

## Evaluation of Actinomycetes Isolates as A Biocontrol Agent Against *Spodoptera littoralis* (Boisd.) and *Fusarium oxysporum*

Abeer M. Mohammad

Faculty of Science & Arts – Al-Dayer , Jazan University

Plant Protection Research Institute, Agric. Ares. Center



### ABSTRACT

According to soil isolation techniques, 23 isolates of actinomycetes were obtained from different soil samples in KSA and Egypt. All isolates were examined for their biological activity against both, the pest *Spodoptera littoralis* (Boisd.), also the plant pathogen *Fusarium oxysporum*. Results illustrated that, three isolates, named DI, DV and FII exhibited high efficacy against the 4<sup>th</sup> instar of *S. littoralis*, with mortality percentage 70, 80 and 60% respectively. Also, other stages' development of *S. littoralis* were affected. Meanwhile, metabolites of 20 isolates showed antagonistic activity against *F. oxysporum*, including the 3 isolates which showed efficacy against the insect pest, the % of inhibition ranged from 67 – 49 %. These 3 isolates were belong to the species *Streptomyces lavendulae* and *Str. clavuligerus*.

**Keywords:** Actinomycetes, biocontrol, *Spodoptera littoralis* and *Fusarium oxysporum*

### INTRODUCTION

Pesticides are designed basically to kill, and because their non-specific mode of action to one organism, they often kill or harm non-target organisms even humans. Also, excessive use of fungicides has led to deterioration of public health, environmental pollution and appearance of pathogen resistance. (Harpreet and Parihar, 2010). Because of this problem a serious efforts is needed to find an alternative method for controlling insect pests and plant pathogens (Canada, 1995). Many groups of microorganisms such fungi, bacteria and viruses have been reported as biocontrol agents. Actinomycetes are aerobic, gram+ve bacteria which widely spread in nature, have been well known for the production of secondary metabolites (Lo *et al.*, 2002). Recently, this organism plays an important role in insect and plant pathogen control, hence they produce metabolites active against many insects and fungi. (Hussain *et al.*, 2002). Chitinase is an enzyme used by insects for degradation the structural polysaccharide "chitin" during molting stages. This enzyme is very important in biological control of insects (Reguera & Leschine, 2001), and plant pathogenic fungi (El-Tarabily, 2003). Actinomycetes are also produce secondary metabolites like hydrocyanic acid (HCN) and indole acetic acid (IAA) (Gopalakrishnan *et al.*, 2011).

So, the main purpose of this research is to isolate local isolates of actinomycetes from soil, later these isolates will be tested for their biological activity against both *Spodoptera littoralis* larvae and the plant pathogen *Fusarium oxysporum*.

### MATERIALS AND METHODS

#### Isolation of actinomycetes from soil.

Soil sample for isolation, were collected randomly from different localities in Egypt's Governorates and farms of Agricultural Research Center, Abo-Areesh & Fayfaa farms, Jazan, KSA. Samples were then packed and stored at 15°C. Samples were then dried for 1 week prior isolation to decrease gram negative population. Starch casein agar (SCA) were used for isolation. One gram of each sample were mixed with 10 ml of sterile distilled water, 100 µl of each sample were

spread onto fresh SCA plates and kept for incubation at 28° C for 7 days. Colonies of actinomycetes were selected by using sterile toothpick then placed on SCA plates and incubated to get pure colonies. (Sinha, *et al.*, 2014).

#### Fungi isolation and growth conditions

The pathogen of vascular wilt disease (*F. oxysporum*) was isolated from diseased vegetables by direct plating method, maintained as pure culture on potato dextrose agar medium at 25 °C for a week and later stored at -20 °C in glycerol. Vicente *et al.*, 2014.

#### Media used for isolation.

Starch- casein agar (SCA). For actinomycetes growth; soluble starch 1%, casein 0.03%, K-NO<sub>3</sub> 0.2%, NaCl 0.2%, K<sub>2</sub>HPO<sub>4</sub> 0.005%, CaCO<sub>3</sub> 0.002%, FeSO<sub>4</sub>·7H<sub>2</sub>O 0.001%, and agar 2.0%.

Potato dextrose Agar (PDA) media; for fungal isolation and growth.

