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Evaluation of entomopathogenic fungi on some biological aspects of cotton leaf worm (*spodoptera Littoralis).*

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Abstract: ***This research aims to investigate the insecticidal effect of some fungal isolates on some biological aspects of Spodoptera littoralis to avoid the harmful impact of chemical insecticides on humans, animals, natural predators, and the whole environment.10 fungi were isolated from the soil and their spore suspension was screened on the 2nd instar larvae of S.littoralis. (isolate NO.9) gave the highest insecticidal effect on S.littoralis even the spore suspension or the filtrate. S.littoralis biological aspects affected by the B.bassiana spore suspension and filtrate respectively were: the highest larval deformation(30%)(23.33%), the highest larval mortality (33.33%)(30%), lowest pupation ratio(66.67%),(70%), Pupal mortality (8.9%) (35.77%) the ratio of adult emergency was (91.07)&(57.57) and both treatments show total injury as (40% and 60%). the fungal isolate NO. 9 is characterized as white mold which seems to be Baeuveria bassiana.***

KEYWORDS: Biological control , Baeuveria bassiana , Spodoptra littoralis, Entomopathogenic fungi.

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I. INTRODUCTION

The Egyptian cotton leafworm, Spodeptera littoralis is one of the most harmful pests which is responsible for great damage in many economical crops such as cotton plants and many vegetable crops in Egypt. Its larva cause defoliation to over than 40 families of important crops like cotton, tomato, potato, and legumes (EPPO, 2008). Great efforts have been made to control this pest. For more than 50 years many chemical pesticides have been used to manage insect pests (Charnley & Collins, 2007). But the continuous use of chemical pesticides cause many problems such as pest resistance and leads to harmful effects on beneficial insects, which are natural enemies of pests, beside toxic residues in food products, water, soil, and air it highly pollutes the whole environment and it also severely affects animals and human (Tudi M et al., 2021) These problems attract worldwide interest to alternate these chemical pesticides with another safe method for pests control. Therefore, bioinsecticides, such as those produced from entomopathogenic fungi, are rapidly emerging as prime substitutes (Zhang et al., 2020). Notably, filamentous fungi considered as a major branch of eukaryotes, emerged during a long evolutionary period. Studies and phylogenetic data over the last four decades indicate that convergent evolution enhanced fungal virulence to most pests and medically important arthropods. This is the prominent feature of several fungal line ages, which has drawn wide attention

Beauveria bassiana is an imperfect entomopathogenic fungus that attacks a wide range of agricultural pests (Feng et al., 1994; Nouh, et al.,2022). It attacks a wide range of both immature and adult insects. The insect disease caused by the fungus is a muscardine. Beauveria bassiana is a soil-borne entomopathogenic fungus found worldwide in diverse habitats (Zimmerman, 2007; Gracian and Mark,2020). B.bassiana is one of the most common fungus-infecting insects (Boucias and Pendland, 1998).

II. MATERIALS AND METHODS

2.1. Isolation of entomopathogenic fungi from soil

A total of 10 fungal isolates were isolated from soil samples soil samples were diluted serially. One milliliter of the diluted soil was spread on Dox medium, which contained 20g Sucrose, 1g K2HPO4, 2g KNO3, 2g Yeast extract, 0.5g Kcl, 0.5g MgSO4-7H2O, 0.002g FeSO4-7H2O, and 20g Agar in 1 liter of water. Four plates were inoculated for each dilution and then incubated at a temperature of 28±1oC and a relative humidity of 50-60% for 7 days. The fungal colonies were selected from each plate and sub-cultured and purified. The pure subcultures were then morphologically examined by a light microscope and characterized according to the key fungal manual of (Hodge,1998).

2.2. Rearing of the tested insect *S. littoralis:*

An original culture of Egyptian cotton leafworm, Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae), which was susceptible to experiments, was maintained at the Department of Cotton Leafworm at the Plant Protection Research Institute at Sharkia Branch. The insect culture was kept in laboratory conditions at a temperature of 26±1°C, a relative humidity of 70±5%, and a 12-hour light:12-hour dark photoperiod. Fresh and clean Castor bean leaves were provided daily to feed the newly hatched larvae, which were kept in glass jars with a one-liter capacity. Feces and debris were cleaned out daily (Gamil, 2011).

