*, *Penicillium expansum, Botryis cinerea* and many other fungi. These results are in agreement with those obtained by [26 -27 -28].

1.3. Evaluation of antagonistic bacteria against the pathogenic fungi *in vitro*:

The efficacy of some bacterial bioagents on the mycelial growth of the tested fungi was investigated to study their antagonistic effect under vitro conditions. Theinhibitory effect of biological control agents, *B.subtilis, B.megaterium, P.fluorescens* and *B.vesicularis* was shown in Table (4). Data presented in the same Table showed that, all culture filtrates of the tested bacterial bioagents suppressed and reduced the mycelial growth of *A.alternata*, *C.herbarum* and *Th.paradoxa* fungi. *B.subtilis, B.megaterium, P.fluorescens* and *B.vesicularis* were the best antagonistic bacteria in suppressing *C.herbarum,* they caused complete suppression of the tested fungi, except *B.subtilis* with all tested concentrations after one week, which gave lower mycelial growth of *A.alternata*, then completely suppressed the mycelial growth after two and three weeks.

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Table (4): Bioassy of the toxins produced by *B.subtilis, B.megaterium, Ps. Fluorescens* and *Brevandomonas vesicularis* later incubated for different periods on growth of the tested fungi.

Incubation periods bacteria	Filtrate concentration (%)	Control	Linear growth of the tested fungi											
			A.alternata				*C.herbarum*				*Thielav.paradoxa*			
			B.su	B.m	P.f	Brev.	B.su	B.m	P.f	Brev.	B.su	B.m	P.f	Brev.
	5	**9.0**	1.95	2.40	1.95	2.0	1.80	0.00	0.00	0.00	2.55	2.20	0.00	1.50
One week	**10**	**9.0**	1.90	2.05	1.50	1.70	1.50	0.00	0.00	0.00	1.95	1.55	0.00	1.30
	15	**9.0**	1.40	1.80	1.30	1.05	1.40	0.00	0.00	0.00	1.55	1.25	0.00	1.00
	5	**9.0**	1.20	1.95	1.40	1.10	0.00	0.00	0.00	0.00	2.40	1.65	0.00	1.45
Two week	**10**	**9.0**	1.20	1.85	1.15	0.90	0.00	0.00	0.00	0.00	1.80	1.35	0.00	1.20
	15	**9.0**	1.00	1.60	1.25	0.90	0.00	0.00	0.00	0.00	1.60	1.00	0.00	0.80
	5	**9.0**	2.35	1.50	2.20	0.00	0.00	0.00	0.00	0.00	1.30	1.50	1.40	1.00
Three week	**10**	**9.0**	1.95	1.60	1.85	0.00	0.00	0.00	0.00	0.00	1.15	1.20	0.95	1.00
	15	**9.0**	1.50	1.40	1.55	0.00	0.00	0.00	0.00	0.00	0.05	1.00	0.45	0.00

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At the same time, *P.fluorescens* was the best bioagent in suppressing the fungal growth of *Th.paradoxa* with all concentrations tested after one and two weeks. Also, *B.vesicularis* gave the same effect in prevention mycelial growth of *A.alternata* after three weeks. The same trend was observed in case of *B.vesicularis* with 15% after three weeks, which entirley prevented the mycelial growth of *Th.paradoxa.* In this respect, all the tested bacterial bioagents at 15% gave the highest inhibition of the mycelial growth of the tested fungi after different periods, as well as all bioagents were active in prevention and reducing the linear growth of the tested fungi. Several bacteria have been identified to play an important role as biological control agents in controlling disease caused by many plant pathogenic fungi. Among different bacteria used as biological control agents, an isolate of *Pseudomonas fluorescens* has been reported to control many different fungi causing fruit rot diseases, [29], A bacterial biocontrol agent of the genus *Bacillus* uses nutrient and space competition, induced resistance, production of diffusible antibiotics, volatile compounds, toxins and cell wall degrading enzymes such as chitinase [30]. [28] who reported that *P. fluorescens* and *B.subtilis* were found to be effective against *B.cinerea, A.alternata* and corynespora cassiicola under vitro conditions.