#### Identification of actinomycetes.

Isolates' identification was done according to spore chain morphology, color of mycelium, production of pigments, colony hardness, odor (earthy/geosmin smell) and gram staining (Gram +ve). Further investigations were done for the isolates to be characterized by physiological and biochemical properties. Bergey's Manual of Determinative Bacteriology (1984).

#### Determination of chitinase producing isolates.

A preliminary experiment was carried out to determine chitinase production by isolated strains, using colloidal chitin agar and observed for a clear zone around colonies suggesting production of the enzyme. (Hsu & Lockwood, 1975).

#### Secondary metabolites extraction

According to the initial observation of chitin degradation, the selected strains were cultured in broth medium and further inoculated (10%) into fermentation medium (glucose 1%, peptone 2% and pH 7.2) then kept for fermentation at 28° C for 7 days. Then the culture was centrifuged at 10,000 rpm for ten min. The cell-free supernatant obtained was added to equal volume of ethyl acetate and shook for 2 hours. The layer containing the metabolites was separated. Ethyl acetate was evaporated and the obtained residue after evaporation was weighed and used for its

antagonistic effect against *F. oxysporum*. (Sinha, et al., 2014).

**Antifungal activity**

Dual culture technique was done to screen the ability of actinomycetes isolates to inhibit plant pathogenic fungus *F.oxysporum*, growth. Fifteen mm disc of a pure culture of *F.oxysporum* was placed at the centre of Petri dishes of different agar plates inoculated with actinomycetes isolates and incubated for 7 days at 28 ± 2°C. The antagonistic efficacy was measured by the colony growth of the pathogen (radius growth towards each strain) and the growth inhibition (%) was estimated in comparison with control plates. Three replications were maintained for each treatment. (Harpreet & Parihar, 2010).

$$(\%) \text{ inhibition} = (\mathbf{R} - \mathbf{r}) / \mathbf{R} \times 100;$$

Where **r**: the radial growth of the fungal colony (mm) against to the antagonistic strain, **R**: the radial growth of pathogen (mm) in control.

**Maintenance of *S. littoralis***

Larvae of cotton leaf worm, *S.littoralis*, were reared in lab, on castor leaves for many generations, at 25 ± 2 °C, as described by El-Defrawiet al., 1964.

**Insecticidal and metamorphic effects of the isolates**

Supernatants of the actinomycete isolates were used to estimate their activity against 4<sup>th</sup> instar larvae of *S.littoralis* with leaf- dipping technique (Makkar & El Mandarawy, 1996) under their optimum natural conditions. For testing the effectiveness of the isolates, fresh castor leaves, were washed with tap water followed by rinsing in sterile water and left for drying

for 5 min. Plant leaves were dipped in isolates supernatants containing their metabolites. The tested plant leaves were left for drying for 5 min and then placed into glass jars. Ten larvae (L2) were put in one jar then the jars were placed at room condition and kept at 27 °C ± 2 °C for 7 days. Three replicates were done for each treatment. In control treatment plant leaves were dipped in distilled water only. Then, mortality was recorded daily. Pupal duration and % adult emergence were also estimated.

**RESULTS**

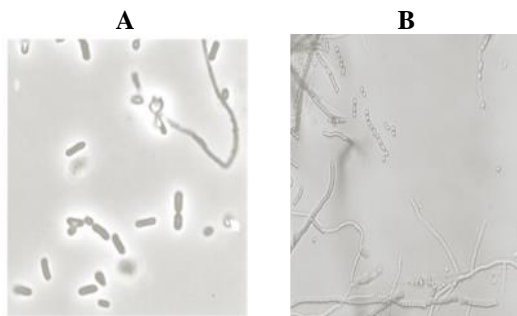
**Isolation of microorganisms:**

**Actinomycetes isolates**

Of all the samples collected, twenty-three actinomycete isolates, were obtained according to soil isolation techniques (Table 1). Nine isolates were obtained from soil in KSA and 14 isolates from Egyptian soil. The isolates were isolated and identified due to morphological, physiological and biochemical characters. Then categorized in Table (1) according to colony color, place of isolation and biological activity against both *S. littoralis* and *F. oxysporum*. Colony color ranged from white, grey and brown. Identification was done according to Bergey's Manual of Determinative Bacteriology, which illustrated that the active isolates were belonging to *Streptomyces lavendulae* and *Str. clavuligerus*. Fig.(1<sub>a,b</sub>) showed that the morphology character of vegetative cells and spore chains of actinomycetes isolate ( DI and FII).