2.3 Screening of fungal spores on some biological aspects of *S.litoralis*.

The production of spores was carried out using Potato Dextrose Agar (PDA) medium. The medium was inoculated with fungal spores and incubated for 14 days at a temperature of 27±1 ^{o}c and a relative humidity of 50-60%. For spores' collection, a sterile solution of 0.01% tween-80 (v/v aqueous solution) was used as a dispersing agent (Hicks **et al.,** 2001). The spores were scraped off and to remove any hyphal debris the resulting suspensions were filtered using a sterilized cheesecloth and Whatman No.1 filter paper. Then the suspensions were centrifuged for 5 minutes at 3000 rpm.The prepared spore suspensions were assessed against 2nd instar larvae of ***S. littoralis*** to evaluate their insecticidal activity. Each spore suspension was sprayed on both sides of castor bean leaves, the leaves of the control were sprayed with water. The treated and control leaves were left to dry and then provided to 10 individuals of second instar larva in a 250 ml capacity jar for each replicate. All treatments were lined with filter paper in an incubator at 25 ± 2 ◦C and 65 ± 5 % RH in 3 replicates for each treatment and so for control groups (10 larvae/replicate). Larval mortality%, pupation%, larval duration, pupal duration, pupal mortality%, deformed pupa%, adult emergence%, and deformed adult% were recorded daily.

2.4. Screening of fungal metabolites on some biological aspects of *S.litoralis*.

The PD broth medium was utilized for the production of ***B.bassiana*** metabolites. The medium was inoculated with fungal spores and incubated at 27±1°C and a relative humidity of 50-60% for seven days, then the mycelia was eliminated from the cultures via filtration through two layers of cheesecloth and subsequently through Whatman's No. 1 filter paper. The mycelia were eliminated from the cultures via filtration through two layers of cheesecloth and subsequently through Whatman's No. 1 filter paper. The fungal filtrates were assessed against 2nd instar larvae of ***S. littoralis*** to evaluate their insecticidal activity. Each filtrate was sprayed on both sides of castor bean leaves, and the leaves of the control were sprayed with water. The treated and control leaves were lifted to dry and then provided to 10 individuals of second instar larva in a 250 ml capacity jar for each replicate. all treatments were lined with filter paper in an incubator at 25 ± 2 ◦C and 65 ± 5 % RH in 3 replicates for each treatment and so for control groups (10 larvae/replicates). Larval mortality%, pupation%, larval duration, pupal duration, pupal mortality%, deformed pupa%, adult emergence%, and deformed adult% were recorded daily.

2.5. Morphological characterization of the most potent fungus:

The most potent fungus against ***S.litoralis*** was morphologically examined by light microscope and characterized according to the Key Fungal Manual of **(Humber, 1998)**.

2.6. Statistical analysis:

Data obtained were statistically analyzed according to a completely randomized design. The appropriate methods were used for the analysis of data according to **(Little and Hills 1975)** and the proper "F" value was calculated as described by (**Fisher** ١٩٥٠ and **Snedecor 1970).**

II. RESULTS

A total of 10 fungal isolates were isolated from soil samples. The spore suspension of these fungal isolates was tasted for their insecticidal activities against the second instar larva of Spodoptera littorales according to the preliminary entomotoxic bioassay.

3.1. Screening of fungal spores on some biological aspects of *S.litoralis*:

Results in **(table 1)** present the effects of different treatments on various biological aspects of ***Spodoptera littorales***. The treatments significantly impacted that isolate (no 9) showed the highest larval deformation (30%) followed by isolate NO 5 (13.33) rest of isolates showed low larval deformation (6.67%)by 4,6 isolates (3.33%)by 3,8 isolates and the isolates NO 7,11 had no deformational effect like control group(0%).

The larval mortality was in the highest percentage in treatment NO9 (33.3%) followed by isolates 8(13.33%) while 6,10 and 3,11 gave the same mortality percentage respectively as follow (10%) and (3.33%) ,isolates NO2,7 showed no mortality effect on larvae seems like the controlled group .

