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Biocontrol Potential of some Entomopathogenic Fungi against The Cotton Leaf Worm *Spodoptera littoralis in vitro*

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ABSTRACT

The objective of this study was to isolate and identify the entomopathogenic fungi as biocontrol agents and to evaluate their pathogenicity against the cotton leaf worm, *Spodoptera littoralis* (Boisd.). Six strains of fungi were isolated from healthy and infected plants using dilution plate method. **Czapek's** agar supplemented with 0.5 % yeast extract and potato dextrose agar, amended with rose bengal (1/15000) and chloramphenicol (50 ppm) was used for primary isolation. *Aspergillus tamarii*, *Aspergillus parasiticus, pencillium* sp., *Trichoderma harzianum*,*Cladosporium* sp .and *Rhizopus* sp. were isolated . Results showed that *A. parasiticus , A. tamarii*, *T. harzianum* and *Pencillium* sp. are the most promonent so in this study we test their virulence against third instar larvae of the cotton leaf worm the mortality percentage were calculated for each fungus using 1×10^6, 1×10^7 and 1×10^8 spore per ml. It is clear from the results that the mortality percentage increased with elapsing time. The high mortality percentage recorded is 86.66% for *A.tamarii* at 1×10^8 spore/ ml then 83.33% with *A.parasiticus* at 1×10^7 spore/ml, 80% for *T. harizianum* at 1×10^6 spore/ml and 40% at 1×10^7spore/ml for *Pencillium* sp. Comparison to these fungi clear that concentration 10^7 give the highest mortality percentage to the cotton leaf worm for all except *A.tamarii* .Then screening to the ability of these fungi to produce protease enzyme reveals that all of them produce protease enzyme except *Pencillium* sp. Gamma irradiation was used to enhance the protease enzyme activity and consequently increase the mortality percentage.

KEYWORDS

Entomopathogenic Fungi, Spodoptera Littoralis, Biocontrol, Czapek›s Agar, Protease Enzyme, Gamma Irradiation.

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The dose level of 1200 Gy increase protease activity of *A. tamarii* from 0.433 to 1.366 so mortality percentage of third instar larvae of *Spodoptera littoralis* increased from 43.33 % to 86.66 % at concentration 1×10^7 of spore suspension irradiated with 1200 Gy dose level..

INTRODUCTION

The Egyptian cotton leaf worm *Spodoptera littoralis* (Boisd.), (Noctuidae, Lepidoptera) is a serious, polyphagous insect, which has a high reproductive capacity. It is an economically important pest of many agricultural crops; it can attack 112 plant species belonging to 44 families (**Mallik arjuna *et al*., 2004)**. Development of an effective control method against the cotton leaf worm, *Spodoptera littoralis* is urgently needed since it causes serious damage to many important agricultural crops in Egypt. An interest in using of microbial insecticides for biological control of insect pests, as alternatives to chemical control, since they neither leave toxic chemical residues in the environment nor induce resistance in their insect hosts **(Evans, 1999)**. And hence, the public awareness and concern for environmental quality, has led to more focused attention on research aiming at developing biological agents (**Hidalgo *et al.*, 1998).** The biological plant protection with entomopathogenic fungi has key role in sustainable pest management program. Entomopathogenic fungi as biocontrol agents have several advantages when compared with conventional insecticides. These include low cost, high efficiency, safety for beneficial organisms, reduction of residues in environment, and increase biodiversity in human managed ecosystems (**Lacey *et al*., 2001)**. Fungal biocontrol agents have unique mode of infection. Incontrast to bacteria and viruses, they are capable of attacking all developmental stages of insects (**Anand *et al.,* 2008; Ferron, 1978(.** They also, do not need to be ingested dy insects and can invade their host directly through the cuticle. That is why entomopathogenic fungi are capable of infecting non feeding mesh like eggs (**Ujian and Shahzad, 2007; Anand and Tiwary, 2009)** and pupae of insects (**Nguyen *et al*., 2007; Anand *et al.,* 2008).** Fungal biological control agents have demonstrated efficacy against a wide range of insect pests including *Spodoptera* species (**Purwar and Sachan, 2005; Lin *et al*., 2007; Amer *et al.*, 2008)**. Entomopathogenic fungi (EPF) have been found in many diverse habitats and associated with a broad range of insect hosts (**Samson *et al*., 1988)**. These habitats include aquatic, forest and agricultural ecosystems that are of direct importance to insect control and crop protection **(Bin and Mitsuaki, 2006 & Palomares *et al*., 2014)** The identification, isolation and characterization of EPF from these ecosystems are driven by a need to fully understanding the roles of these fungi within their natural environments. Only with this knowledge we can attempt to utilize EPF for the control of insect pests. Enzyme secretion is believed to be a key factor in determining the virulence of the isolate and this is considered as a rationale for the enhance virulence of certain isolates (**Mustafa and Kaur, 2009)**. Highly pathogenic strains show detectable amounts of extracellular chitinase, lipase, and protease activities (**Samuels *et al.*, 1989)**.