**Table (1): Actinomycetes isolates: colony color, place of isolation and biological activity**

Isolate	location	Colony color	% mortality <i>S. littoralis</i>	% inhibition of <i>F. oxysporum</i>
DI	Dakahliya Governorate	whitish	<u>70</u>	67
DII	---	Grey	10	51
DIII	---	Dark grey	0	60
DIV	---	Dark grey	0	56
DV	---	Grey	<u>80</u>	62
KSI	Kafr-Shiekh Governorate	Brownish	0	62
KSII	---	Yellowish	0	65
KSIII	---	Dark brown	0	60
KSIV	---	Grey	0	56
KSV	---	Dark brown	0	58
GI	Giza Gov.	Yellowish white	0	49
GII	---	Grey	0	53
GIII	---	Grey	0	51
AbI	Abo Areesh	Brownish	0	58
AbII	---	Dark brownish	0	60
AbIII	---	Grey	0	58
FI	Fayfaa	whitish	0	56
FII	---	Yellowish white	<u>60</u>	60
FIII	---	Grey	0	58
FIV	---	Dark brownish	0	56

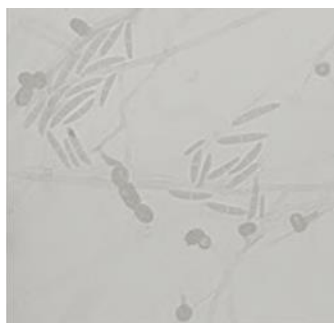


**Fig.(1): Vegetative and spore chains of the isolates; A) DI ;B) FII**

**Fungal pathogen**

*Fusariumoxysporum*, causes serious problems all over the world, for many field crops. For isolation, diseased tomato plants were tested for vascular wilt symptoms' characteristics and then the isolation procedures were done for the fungus. The lesion root areas with brownish rot showed existence of fungi

which were isolated according to Vicente *et al.*, 2014, then identified as *F.oxysporum*. The isolation was done on PAD media. Then the fungi was examined microscopically. Fig.(2) illustrated the morphology of macroconidia and microconidia (asexual spores) of *F.oxysporum*.

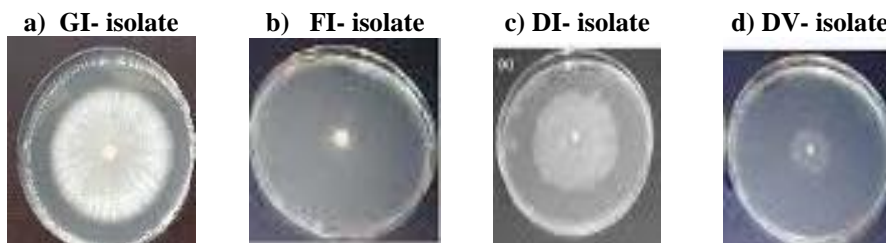


**Fig.(2): Macroconidia and Microconidia of *Fusariumoxysporum***

**Enzymatic screening and antifungal activity**

Enzymatic tests were done to determine the actinomycetes' ability to act as a degrader of organic compounds (chitin) when used in agricultural applications. *In vitro*, tests using the dual culture technique to examine the potential of actinomycetes isolates as bio-control agents, were done against the fungal pathogen *F.oxysporum*. The test organism were isolated from the diseased tomato plants. Results revealed that, from all the 23 isolates, there are 20 able to inhibit the growth of the pathogen. The antagonists isolated bacteria grew faster than the fungus, limiting the growth of the pathogen in

varying, which appeared in various diameters. Looking at the growth diameters, it is clearly appeared, as indicated in Table (1) and Fig. (3), the percentage of inhibition ranged from 67 – 49 %. There was significant differences ( $P < 0.05$ ) in percentage inhibition of radial growth of pathogen by all the isolates (Table 2). Inhibition were clearly observed by all treatments. (Fig. 3). Also, the metabolite extracts of the isolates were used to examine their antifungal activity against the pathogen. Metabolic extracts showed the ability to inhibit the pathogenic fungal growth.