Additionally, the larval duration with various fraction treatments, as compared to the control (12.27 days), exhibited a low level of significant variation. The majority of fractions displayed durations ranging from 12.8 to 14.63 days, except the no 9 isolates, which recorded 15.4 days.

The pupation rate showed the lowest percentage in isolate NO9 treatment (66.67%) followed by isolate NO 8 (86.67%), the rest of treatments showed low significantly differenced pupation rates ranging from (90%) for isolates NO.6&10,(96.67%) for 3&11 isolates and (100%) for 2,7 isolates and the control group.

Pupal deformation for the whole treatments showed non-significant differences as most of them gave (0%) except treatments (10, 11) showed notable deformation (4.67,6.76%) but not significantly different from the control. The highest pupal mortality was observed in 5,11 treatments (14.07%) Pupal duration respectively indicating highly significant differences.

Treatment 4 and Treatment 11 had the longest pupal duration (9.9 day) and (9.5 day) respectively while treatment 2 (6.3 day) had the shortest duration, and treatment 9 showed no significant differences with the control group (8.77 and 8.87 day).

About adult Emergence (emergence%) Treatments 2, 7, 10, and 11 (100%) showed the highest adult emergence rates, followed by Treatment 8 (88.43%) and 9 (91.07%). Treatment 5 had the lowest emergence rates (85.93).

Only treatments 3,11 showed partial emergence (6.67,17.41%), while the rest treatments and the control group showed no partial emergence (0.0%)

Total Injury rates were highest in treatment 9 (40%), followed by treatment 8(23.33%), 5(20%), 11(10%), Control and the rest treatments showed very low injury rates as treatments 3,4(6.67%), treatments 2,11and control group(3.33%) instead to treatment 7 showed 0% injury ratio.

Table (1): Efficacy of fungal spore suspension on some biological aspects of second instar larva of *S. littoralis* .

Treatmen	Larval deformation	Larval mortality%	Larval duration(day)	Pupation%	Pupal deformation	Pupal mortality%	Pupal duration	Ememrge-ncy%	eme
Control	0^{a}	0^{c}	12.27^{e}	100^{a}	0^{a}	3.33^{bc}	8.87^{bc}	96.67^{ab}	
2	7.04^{b}	0^{c}	$^{13.13\ cde}$	100^{a}	0^{a}	3.33^{bc}	6.3^{f}	96.67^{ab}	
3	3.33^{b}	3.33^{bc}	13.6^{bcd}	96.67^{ab}	0^{a}	3.70^{bc}	7.8^{dc}	96.29^{ab}	
4	6.67^{b}	0^{c}	14.57^{ab}	100^{a}	0^{a}	6.67^{abc}	9.9^{a}	93.33^{abc}	
5	13.33^{b}	6.67^{bc}	13.63^{bcd}	93.33^{ab}	0^{a}	14.07^{a}	8.27^{cd}	85.93^{c}	
6	6.67^{b}	10^{bc}	12.8^{de}	90^{ab}	0^{a}	0^{c}	9.27^{ab}	100^{a}	
7	0^{b}	0^{c}	13.97^{bc}	100^{a}	0^{a}	0^{c}	7.23^{e}	100^{a}	
8	3.33^{b}	13.33^{b}	13.43^{cd}	86.67^{b}	0^{a}	11.57^{ab}	8.43^{cd}	88.43^{c}	
9	30^{a}	33.33^{a}	15.4^{a}	66.67^{c}	0^{a}	8.9^{abc}	8.77^{bc}	91.07^{bc}	
10	6.67^{b}	10^{bc}	12.87^{de}	90^{ab}	4.76^{a}	7.5^{abc}	7.33^{e}	100^{a}	
11	0^{b}	3.33^{bc}	14.63^{ab}	96.67^{ab}	6.67^{a}	14.07^{a}	9.5^{ab}	100^{a}	
F value	3.3263	6.8857	6.8247	6.8857	0.9054	2.6575	16.9828	3.6065	
P≥0.05	0.0090 **	0.0001 ***	0.0001 ***	0.0001 ***	0.5446 ns	0.0269 *	0.0000 ***	0.0058 **	***
LSD 0.05	13.8646	11.0292	1.0371	11.0292	7.2448	9.1653	0.7732	7.7213	

control is untreated with any fungle isolate .