The objective of the present study was to isolate and identify the entomopathogenic fungi species as biocontrol agents, and to test the efficacy of the isolated native strains to produce protease enzyme and consequently killing activity to *Spodoptera littoralis* under laboratory conditions.Finally enhancement of these activity by gamma irradiation.

MATERIALS AND METHODS

Insect Rearing

The culture of the tested insect of ***Spodoptera littoralis*** was initiated from egg-masses collected from infested cotton field at Qualubia Governorate and reared in the Nuclear Research Center (Inshas), Sharkia Gover-

norate. ***Ricinus communis***, leaves were introduced as food for the newly hatched larvae. Cotton leaf worm larvae were transferred daily to new clean jars, provided with fresh castor oil plant leaves and kept under the laboratory conditions of 27± 2ºC and 60-65 R.H. Daily observation was carried out until the 3th larval instar.

Isolation of Entomopathogenic Fungi From plants

Collection of Plant samples

Plant samples were collected from different places. Healthy and diseased **Cabbage**, **Alfafa, Maize** and ***Ricinus communis*** leaves were transferred to the laboratory in tight sterilized polyethylene bags and kept at low temperature until plating. All collected samples were from Abou Zabal AlQualubia Governorate except the last sample which is from Abou Zabal AlQualubia Governorate and Egyption Atomic Energy Authority Inshas.

Isolation of Fungi

Fungi were isolated by using dilution plate method **Johnson *et al.* (1960)** in which six plates were used for isolating/sample. **Czapek's** agar supplemented with 0.5 % yeast extract (CYA) and potato dextrose agar (PDA), amended with rose bengal (1/15000) and chloramphenicol (50 ppm) was used for primary isolation. Plates were incubated at 28 ºC for 10 days and developing fungi were counted. For maintaining cultures and for proper identification, pure cultures of isolated fungi were grown on standard media such as Vegetable Agar (VA), Oatmeal Agar (OA), Malt Extract Agar (MEA) Potato Dextrose Agar (PDA) and Potato Carrot Agar (PCA).

Identification of the isolated fungi

Taxonomic identification by morphology of fungal isolates was mainly based on the following identification keys: **Raper and Thom (1949)**, **Pitt (1980)** for *Penicillium* **;Raper and Fennell (1965)** for ***Aspergillus*** ; **Ellis (1971 and 1976)** for dematiaceous hyphomycetes; **Arx (1981)**; **Domsch *et al.* (1980)** for miscellaneous fungi. The systematic arrangement follows the last system of classification appearing in the 9th edition of Anisworth & Bisby's Dictionary of the fungi (**Kirk *et al.*, 2001**).

Media used for growth of fungal microorganisms

Abasal medium (**Czapek's-Dox**) of the following composition (g / l) was used: Sodium nitrate, 2.0; potassium dihydrogen phosphate, 1.0; potassium chloride, 0.5; ferrous sulphate, 0.01; magnesium sulphate, 0.5 and sucrose, 30; made up in distilled water **(Thom and Raper, 1945)**.