**Fig. (3 ) Antagonistic reaction against *Fusariumoxysporum* growth.**

**Table (2): Radial growth (mm) and inhibition (%) of pathogen in dual culture test**

Isolate	*Radial growth of fungus (mm)	% growth inhibition
DI	1.4 <sup>d</sup>	67
DII	2.1 <sup>a</sup>	51
DIII	1.7 <sup>b</sup>	60
DIV	1.9 <sup>b</sup>	56
DV	1.6 <sup>c</sup>	62
KSI	1.6 <sup>c</sup>	62
KSII	1.5 <sup>c</sup>	65
KSIII	1.7 <sup>b</sup>	60
KSIV	1.9 <sup>b</sup>	56
KSV	1.8 <sup>b</sup>	58
GI	2.2 <sup>a</sup>	49
GII	2.0 <sup>b</sup>	53
GIII	2.1 <sup>a</sup>	51
AbI	1.8 <sup>b</sup>	58
AbII	1.7 <sup>b</sup>	60
AbIII	1.8 <sup>b</sup>	58
FI	1.9 <sup>b</sup>	56
FII	1.7 <sup>b</sup>	60
FIII	1.8 <sup>b</sup>	58
FIV	1.9 <sup>b</sup>	56

R = 4.3 mm; \* = Means of three replicates; Means followed by different letters differ significantly at P = 0.05

**Insecticidal activity**

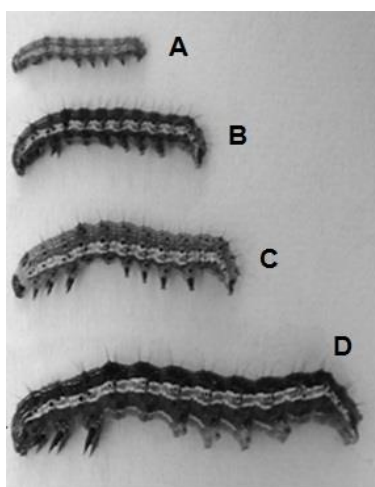
In order to study the efficiency of the actinomycetes isolates against the insect pest *S.littoralis*, the secondary metabolites were introduced to the larval food. Data of this experiment are presented in Tables (1 &3). Three isolates represented high efficacy against the larvae. DI isolate caused 80 % mortality in tested larval group, and DV isolate caused

80 % while FII caused 60%. Also, the next stages of the survived larvae are affected, larval duration changed, the pupal duration and adult emergence changed, consequently. Prolongation of larval stages were observed, also pupal duration. Not all pupae survived, succeed to transform to adult stages (moth).

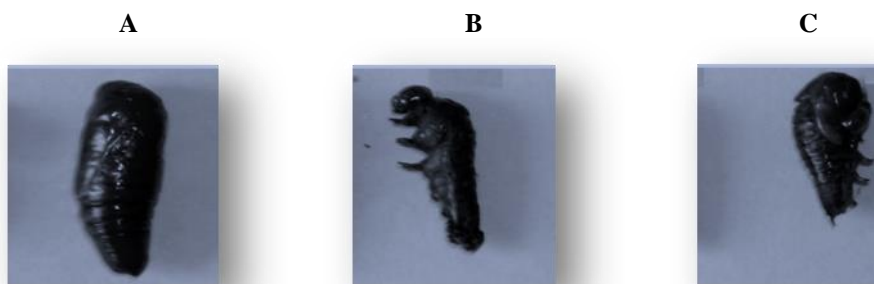
**Table (3): Stages' development of *S. littoralis* exposed to actinomycetes secondary metabolites.**

Isolate	% Mortality	Pupal duration(days±SD)	% Adult emergence
DI	70b	9.69±0.84ns	38
DV	80a	8.05±1.2	21
FII	60c	9.08±0.56ns	40

Means±SD followed by ns; not significantly different (p>0.05).