2. *In vivo* experiments:

2.1. Effect of certain preharvest Biocides treatments on date fruit rots:

Preharvest applications were carried out to study the effect of commercial biocides on the development of date fruit rots caused by *A.alternata*, *C.herbarum* and *Th.paradoxa*. Date presented in Table (5) showed that T.34 *(T.asperellum)* with all concentrations tested was the most effective biofungicide in decreasing fruit rots caused with the three tested

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fungi, when applied as preharvest treatment. Plant guard biofungicide at 3g/L.W, followed with Bio-Arc at the same concentration were effective in minimizing date fruit rots caused with the same fungi to varying degrees. Also, Bio-z-Zeid was very effective at 3g/L.W in reducing fruit rots caused with *Th.paradoxa* and *C.herbarum*, while on *A.alternata* exhibited moderate effect Meanwhile, Rizo-N 3g/L.W gave slight decrease in disease severity caused with the pathogenic fungi *C.herbarum* and *Th.paradoxa,* while on fruit rot caused with *A.alternata* was the least effective bio-fungicide.

It was clear from the same data that Serrando bio-bacteriocide with all tested concentrations was the least effective in limiting fruit rots of dates. These findings are in agreement with those obtained by [31 -32- 19]. Biofungicides maybe microorganisms such as bacteria or fungi based product like secondary metabolites Biofungicides i.e.

Table (5): Evaluation of commercial bioagents as preharvest treatments on the incidence of postharvest rots of Bartamoda dates during 2019 season.

Commercial biocides	Conc. With g or cm/L.W	Severity of infection Artificial inoculation with 2019		
		A.alternata	*C.herbarum*	*Th.paradoxa*
Plant guard	**1cm**	20.85	26.35	19.87
	2cm	13.19	21.87	9.67
	3cm	7.29	9.25	3.71
Bio Arc	**1g**	17.19	13.54	18.90
	2g	14.92	11.75	14.55
	3g	8.33	9.77	9.37
Bio Zeid	**1g**	22.91	18.68	19.49
	2g	15.62	14.35	10.75
	3g	11.10	9.58	8.33
T-34	**1g**	17.49	17.92	13.10
	2g	8.33	11.85	7.29
	3g	2.77	4.29	3.45
Rizo-N	**1g**	28.34	24.65	24.98
	2g	22.90	15.25	18.54
	3g	15.80	11.10	11.80
Commercial *B.subtilis* (Serrando)	**1cm**	31.59	33.33	26.38
	2cm	25.04	26.0	20.87
	3cm	17.45	18.77	15.62
Untreated fruits	**---**	100.0	96.0	97.25
L.S.D. at 5% for	**B**	1.35	1.56	0.58
	C	1.40	1.04	0.95
	B x C	1.85	1.92	1.65

Plant guard, Bio-Arc, Bio-Zeid, T34 and Rizo-N suppressed the different growth by the producing secondary

metabolites like antibioties, cell wall degrading enzymes and hydrogen cyanide [33]. Also, many investigators showed that *Trichoderma spp.* have antagonistic effect against a wide range of the pathogens. The inhibitory effect of plant guard is related to the antagonistic action exerted by *T.harzianum*. No single mechanism of how *T.harzianum* is able to inhibit the growth of fungal plant pathogen is known. Metabolites produced by *T.harzianum* may also play a role in mycoparasitism of the hyphae. In addition, *T. asperellum* has been recently shown to induce systemic resistance in plants through a mechanism that employs Jasmonic acid and ethylene signal-transduction path ways [34].

2.1. Effect of different Biocides on date fruit rots:

Different concentrations of four bio-fungicides and bacteriocides were evaluated for their effectiveness to control date fruit rots under cold storage during 2020 and 2021 seasons. It was shown from data in Table (6) that all tested biocides significantly suppressed and decreasing the fungal infection of date fruit rots during 2020 season. The decreasing of fungal infection increased with increasing biocides concentrations from 1to 3g/L.W. Each of T.34 (bio-control) followed by plant guard and Bio-Arc at 3g/L.W were the most effective biocides which minimized date fruit rot caused with *A.alternata* to a large degree compared with untreated date fruits. Also, Bio-Zeid at the same concentration gave higher effect in reducing the fungal infection caused with the same fungus. As means, plant guard and T.34 (bio-control) at 3g/L.W entirely inhibited date fruit rots caused with *C.herbarum*. At the same time, Bio-Arc at 3g/L.W gave the highest reduction of date fruit rot caused with the same fungus, as recorded 2.4% , respectively. While, Bio-Zeid came in the last, as recorded 6.33% , relatively the reduction of disease severity increased with increasing the biocides concentration, Wherever, T.34 (bio-control), followed by Bio-Arc and plant guard each at 3g/L.W were more effective in limiting disease severity caused with *Th.paradoxa,* as recorded 2.65, 3.75 and 3.77% , respectively. Also, Bio-Zeid at 3g/L.W exhibited clear effect in reducing disease severity caused by *Th.paradoxa,* as reached 4.81%.