Preparation of fungal inocula for fungal bioassays

Conidia were harvested by scraping the sporulating colonies and suspended in sterile distilled water containing 1.0% Tween80 (v/v aqueous solution, as wetting agent) **(Hicks *et al*., 2001)**. The resulting conidial suspensions were first cleared for hyphal debris by filtration, using a suitable sterilized piece of clothes, and centrifugation for 5 min at 3000 rpm then washed twice with 0.05% Tween80 with intervening centrifugation. Resulting conidia were resuspended in 1.0% Tween80 and the concentrations were determined using a haemacytometer before they were diluted with sterile water containing 1.0% Tween80 to reach the appropriate concentrations (1×10^6, $1 \times 10^7$and 1×10^8conidia / ml). Conidial viability was examined prior to the experiments by placing three droplets of a 1×10^6conidia spores ml-1suspension on to suitable agar plates followed by incubation for 24h at 30°C, after which, their germination was examined under a light microscope by observing less than 90% growth for all isolates.

Bioefficacy of the isolated entomopathogenic fungi against 3rdinstar Larvae of Spodoptera littoralis

S. littoralis larvae were immersed individually for 30 seconds into a fungal suspension containing

($1x10^6$, $1x10^7$, $1x10^8$ conidia / ml). For the control treatment, larvae were dipped into a 0.1% Tween (80) solution. Treated larvae were allowed to crawl freely on tissue paper in a Petri dish to remove excess moisture. Then treated larvae were placed individually in small plastic containers (3.5x1.3cm). These containers were placed in crispers having wet towel paper to maintain humidity. All treated larvae were incubated at 27±1ºC, 80±5%, R.H. and photo phase of 12 hours. Excised parts of fresh ***Ricinus Comminus*** leaves surface sterilized with aqueous solution of sodium hypochlorite (0.5%v/v) then washed twice with distilled water were introduded as a food source for the larvae. Leaves were regularly replaced with fresh ones at an interval of 24 hours. Each treatment had batch of 10 larvae and replicated three times. Mortality data was recorded up to 10 days. The cadavers were incubated at 27±1ºC in an incubator to investigate mycosis and sporulation of them. All fungi were isolated in Bioinsecticide laboratory, Biological Application Department, Nuclear Research Center (Inshas), Sharkia Governorate and identified in microbiology laboratory, Faculty of Science, Port Said University.

Protease assay

Protease activity was determined by Plate assay method (**Anagnostakis, 1975).** Production of proe teolytic enzymes was detected by using gelatin as protein source in growth medium. The fungal strain was spot inoculated in petri dishes with nutrient agar medium supplemented with 1% gelatin (Peptone, 5g; Beef extract, 3g; NaCl, 5g; Agar, 15g; Distilled water, 1 liter, pH 6). Prior to inoculation, the petri dishes were incubated at 28 ±1oC for 7 days. After a week of incubation, gelatin degradation was observed as a clearing zone around fungal colonies. This zone of gelatinolysis was seen clearly upon flooding the plate with aqueous saturated solution of mercuric chloride reagent (15g $HgCl_2$ dissolved completely in 20 ml 7M conc. HCl, then raised to 100 ml with sterile distilled water). Mercuric chloride solution reacted with gelatin to produce a white precipitate which made the clearing zone visible. The clearing zone was measured as indicative of the extracellular protease activity of the fungal strain. Enzyme activity was measured by the following formula: EA = D-d; D-diameter of colony plus clearing zone; d-diameter of colony.

Effect of gamma radiation on protease enzyme activity

Source of Gamma rays for irradiation studies

Gamma cell (Cobalt-60), located at Nuclear Research Center, Egyptian Atomic Energy Authority at Inshas, Egypt was used as a source of gamma rays at dose rate of 0.855 KGy/h.

Statistical analysis

Data were analyzed statistically following standard procedures for analysis of variance. Differences among means were evaluated for significance according to Duncan's New Multiple Range Test at the 5% level of probability using COSTAT.

RESULTS AND DISCUSSION

Entomopathogens have been suggested as controlling agents of insect pests for over acentury. There has been an increasing interest in employing fungal pathogens to combat insect pests. New application and production in combined with a greater understanding of both fungal and insect ecology have shown that biological insecticides can now compete with traditional chemical pesticides much faster.