-The mean value followed by different letters (a ,b ,c ,d &e) with in the same row are significantly different (One-way ANOVA. , $P \leq 0.05$) .

- L.S.D : the least significant difference . - *** means highly significant .

3.2. Screening of fungal filtrate on some biological aspects of *S.litoralis*.

The obtaianed rssults in**(Table 2)** shows the insecticidal activities of fungal filtrate of the four promising fungi(9,8,5and 10) on the second instar larva of ***S.littoralis.***

Treatments 2 to 11 caused varying degrees of larval deformation, while treatment 9 showed the highest deformation at 23.33% followed by 11(14.07%). The other treatments showed very low percent of larval deformation5(6.67%) , 8(3.335) and Control showed no larval deformation.

Larval mortality rates range from 0% (Control) to 30% (for the treatment 9), followed by 11(17.41%), 5(16.67%), and 8(6.67%) while the control had no larval mortality.

Larval duration ranges from 15.4 days (Control) to 16.3 days (treatment 9). Differences in duration are not statistically significant

Pupation rates range from 70% (treatment 9) to 100% (Control) Treatment 9 significantly reduces pupation rates compared to others followed by 5(80%) and 11(82.6).

Only treatment 9 showed pupal deformation (33.77%) while the other treatments and the control showed no pupal deformation at all.

Pupal mortality ranges from 3.70% (treatment 8) minimal effect to 35.77% (treatment 9) maximum effect, followed by 15.07% (treatment 11) and 4.16% for the control group.

Pupal duration ranges narrowly from 6.33 days (treatment 9) to 7.33 days (treatment 5) and the control group last for 6.97 days.

Most treatments showed high emergence rates except treatment 9 (57.57%). And the Partial emergence ranges from 0% (Control, 8) to 41.11% (treatment 9) followed by 21.75% for (treatment 11).

Treatment 9 showed the highest total injury percent .this result and the whole result indicate that isolate NO.9 had the most promising insecticidal effect on ***S.littoralis.***

The results of this experiment show that the fungus No.(9) shows the highest insecticidal effect on the biological aspects of s.littoralis, larval mortality (30%), pupal deformation (35.77%), pupal mortality(35.77%), adult emergence(57.57%), partial emergence(41.11%) and resulted in (60%) total injury comparing with the control which gave (0,0,4.16,95.83,0%)for the same aspects and the control total injury percent was 4.17%. So this fungus was forwarded for morphological characterization.

Table(2): Efficacy of secondary metabolites of the 4 promising fungi on some biological aspects of the second instar larva of *S. littoralis* .

Treatment	Larval deformation	Larval mortality%	Larval duration	Pupation%	Pupal deformation	Pupal mortality%	pupal duration	Ememrgence %
Control	0^{C}	0^{b}	15.4^{b}	100^{a}	0^{b}	4.16^{b}	6.97^{a}	95.83^{a}
5	6.67^{bc}	16.67^{ab}	16.1^{a}	80^{bc}	0^{b}	4.76^{b}	7.33^{a}	95.24^{a}
8	3.33^{c}	6.67^{b}	15.8^{ab}	93.3^{ab}	^{0b}	3.70^{b}	7.17^{a}	96.29^{a}
9	23.33^{a}	30^{a}	16.3^{a}	70^{c}	35.77^{a}	35.77^{a}	6.33^{a}	57.57^{b}
11	14.07^{ab}	17.41^{ab}	15.8^{ab}	82.6^{abc}	0^{b}	15.07^{b}	7.07^{a}	89.68^{a}
F value	8.7266	3.8630	2.3846	4.3361	18.7484	8.0787	0.7065	16.7490
P≥0.05	0.0027 **	0.0378 *	0.1208 ns	0.0273 *	0.0001 ***	.0036 **	0.6055 ns	0.0002 ***
LSD 0.05	9.9577	18.3566	0.6775	17.7455	10.0908	15.2262	1.4340	16.7489

control is untreated with any fungal isolate.