The same experiment was carried out the next season 2021 for confirmation of the obtained results. It was noted from data in Table (6) that disease severity decreased with increasing the biocides concentration during 2021 season. All biocides significantly reduced the fungal infection; however T.34 and Bio-Arc with all tested concentrations gave the best results in controlling date fruit rots caused by *A.alternata.* As regards, plant guard at 2 and 3g/L.W was effective against *A.alternata*, as recorded 8.66 and 3.45%, respectively. Also, Bio-Zeid at 2 and 3g/L.W exhibited moderate effect, while Bio-Zeid at 1g/L.W was the less effective in reducing the decay. The same data showed that plant guard and T.34 at 2and 3g/L.W were the most effective biocides in reducing disease severity caused with *C.herbarum.* The same trend was observed by using Bio-Arc at 3g/L.W. On the contrary, Bio-Zeid with all tested concentration was less effective in reducing disease severity. Data also revealed that T.34 at2g/L.W significantly decreased disease severity caused by *Th.paradoxa* as recorded 4.33%, while at 3g/L.W completely prevented fruit rot caused with the same fungus. At the same time, Bio-Arc followed by plant guard each at 3g/L.W were very effective in reducing disease severity caused by *Th.paradoxa*, as reached 2.77 and 3.33%, respectively. Meanwhile, Bio-Zeid at 3g/L.W gave the same effect. Generally, all biocides tested with high concentration 3g/L.W gave the highest reduction in disease severity caused with the tested fungi.

Table (6): Effect of the biocides on the incidence of postharvest diseases of Bartamoda dates under storage conditions during 2020 and 2021 seasons.

Biocides treatments	Conc. cm or g/L.w	Severity of infection					
		2020			2021 seasons		
		A.alternata	*C.herbarum*	*Th.paradoxa*	*A.alternata*	*C.herbarum*	*Th.paradoxa*
Plant guard	**1**	18.33	13.80	14.80	16.85	11.90	12.54
	2	6.25	5.15	8.33	8.66	4.25	7.95
	3	2.40	0.0	3.77	3.45	1.77	3.33
Bio-ARC (biocontrol)	**1**	16.40	13.10	13.19	14.90	13.10	10.25
	2	8.33	7.24	6.66	7.66	8.33	7.29
	3	2.77	2.42	3.75	1.33	4.15	2.77
T-34	**1**	10.75	11.45	13.66	13.75	10.25	9.80
	2	7.33	6.66	7.42	4.85	6.29	4.33
	3	1.65	0.0	2.65	2.77	2.33	0.0
Bio-zeid	**1**	13.45	13.90	16.65	17.10	15.33	14.33
	2	8.33	9.25	10.75	8.24	11.10	10.15
	3	4.15	6.33	4.81	5.33	7.29	5.90
Untreated fruits	**---**	100.0	100.0	96.33	100.0	98.0	97.66
L.S.D. at 5% for	**B**	1.55	1.35	1.45	1.87	1.54	0.92
	C	1.94	1.75	1.65	1..90	0.80	1.40
	B x C	2.85	3.10	3.30	2.45	1.75	2.58

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Most postharvest rots of several fruits could be reduced considerably by spraying with spores of antagonistic fungi at different stages of fruit development or by dipping the harvested fruit in their suspensions, [8]. When the antagonistic fungal cells come into contact with the fruit surface, they also occupy the wounds and affect the germination of pathogenic fungal apores [35]. These findings were in agreement with those previously obtained by [36 -32] who reported that biocides i.e. Bio-Zeid, Bio-Arc, plant guard and Rizo-N were significantly effective in controlling disease incidence these fore, Bio-Arc (*B.megaterium*) and Bio-Zeid (*T.album*) as biocides play a very useful role as effective and safe means in controlling root rots.