**Fig. (4): Effects of different secondary metabolites (isolates) on larval developments ( 6<sup>th</sup> instar): a) DV-isolate; b) DI-isolate; c)FII-isolate and d) control.**



**Fig. (5):Metamorphic effects of the isolates, a) control treatment; b) FII-isolate c) DV- isolate**

## DISCUSSION

Insect pests and fungal pathogens pose serious problems worldwide for economically important plants. *S.littoralis* considered as one of the major economic pests all over the world, infesting more than 40 plant families. Also the plant pathogen, *F.oxysporum*, which cause vascular wilt of many crops, occurs in most major crops production regions worldwide.

And because of the hazard effects of insecticides and fungicides, which cause toxicity for human and other organisms, an alternative methods environmentally friendly must be used. The microorganisms considered as a powerful agent could be used for biological control. Since then , there were many trials for using and developing microorganisms in this field. Recently, studies on chitinolytic microorganisms have introduced an increase of knowledge including their role in inhibition of growth of fungal plant pathogen and insect pests.

Actinomycetes which secrete metabolites (enzymes and antibiotics), have both mycolytic and insecticidal activity. This suggests that, it can be possible for using as biological control agent. Harpreet & Parihar (2010) isolated actinomycetes from soil and proved that, the isolates were effective against the test fungi, including *Fusarium* spp. Similar results have been recorded by many authors such, Kharma et al., 2009, who reported that, the crude extracts of antifungal compounds was active against test fungi.

Last decades actinomycetes have proved, by many authors, to be a powerful source for biological control of insect pests, where it is producing metabolites have insecticidal efficacy. Bream et al., 2001, mention the possibility of using actinomycetes metabolites for controlling *S.littoralis*. While, Gad El Hak et al., 2005 & El-Khawaghet et al., 2011, made investigation confirmed the ability of actinomycetes for controlling *Drosophila* and mosquito larvae.

## CONCLUSION AND FUTURE PROSPECTS

Secondary metabolites of the fermentation media from isolated actinomycetes were toxic to both , the plant pathogen *F. oxysporum* and the larvae of *S. littoralis*. Therefore, these metabolites show considerable

potential for sharing in pest management procedures, as new biopesticidal formulation. Also it can be used as an environmentally friendly fungicides.

In the further studies into the use of the actinomycetes for agricultural industry will be done to benefit from these important microbes for agricultural development. Many insect pests and plant pathogens may be controlled by using these organisms safely.

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## REFERENCES

- Bergey's Manual of Determinative Bacteriology (1984). Vol. 4 2nd Edition.
- Bream, A.S.; S.A. Ghazal; Z.K. Abd El-Aziz and S.Y. Ibrahim (2001). Insecticidal activity of selected actinomycetes strains against the Egyptian cotton leafworm. Med. Fac. Landbouww. Univ. Gent, 66/2.
- Canaday, C.H. (1995). Biological and cultural tests for control plant diseases. American Photopathological Society, St. Paul, MN.
- El-Defrawi, M.E. ; A. T. Topozada; A. Salama and S. A. El-Kishen (1964). Toxicological studies on the Egyptian cotton leaf worm, *Prodenia litura* L. II Reversion of toxaphene resistance in the Egyptian cotton leaf worm. J. Econ. Entomol. 57: 593-599.
- El-Khawagha, M.A.; Kh. Sh. Hamadah and T. M. El-Sheikh (2011). The insecticidal activity of actinomycetes metabolites against the mosquito *Culex pipiens*. Egypt. Acad. J. biolog. Sci. 4(1):103-113.
- El-Tarabily, K.A. (2003). An endophytic chitinase – producing isolate of *Actinoplanes missouriensis*, with potential for biological control of root rot lupin caused by *Plectosporium tabacinum*. Australian J. Botany, 51: 257-66.
- Gad El Hak , G.G.; K.A. El Tarabily and F. K Al-Kaabi (2005). Insect control using chitinolytic soil actinomycetes as bio-control agents. International Journal of Agriculture and Biology. 7(4):627-633.