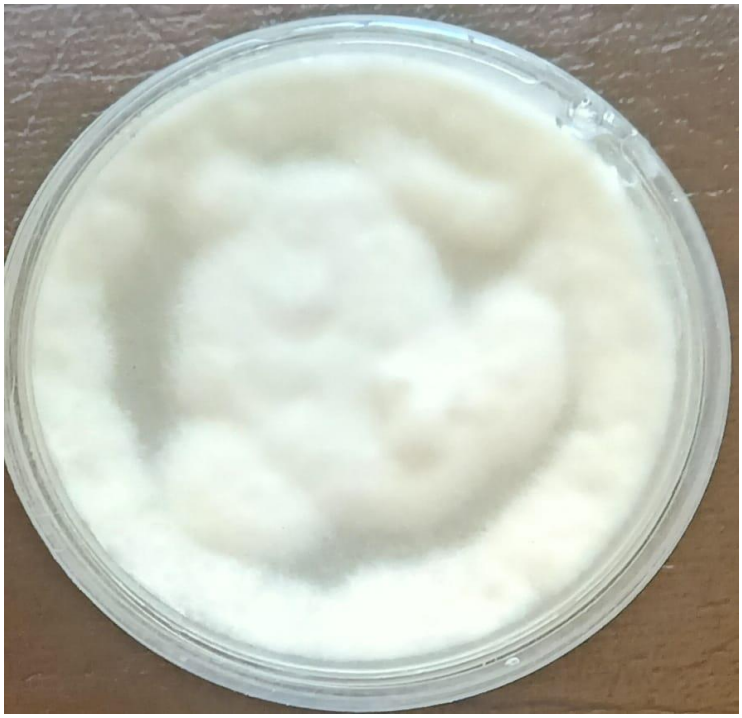
-The mean values followed by different letters (a,b,c & d) within the same row are significantly different (One-way ANOVA., *P* ≤0.05).

- L.S.D: the least significant difference. - * means highly significant.**

High F values and P values below 0.05 for most metrics (except larval duration and pupal duration) indicate significant differences among treatments.

3.3. morphological characterization and identification of the most potent fungus:

Figure (1) shows the most potent fungal isolate NO.(9) . It grows as a white mold that appears to be ***Beauveria bassiana.***



Figure(1) plate culture of *Bauveria bassiana*

Figure (2) shows the microscopic examination of Baeuveria bassiana which appears as many dry, powdery conidia in distinctive white spore balls. Each spore ball is composed of a cluster of conidiogenous cells. The conidiogenous cells of the fungal are short and ovoid; the conidia are single-celled, and haploid. This morphological structure indicated that this fungal isolate seems to be ***Beauveria bassiana.***