Conclusion

It is very clear that in recent years the interest in biocontrol strategies that minimize the use of chemical pesticides is a worldwide trend, which has driven research in this field. Biological control of postharvest date fruit rots using microbial bioagents and bio-fungicides associated with plants is an efficientand effective approach to control diseases and is considered environmentally friendly. A successful biocontrol agent is generally equipped with several mechanisms which often work in concert, and may be crucial in controlling disease development**.**

References

[1] Parvin, S.; Easmin, D. and Sheikh, A. (2015). Nutritional analysis of date fruits (*Phoenix dactylifera* L.) in perspective of Bangladesh, Asian J. Bio. Life Sci., 3 (4), pp. 274-278.

[2] Chao C.C.T. and Krueger R.R. (2007). The date palm (*Phoenix dactylifera* L.): overview of biology, uses, and cultivation. Horticultural scientists 42 (5): 1077–1082.

[3] Eoin, L.N. (2016). Systematics: blind dating. Nat. Plants 2:16069. Doi: 10.1038/nplants.2016.69.

[4] M.A.L.R. 2020

[5] Baraka, M.A., and Abdel-Sattar, M. A. (1985). Reaction of cantaloupe varieties to infection with fruit rot fungi. The first national conference of pests and diseases of Vegetables and Field crops in Egypt. Vol. II.Ismaialia,21-23.

[5] Baraka, M.A.; El-Tobshy, Zeinab, M. and Hanafy, S.M.(1985). Control of postharvest diseases of dates in Egypt. Annals of Agricultural science, Moshtohor.23(2):723-730.

[6] Attia,M.M.M. (2011). Efficiency of physical treatment and essential oil in controlling fungi associated with some stored date palm fruits. Australion journal Basic Applied science,5(6):1572.

[7] Edoardo, P.; Abdelfattah, A.; Danino yaara, S.S.; Feygenberg, O.; Spadaro, D.; Wisniewski, M. and Droby, S. (2020).characterizing the fungal microbiome in date fruit pulp and peel from early development to harvest. Microoganisms Journal,8:641

[8] Karabulut O.A. and Baykal N. (2003). Biological control of postharvest diseases of peaches and nectarines by yeasts.- Journal of phytopathology 151(3): 130-134

[9] Kasem, Aya, H.H. (2018). Studies on date palm root rot diseases under Aswan Governorate conditions. M.Sc. Thesis, Fac. Of Agric. South valley Univ.137pp**.**

[10] Mahmoud, S.; Ziedan, E.; Farrag, E. and Elymani, A. (2016). Antifungal activity of pyocyanin produced by *Pseudomonas aeruginosa* against *Fusarium oxysporum* schlecha root-rot phytopathogenic fungi. International Journal of pharm. Tech. Research,9(8):43-50.

[11] Tekiner, N.; Kotan, R.; Tozlu, E. and Dadasoglu, F. (2019) . Determination of some biological control agents against *Alternaria* fruit rot in Quince. Allan Teri J. of Agricultural Sciences, (Aliteri ziri Bilimler Dergisi) 34(1):25-

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(ASWJST 2023/ printed ISSN: 2735-3087 and on-line ISSN: 2735-3095) https://journals.aswu.edu.eg/stjournal

31.

[12] Barnett, H.L. and Hunter, B.B. (1986). Illustrated genera of imperfect fungi. 4th Ed., Macmillan Publishing Co., New York, 218 pp.

[13] Abdel-Sattar, M.A. (1978). Studies on banana fruit rot during development , harvesting and ripening in A.R.E. Ph.D. Thesis, Fac. Of Agric., Cairo univ. 213PP.

[14] Brain, P.W. and Hemming, H. G. (1945). Gliotoxin, afungistatic metabolic product of *Trichoderma Viride.* Ann. Appl. Biol., 32:412-420.

[15] Gomez, K.A. and Gomez, A.A. (1984): Statistical procedures for agricultural research, Second Edition, John Wiley & Sons, Inc., New York, 680 pp.

[16] Lugtenberg, B.J.J.; Bloemberg, G.V.; Woeng C.A, and Thomas F.C. (2003). Phenazines and their role in bio-control by Pseudomonas bacteria. New Phytologist 153: 503-523.

[17] Eshetu, B.; Amare, A. and Seid, A. (2015). Evaluation of local isolates of *Trichoderma*spp. against black root rot (*F. solani*) on faba bean. J. Plant Pathol. and Micro., 6 (6): 1-5.

[18] Akhtar, R. and Javaid, A. (2018). Biological management of basal rot of onion by *Trichoderma harzianum* and withania somnifera. Planta Daninha, 36.