- Gopalakrishnan, S.; S.Pande; M. Sharma, P.Humayun; KeerthiKiran; D.Sandeep; M.Vidya; O.Rupela(2011).Evaluation ofactinomyces isolates obtained fromherbal vermin compost for thebiological control of *Fusariumwiltchickpea*. Crop Prot., 30: 1070 -1078.
- Harpreet, S. and Parihar, L. (2010).Antifungal activity of extracts obtained from actinomyces .J. Yeast and Fungal Res. 1(10), 197-200.
- Hsu, S. C. and J. L. lockwood (1975). Powdered chitin agar as a selective medium for enumeration of actinomyces in water and soil. Appl. Microbiol. , 29(3): 422-426.
- Hussain,A.A.; S.A. Mostafa; S.A.Ghazal and S.Y. Ibrahim, (2002). Studies on antifungal antibiotic and bioinsecticidal activities of some actinomyces isolates. African J. Mycol. Biotechnol.,10: 63-80.
- Kharmna, S. ; A. Yokota and Jf. Peberdy (2009). Antifungal activity of Streptomyces spp. Isolated from rhizosphere of Thai medicinal plants. Int. J. Integr. Biol. 6(3): 143-147.
- Lo, C.W.; N.S.Lai;H.Y.Wong and N.S.Cheah(2002). Actinomyces isolated from soil samples from the crocker range Sabah. ASEAN Rev. Biodiversity and Environmental conservation. July-Sept. 2002.
- Makkar AW, El Mandarawy, Monna BR. 1996. Laboratory studies for increasing the efficacy of abioinsecticide against *Spodopteralittoralislarvae*. Ann AgrSci, Moshtohor. 34:1925–1934.
- Reguera, G. and S.B. Leschine(2001). Chitin degradation by cellulolytic anaerobes and facultative aerobes from soils and sediments.FEMS Microbiol.Lett., 204:367-74.
- Sinha, K. ; R. Hegde; and A. Kush(2014).Exploration on native actinomyces strains and their potential against fungal plant pathogens. Int.J.Curr.Microbiol.App.Sci, 3(11) 37-45.
- Vicente, P.; M.A. Dita and M. de-la-Parta (2014).Prevention and diagnostic of Fusarium wilt of banana caused by *Fusariumoxysporum*. Technical manual prepared for the regional training workshop of the diagnosis of Fusarium wilt organized by FAO regional office of the Caribbean and CARDI. 5-9 May, 2014 in st.Augustine , Trinidad and Tobago.

## تقييم عزلات محلية من البكتيريا الخيطية كعامل مكافحة حيوية ضد دودة ورق القطن *Spodoptera littoralis* و

### الممرض الفطري *Fusarium oxysporum*

عبيد محمود محمد

كلية العلوم و الآداب .الداير. جامعة جازان

معهد بحوث وقاية النباتات - مركز البحوث الزراعية

تعتبر الكائنات الدقيقة عامل قوي من الممكن استخدامه في مجال مكافحة الحويية. و قد جرت محاولات عديدة لاستخدامها و تطويرها لهذا الغرض. لذلك يهدف هذا البحث لعزل عدة عزلات محلية للبكتيريا الخيطية ( الاكتينومييسيتات)، من التربة و تجربتها حيويًا ضد كل من دودة ورق القطن *Spodopteralittoralis* وكذلك الممرض الفطري *Fusariumoxysporum*. تبعًا للطرق المتبعة للعزل من التربة ، تم عزل ٢٣ عزلة بكتيرية من البكتيريا الخيطية من التربة الزراعية بمصر و بالمملكة العربية السعودية. ثم تم اختبار الفعالية الحيوية للعزلات ضد كل من يرقات العمر الرابع للأفة *S. littoralis* و كذلك الممرض الفطري *F.oxysporum* المسبب المرضي لمرض الذبول الوعائي الفيوزارمي. و قد أظهرت النتائج أن هناك ثلاث عزلات ( DI, DV and FII ) ذات فعالية ضد يرقات العمر الرابع لدودة ورق القطن بنسبة وفيات ٧٠ ، ٨٠ ، ٦٠ % على التوالي. كذلك فقد ظهرت فعالية على الأطوار الأخرى في صورة تشوهات و فشل في التعذر و خروج الفراش. بينما أثبتت النتائج أن ٢٠ عزلة من الكل ( بما فيها الثلاث الفعالة ضد الأفة) ذات فعالية في تثبيط النمو للمرض الفطري بنسب تفاوتت من ٤٩ – ٦٧ % . كانت العزلات الثلاث تنتمي للأنواع البكتيرية *Stryptomycelavendulae*, *Str. clavuligerus*.