Isolated Fungi

In this study entomopathogenic fungi isolated from healthy and infected plants. In order to select the most proper fungal strains for controlling cotton leaf worm; fungal biota of healthy and infected leaves of host plants of that insect were surveyed. During this part of study, a total number of 11 species belong to 6 genera, has been isolated. Isolated

fungi belong to three classes (**Table 1)** of which **Hyphomycetes** comes first where represented by 8 species accounting for 83.33% of the total isolated taxa. It is followed by classes **Ascomycetes** and **Zygomycetes** which is represented by only one species constituting 8.33% each. Many authers confirm these results of isolating fungi from the plant tissue as **Saikkonen *et al.* (2004); Elliot *et al*. (2000) and White *et al.* (2002)** who stated that endophytic fungi are often regarded as plant defending mutualists and discussed the presence of *B. bassiana* in internal plant tissue as an adaptive protection against herbivorous insects. **Amin *et al.* (2014)** who Studied the isolation and identification of fungal endophytes from healthy pods of cocoa plant and investigated their effects against the egg of cocoa pod borer ,his results also indicated that there were five fungal endophytes genera as, *Trichoderma* sp., *Fusarium* sp., *Beauveria* sp., *Aspergillus* sp.

Table (1) : *Number of isolated species.*

Classes	Healthy plants	Infected plants	Total	%
Hyphomycetes	7	8	8	80
Ascomycetes	-	1	1	10
Zygomycetes	1	-	1	10
Total number of species	7	9	11	100

Species richness

Species richness means the number of species belonging to each genus isolated throughout the current investigation. The genera recorded are given in Table (2). It is obviously evident, from the Table, that *Aspergillus* is the richest by showing a spectrum 6 species. However, the remaining genera represented only by one species each.Another work concide with these results were obtained by **Leatherdale (1970), Zimmerman (1993) and Smith *et al.* (1999)** who stated that Hyphomycete fungal species, for example *B. bassiana*, were reported to infect insect pests from several orders and thereby play an important role in the regulation of pest populations. **Ismail and Abdel-Sater (1993)** also found that *Aspergillus*, the most frequent genus, was associated with all different insect stages.

Table (2) : *Genera and species richness of isolated fungi.*

No. of genera	Healthy plants No. of species	Infected plants No. of species	Total no. of species
Aspergillus	5	6	6
Penicillium	1	1	1
Trichoderma	1	1	1
Rhizopus	1	0	1
Cladosporium	1	0	1
Total	9	8	11

Total fungal count

Fungal counts were expressed as total number of colony forming units per gram dry sample (cfu/g) of each species. In Table (3) a total number of six species was isolated from plants. In view of mean counts , the most domineny texa found in decreasing order were *Penicillium* sp.,*Trichoderma harizianum*, *Aspergillus tamarii*,*Aspergillus parasiticus*, *Rhizopus* sp. and *cladosporium* sp.

Table (3) : *Mean count (cfu/g)* of fungi isolated from healthy and infected plants (infected by cotton leaf worm).*

Plant / Species	Cabbage		Alfalfa		Ricinus		Maize		Mean
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	
***Penicillium* sp.**	25166	16666	31166	-	-	-	-	-	9124±4.7
Trichoderma harizianum	36667	167	-	-	-	-	-	-	4604±4.6
Aspergillus tamarii	333	166	166	-	333	-	-	-	124±0.05
Aspergillus parasiticus	-	-	-	-	-	833	-	-	104±0.1
***Rhizopus* sp.**	333	-	-	-	-	-	-	-	41±0.04
***Cladosporium* sp.**	-	-	332	-	-	-	-	-	41±0.02

(cfu/g) = colony forming unit.*

After isolation and counting of the colonies was done *A. parasiticus, A. tamarIi*, *T. harzianum* and *pencillium* sp. are selected to test their virulence against the third larval instar of the cotton leaf worm through recording the mortality percentage for each fungus using (1×10^6, 1×10^7 and 1×10^8 conidial spore/ml).

Virulence test of the isolated entomopathogenic fungi against 3rd instar larvae of Spodoptera littoralis.

In the current study four of well-known entomon pathogenic fungi were tested for virulance against *S. littoralis* larvae. These are *A.tamarii, A.parasiticus, Pencillium* sp.*, and T.* harzianum.