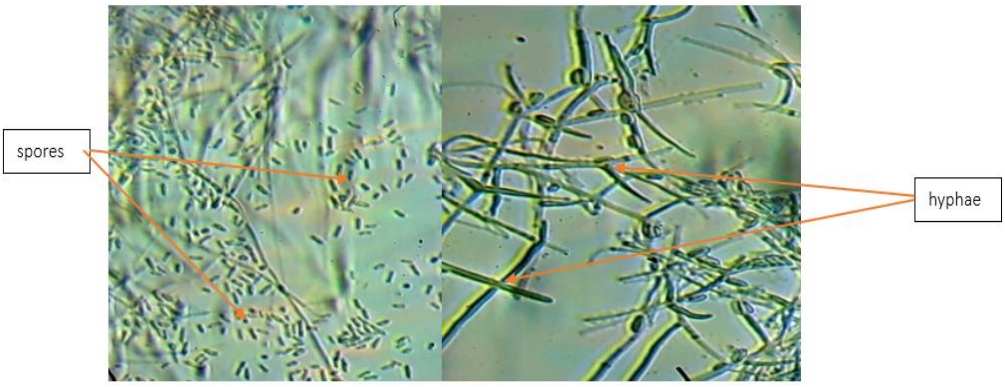


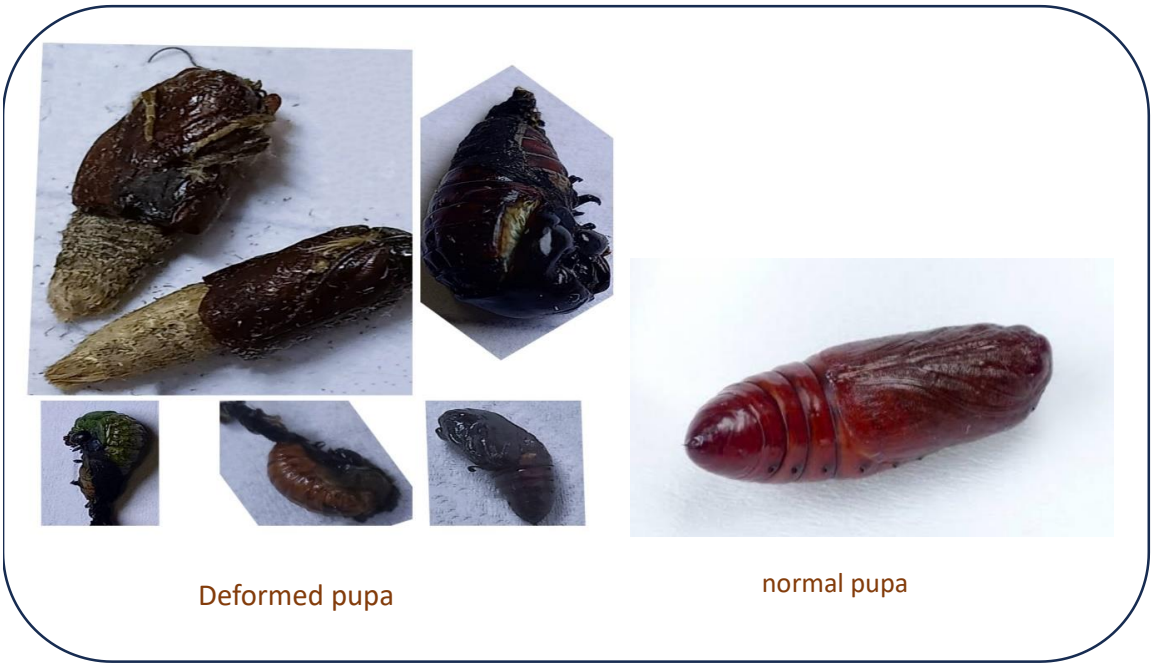
Figure (2) Microscopic examination of ***Baeuveria bassian***

Figure(3): *shows some* death symptoms and some biological effects of ***Baeuveriria bassiana*** on the larval stage of ***Spodoptera Littoralis***.

Death symptoms and some biological effects of bioactive fractions of *B. bassiana* against *s. littoralis* different stages.

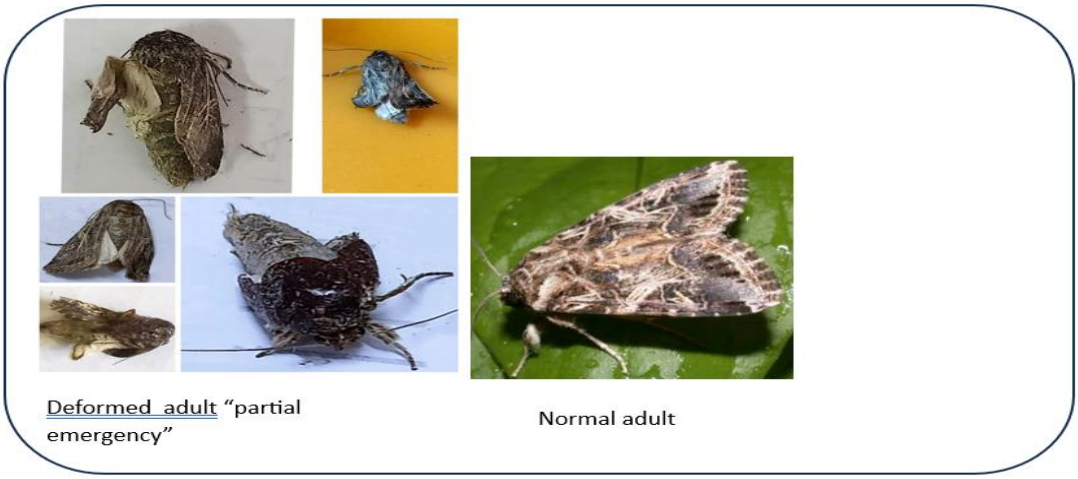
Figure(3):Death symptoms and some biological effects of ***Baeuveriria bassiana*** on larval stage of ***Spodoptera Littoralis.***