[19] Abdelaziz, M, A. (2021). Microbiological and pathological studies on some garlic diseases. Ph.D. Thesis. Fac. of Agric. Minia Univ., 167pp.

[20] Gravel, V.; Martinez, C.; Antoun, H. and Tweddell, R.,J, (2004). Evaluation of antagonistic microorganisms as biological control agents (BCAs) of root rot *Pythium ultimum* of greenhouse tomatoes in rock wool. Canadian Journal of plant pathology 26:152-159.

[21] Haggag, Karima, H.E.; Elshahawy, I.E. and Abd-El-Khair, H. (2015). Antagonistic Activity of *Bacillus* and *Pseudomonas* Isolates Alone or in combination with fungicides against some soil borne plant pathogen under laboratory and greenhouse conditions. Middle-East Journal of Scientific Research,23(10):2354-2365.

[22] El-Meneisy, Afaf, Z. A.; Abdelaziz, M. Samah and Yaseen, Y.K. Rabaa (2019). Biological control of onion white rot disease using different *Bacillus spp.* Am-Euras. J. Agric. & Environ. Sci., 19(1):64-73.

[23] Bhattacharjee, R. and Dey, U. (2014). An overview of fungal and bacterial biopesticides to control plant pathogens/diseases. African Journal of Microbiology Research, 8(17), 1749-1762.

[24] Motlagh, M.R.S. and Samimi Z. (2013). Evaluation of *Trichoderma* app., as biological agents in some of plant pathogens.- Annals of Biological Research 4(3):173-179.

[25] Hafez.E.E.; Meghad A.; Elsalam H.A.A. and Ahmed S.A. (2013). Biological and molecular studies on *Trichoderma viride*- plant pathogenic fungi interactions.- Journal of world Applied sciences 21(12):1821-1828.

[26] Batta, Y.A. (2004). Effect of treatment with *Trichoderma harzianum* Rifai formulated in invert emulsion on postharvest decay of apple blue mold.- International Journal of Food Microbiology 96:281-288.

[27] Shaikh, F.T. and Nasreen S. (2013). Biocontrol efficacy of *Trichoderma kaningii* against some plant pathogenic fungi.-Indian Journal of Research 2(3):9-10.

[28] Salim, A.B. (2011). Effect of some plant extracts on fungal and aflatoxin production Int. Acad. Res., 3:166-120.

[29] Trias, R.; Baneras, L.; Montesinos, E. and Badosa E. (2010). Lactic acid bacteria from fresh fruit and vegetables as biocontrol agents of phytopathogenic bacteria and fungi.-International Microbiology 11(4):231-236.

[30] Nunes, C. (2012). Biological control of postharvest diseases of fruit.-European Journal of plant pathology 133:181-196

[31] Salama, O.U.F.; Mohamed, I.A. and Ahmed, E.I. (2008). *In vitro* evaluation of the biocontrol activity of some biofungicides on S.cepivorum. Int. J. Biol., Vol. 10, No.3.

[32] El-Naggar, M. A., Zaki, M. F. and El-Shawadfy, M. A. (2018). Management of onion root-rot diseases caused by soil born fungi under Middle Sinai conditions. Middle East J. Appl. Sci., 8(1):91-99.

[33] Bakker, A. W. and Schippers, B. (1987). Microbial cyanide production in the rhizosphere in relation to potato

yield reduction and *Pseudomonas sp.* plant growth stimulation soil Biology biochemistry, 19:551.

[34] Zlata, D.K.; Jelena, T.L.; Stevan, N.M.; Jelica, M.G.V.; Mirjana, A.V. and Svjetlana, R.A. (2008). *Fusarium* rot of onion and possible use of bioproduct. Proc. Nat. Sci, Matica Srpska Novi Sad, 114-148.

[35] Liu J.; Wisniewski M.; Droby S.; Norelli J.; Hershkovttz V.; Tian S. and Farrell R. (2012). Increase in antioxidant gene transcripts, stress tolerance and biocontrol efficacy of *Candida oleophila* fol-lowing sublethal oxidative stress exposure.-FEMS Microbiol. Ecol. 80:578-590

[36] Abou-Zeid, N.; Mohmoud, N. and Saleh, R. (2016). Effect of some Biotic and Abiotic Applications on control of Fusarium Wilt of pepper plants. Egyptian Journal of Phytopathology. 44(2).103-118.

.