The virulence of fungal entomopathogens involves four steps: adhesion, germination, differentiation and penetration. Each step is influenced by a range of integrated intrinsic and external factors, which ultimately determine the pathogenicity. The virulence of an entomopathogenic fungus is recognized first by adhesion to an insect body. The failure of a pathogen to adhere with the epidermis is considered a feature of virulent strains (**Al-Aidroos and Roberts, 1978**). After adhesion, the next factor for the virulence of a strain is the enzymes that hydrolyze the epidermis of the insect. The most important enzymes secreted by entomopathogenic fungi are lipases, proteases and chitinases, which are produced sequentially, reflecting the order of the substrates they encounter **(Smith *et al.*, 1981)**. A wide range of factors such as water, ions, fatty acids and nutrients on the cuticle surface and the physiological state of the host, influence spore germination and behavior (**Hassan *et al.*, 1989**). Successful germination requires the assimilation of utilizable nutrients and

a tolerance to any toxic compound present on the surface. After germination, appresoria appear at the end of short germ tubes, subterminally or on the side branches. Penetration of the cuticle is accomplished by the germ tube itself or by the formation of an appressorium that attaches to the cuticle and gives rise to a narrow penetration peg (**Boucias and Pendland, 1982; Roberts and Humber, 1981; Wraight *et al.*, 1998; Zacharuk, 1973**). The penetration process is considered to be a combination of enzymatic and mechanical forces. The exact mechanism for entry is usually peculiar to the species. A range of cuticle-degrading enzymes is produced during penetration into the host (**Gillespie *et al.*, 1988**).

Eventually, it emerges into the haemocoel of the host, and consequently, grows inside the haemocoel as yeast-like blastospores, hyphal bodies or protoplasts. This may occur if the fungus has the ability to produce immune suppressors that block the host defensive immune responses, and consequently,kills the host by disrupting its physiological processes and/or consuming nutrients in the haemolymph (**Samson, *et al*., 1988**), as well as hydrophobic and electrostatic forces also play a role (**Boucias *et al.*, 1998)**.

Table (4) show that the mortality percentage in the 3rdinsrar larvae of ***S.littoralis*** increased with increasing time elapsed after treatment with ***T. harizianum.*** Highest mortality percentage 80% recorded at conidial concentration ($1x10^6$ spores/ ml). The observed mortality may be because *Trichoderma* are known to produce a number of antibiotics, such as trichodermin , trichodermol, harzianum A, harzianolide and peptaibols **(Claydon, *et al.*, 1991, Dickinson, *et al.*, 1995 and Hoell *et al*., 2005).** These components are proved to be insecticidal when fed to larvae of ***S.littoralis*** or when applied to the cuticle together with the serine protease. Thus **Shakeri and Foster (2007**) suggested these components as virulence factors involved in insect pathogenicity. Therefore, it suggested that enzymatic and antibiotic activities detected from *Trichoderma* could have occurred by one or all of these compounds. Also, **Ahmed and El-Katatny (2007)** results confirm this study, the results stated that 80% larval mortality occurred at a minimum concentration of 1×10^8 conidia ml-1 of *Trichoderma harizianum*.

Table (4) : *Mortality percentage of third instars larvae of Spodoptera littoralis after feeding on treated castor bean leaves immersed in different concentrations of Trichoderma harizianum after different periods.*

Conc. (spore /ml)	Mortality % Indicated days after treatments							Total Mortality%
	2	4	6	8	10	12	14	
Control	0	0	0	1	8.33	9.66	9.66	**9.66** [b]
10^6	0	3.33	16.66	26.66	33.33	63.33	80	**80** [a]
10^7	3.33	13.33	23.33	30	40	53.33	73.33	**73.33**[a]
10^8	3.33	3.33	13.33	33.33	33.33	43.33	50	**50**[a]

Values followed by the same letter in last column are not significantly different at P>0.05.
LSD 0.05=36.4651778163

Tables (5) show the mortality percentage in the 3rdinsrar larvae of ***S.littoralis*** *using A.parasiticus* spore suspension. Comparison between total mortality percentage recorded with three diffferent concentrations (10^6,10^7,10^8) spore suspension of *Aspergillus parasiticus* and the control (treating third instar larvae of ***S. littoralis*** with distilled water) indicated that the spore suspension concentration 10^7 give the best mortality percentage (83.33%). Also there is not any significant difference between mortality percentages recorded at different fungus concentrations, while they are significantly differ from control. So ***A. parasiticus*** can be used as an important biological control agent against ***S. littoralis*** where it recorded the highest mortality percentage**. Shoulkamy *et al.* (2014)** results also agree with these results.