Figure(4): shows some Death symptoms and some biological effects of ***Baeuveria bassiana*** on the pupal stage of ***Spodoptera Littoralis.***



Figure(4):Death symptoms and some biological effects of ***Baeuveria bassiana*** on pupal stage of ***Spodoptera Littoralis***

Figure(5): shows some death symptoms and some biological effects of ***Baeuveria bassiana*** on the adult stage of ***Spodoptera Littoralis.***



Figure(5):Death symptoms and some biological effects of ***Baeuveria bassiana*** on adult stage of ***Spodoptera Littoralis.***

IV. Discussion

The cotton leafworm; *S. littoralis* was considered one of the most serious and destructive pests. Great efforts have been made to control this pest chemically (**Zohdy *et al.*, 2000)**. For many years, chemical insecticides have been used to control insect pests, but these pesticides have caused various health issues for all living organisms **(Sabry *et al.*, 2023)**. Several studies have been conducted to explore the most efficient control methods without using pesticides to overcome the harmful effects of chemical residues on animal and human health. Entomopathogenic fungi, such as ***Beauveria bassiana, Metarhizium anisopliae***, and ***Lecanicillium lecanii,*** are widely studied for their insecticidal properties. These fungi infect their hosts by penetrating the insect's cuticle and proliferating within, eventually causing the insect's death through mechanical damage and toxin production **(Sabbour and Soliman,2014).**

In the present study, a total of 10 fungal isolates were isolated from the soil. The spore suspensions of these fungal isolates were tested for their insecticidal activities against the second instar of ***Spodoptera littoralis***. Only 4 fungal isolates gave promising results on ***S. littoralis.*** Consequently, filtrates of the most bioactive fungi were tested for their insecticidal effect on ***S. littoralis***.

The result indicated that isolate (No. 9) had the highest insecticidal effect. Using morphological characterization by the light microscope, the most potent fungus (isolate No. 9) was identified as ***Beauveria bassiana***. This result agreed with **El-Katatny (2010)** who revealed that ***B. bassiana*** isolates among other fungal isolates, caused a high mortality rate in *S. littoralis* larvae. Similarly, **El-Hawary and Abd El-Salam (2009)** documented that ***B. bassiana*** was more effective than ***Paecilomyc fumosoroseus*** against *S. littoralis*. On the contrary, **Funda Şahin and Yusuf Yanar (2021).** showed that ***B. bassiana*** isolates explored a lower mortality rate than *Metarhizium spp*. these findings confirmed that different results can be obtained using different isolates of the same species as observed in this study.

The high insecticidal effect of B.bassiana may be owing to biochemical composition of the fungal exo and endo metabolites as shown by ***(M. Chaithra et al., 2022).***

Conclusion

The main objective of this research is to use entomopathogenic fungi in the biocontrol of ***spedoptera littoralis*** to reduce chemical insecticides usage and decrease its harmful effect on human, animals and the whole surrounding environment. And it was concluded that. The most effective fungal isolate of 10 isolates from different soil samples was isolate No 9 which gave the highest insecticidal potential on S. littoralis under laboratory condition.The most active fungal isolate was Identified as ***Beauveria bassiana***. under light microscope. As aresult of this study ***Baeuveria bassiana*** is a promising biopesticide to be used in I.P.M. programs.

References

Bara, G. T., & Laing, M. D. (2020). Entomopathogens: potential to control thrips in avocado,with special reference to Beauveria bassiana. *Horticultural Reviews*, *47*, 325-368. https://doi.org/10.1002/9781119625407.ch7.

Boucias, D. G., Pendland, J. C., Boucias, D. G., & Pendland, J. C. (1998). Entomopathogenic fungi: fungi imperfecti. *Principles of insect pathology*, 321-364.