Table (5) : *Mortality percentage of third instars larvae of Spodoptera littoralis after feeding on treated castor bean leaves immersed in different concentrations of Aspergillus parasitcus.*

Conc. (spore /ml)	Mortality % Indicated days after treatments							Total Mortality%
	2	4	6	8	10	12	14	
Control	0	0	0	1	8.33	9.66	9.66	**9.66** [b]
106	0	0	0	3	3	33	66.33	**66.33** [a]
107	0	0	0	6.66	30	56.66	83.33	**83.33** [a]
10^8	3.33	3.33	6.66	6.66	16.66	40	76.66	**76.66**[a]

Values followed by the same letter in last column are not significantly different at P>0.05
LSD 0.05 = 38.819638873

Table (6) Show the recorded mortality percentages of third instars larvae of ***S. littoralis*** after feeding on treated castor bean leaves immersed in different concentrations of *A. tamarii.* comparison between total mortality percentages recorded cleared that a spore suspension concentration 10^8 give the best mortality percentage (86.66%) then 10^6 give (60%) and 10^7give (43.33%) incrossponding to control (9.66%),and increasing the elapsed time from-12to14 days doesn't increase the mortality percentage, it remain the same.

Table (6) : *Mortality percentage of third instars larvae of Spodoptera littoralis after feeding on treated castor bean leaves immersed in different concentrations of Aspergillus tamarii.*

Conc. (spore /ml)	Morality % Indicated days after treatments							Total Mortality%
	2	4	6	8	10	12	14	
Control	0	0	0	1	8.33	9.66	9.66	**9.66** [b]
10^6	0	10	10	13	36.66	60	60	**60** [a]
10^7	0	0	3.33	3.33	30	43.33	43.33	**43.33**[ab]
10^8	0	10	10	26.66	46.66	86.66	86.66	**86.66** [a]

Values followed by the same letter in last column are not significantly different at P>0.05
LSD 0.05 = 45.801909629

Table (7) show mortality percentage of third instars larvae of ***S.littoralis*** after feeding on treated castor bean leaves immersed in different concentrations of *Pencillium* sp. The obtained results indicated that by increasing both concentrations and elapsed time the mortality recorded did not significantly increase and the highest one was 40% at concentration of 10^7 and14 days post treatment.These results also show that *Pencillium* sp. has the lowest mortality percentage recorded compared to the remaining fungal strains .

Generally, the results of four tested fungus indicated that the mortality percentage increased with time, this is because the fungi need more time to adhere the insect cuticle, penetrate and complete its cycle inside the insect body**. Asi *et al.* (2009)** results agree with our results, he found that the mortality of the aphids increased with increase in spore concentration and exposure time. Also, **Ansari *et al.* (2004)** found that recorded mortality depended on the concentration of conidial suspension, exposure time and temperature. The susceptibility of same aphid species may vary to different fungal strains.

Table (7) : *Mortality percentage of third instars larvae of Spodoptera littoralis after feeding on treated castor bean leaves immersed in different concentrations of Pencillium sp.*

Conc. (spore /ml)	Mortality % Indicated days after treatments							Total Mortality%
	2	4	6	8	10	12	14	
Control	0	0	0	1	8.33	9.66	9.66	**9.66** [a]
10^6	3.33	3.33	6.66	20	30	36.6	36.6	**36.6** [a]
10^7	0	0	6.66	13.3	30	40	40	**40** [a]
10^8	0	0	3.33	10	30	33.33	33.33	**33.33**[a]

Values followed by the same letter in last column are not significantly different at P>0.05
LSD 0.05 = 51.279434795

Primary screening of the isolated fungi for protease enzyme production

It is clear from the screening that all of the isolates produce protease enzyme except *Penicillium* sp. *Aspergillus tamarii* gave the highest percentage recorded. comparison between total mortality percentages recorded cleared that a spore suspension concentration 10^8 give the best mortality percentage (86.66%) then 10^6 give (60%) and 10^7give (43.33%) incrossponding to control (9.66%). The lowest mortality percentage was at concentration of 1×10^7 so, gamma irradiation was used to enhance the protease enzyme activity and concesequantly increase the lowest mortality percentage. From the results it is clear that the dose 1200 Gy increase protease activity of *A. tamarii* from 0.433 to 1.366 so mortality percentage of third instar larvae of *Spodoptera littoralis* increased from 43.33 % to 86.66 % at concentration 1×10^7 of spore suspension irradiated with 1200Gy.

Table (8): show the effect of exposing spore suspension (1×10^7) of *A.tamarii* to gamma-irradiation at 400, 600, 800, 1000 and 1200 Gy. It appears that exposing *A. tamarii* to gamma-irradiation at dose 400,600 and 1200 Gy increase production of protease enzyme. A dose of 1000 Gy decrease protease enzyme activity and 800 Gy make the fungus unable to produce protease enzyme, while it was still not differ significantly from each other .So dose level 1200 Gy is the best one that increase protease enzyme activity and thus was significantly differ from other treatments and control.

Table (8) : *Effect of gamma radiation on Aspergillus tamarii protease enzyme production and activity.*

Dose	Enzyme activity
control	0.433[c]
400Gy	0.811[b]
600Gy	0.693[bc]
800Gy	0 [d]
1000Gy	0.3[cd]
1200Gy	1.366[a]

LSD 0.05= 045275853315

From data in table (8) it is clear that gamma radiation was used to enhance the protease enzyme activity, the dose of 1200 Gy increase protease activity of *A. tamarii* from 0.433 to 1.366 .Mortality percentage of third instar larvae of ***Spodoptera littoralis*** increased from 43.33 % to 86.66 % at concentration 1×10^7 of spore suspension irradiated with 1200 Gy (Table 9).

Table (9) : *Comparison between the mortality percentage of third instar larvae of Spodoptera littoralis treated with normal and irradiated spore suspension of A. tamarii.*

Conc. (spore /ml)	Mortality % Indicated days after treatments							Total Mortality%
	2	4	6	8	10	12	14	
10^7normal spore suspension	**0**	**0**	**3.33**	**3.33**	**30**	**43.3**	**43.3**	**43.3**
10^7 irradiated spore suspension (1200GY)	**23.33**	**26.66**	**26.66**	**40**	**56.66**	**63.33**	**86.66**	**86.66**

Protease activity can determine the virulence of entomopathogenic fungi to some degree, thus can be used as a virulence index (**St-Leger *et al*., 1987; St-Leger *et al*., 1996; Feng, 1998; Gillespie *et al*., 1998; Castellanos-Moguel *et al*., 2008**). However, there are other studies in which, no reliable relationship between the protease activity and virulence of studied entomopathogenic fungi has been estabe lished (**Gillespie *et al*., 1998; Vargas *et al*., 2003; Dias *et al*., 2008**).

Gamma radiation used to increase the activity of protease enzyme and consequently to increase the mortality percentage of the fungi that has lower mortality percentage. **Smith (1958**) compiled a paper on using of radiation to produce useful mutations based on papers presented in three symposia in the United States from August 1956 to January1957. **Casarett (1968)** reported that, radiation doses required to proo duce measurable change in the common catabolic processes are higher than that necessary to decrease

survival cells of microorganisms. **Olive (1998)** proved that, ionizing radiation produces many types of DNA lesions that have the potential of killing microbial cells. **Gherbawy (1998)** studied the effect of gamma irradiation on the production of cell wall degrading enzymes by *Aspergillus niger*.The impact of gamma radiation on the metabolic activities of microorganisms differs as it may be negative **(Jones *et al*., 2004)** or positive effect **(Haggag and Mohamed, 2002)**.

CONCLUSION

The results obtained in this study indicated that the high mortality percentages recorded for *A. tamarii* is 86.66% at concentration of 1×10^8spore / ml ,83.33% for *A. parasiticus* at 1×10^7spore/ml, for *T. harizianum* 80% at 1×10^6spore /ml and 40% at 1×10^7spore/ml for Pencillium sp. It is clear form mentioned results that the best fungus that kills the cotton leaf worm is *A. tamarii* and the least one is *Pencillium* sp. Gamma irradiation can be used to enhance the activity of the fungal enzyme and this will increase their virulence against cotton leaf worm. Thus we can conclude that entomopathogenic fungi play a major role in the regulation of *Spodoptera littoralis* pest population and it's management.

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