Chaithra, M., Prameeladevi, T., Prasad, L., Kundu, A., Bhagyasree, S. N., Subramanian, S., & Kamil, D. (2022). Metabolomic diversity of local strains of Beauveria bassiana (Balsamo) Vuillemin and their efficacy

against the cassava mite, Tetranychus truncatus Ehara (Acari: Tetranychidae). *Plos one*, *17*(11), e0277124. https://doi.org/10.1371/journal.pone.0277124.

Charnley, A. K., & Collins, S. A. (2007). Ten entomopathogenic fungi and their role in pest control. *Environmental and microbial relationships*, *4*, 159.

El-Hawary FM, Abd El-Salam AME (2009) Laboratory bioassay of some entomopathogenic fungi on *Spodoptera littoralis* (Boisd.) and *Agrotis ipsilon* (Hufn.) larvae (Lepidoptera: Noctuidae). Egypt Acad J Biol Sci 2(2):1–4. https://doi.org/10.21608/eajbsf.2009.17547

El-Katatny MH (2010) Virulence potential of some fungal isolates and their control-promise against the Egyptian cotton leaf worm, *Spodoptera littoralis*. Arch Phytopathol Plant Prot 43(4):332–356. https://doi.org/10.1080/03235400701806278.

EPPO. (2008): European and Mediterranean Plant Protection Organization. *Spodoptera litura* fabricious. EPPO Bull., (9):142-146.

Feng M G, Poprawski T J, Khachatourians G G. (1994). Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. *Biocontrol Science and Technology* **4**: 3–34.https://doi.org/10.1080/09583159409355309.

Fisher, R.A. (1950): Statistical methods for research workers. IInd. Rev. ed., Oliver and Boyed, London.

Gamil, W. E., Mariy, F. M., Youssef, L. A., & Halim, S. A. (2011). Effect of Indoxacarb on some biological and biochemical aspects of *Spodoptera littoralis* (Boisd.) larvae. *Annals of Agricultural Sciences*, *56*(2), 121-126. https://doi.org/10.1016/j.aoas.2011.07.005.

Hicks, B.J., Watt, A.D. and Cosens, D. (2001): The potential of *Beauvaria bassiana* (Hyphomycetes: Moniliales) as a biological control agent against the pine beauty moth, *Panolis ammea* (Lepidoptera: Noctuidae). Forest Ecology and Management. (149):275-281. https://doi.org/10.1016/S0378-1127(00)00561-2.

Hodge, K. T., Humber, R. A., & Wozniak, C. A. (1998). Cordyceps variabilis and the genus Synglíocladium. *Mycologia*, *90*(5), 743-753.https://doi.org/10.1080/00275514.1998.12026966.

Little, T.M. and Hills, F.J. (1975): Statistical methods in agriculture research. Available from V.C.D.Book Store, University of California, Davis: 241 pp.

Nouh, G. M., Eid, A. E., Kamel, H. M., & Adly, D. (2022). Efficacy of the entomopathogenic fungus, Beauveria bassiana and certain insecticides on some cabbage insects in field. *Egyptian Journal of Agricultural Research*, *100*(4).on Spodoptera littoralis (Boisd.) and Agrotis ipsilon (Hufn.) larvae (Lepidoptera: Noctuidae). Egypt Acad J Biol Sci 2(2):1–4. https://doi.org/10.21608/eajbsf.2009.17547.

Raheja, K. L., & Snedecor, J. G. (1970). Comparison of subnormal multiple doses of l-thyroxine and l-triiodothyronine in propylthiouracil-fed and radiothyroidectomized chicks (Gallus domesticus). *Comparative Biochemistry and Physiology*, *37*(4), 555-563. https://doi.org/10.1016/0010-406X(70)90100-3.

Sabbour, M. M., & Soliman, E. A. (2014). Efficacy of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* against some insect pests infesting tomato plants in Egypt. *Egyptian Journal of Biological Pest Control*, *24*(2), 421-426.

Sabry, H. M., El-Medany, W. A., El-Tahawe, H. S., & Abd-ElAzeem, E. M. (2023). Biochemical Evaluation of Acremonium sp. as Biological Control Agent Against the Spiny Bollworm, Earias insulana by Scanning Electron Microscopy. Arab Journal of Plant Protection, 41(2). https://doi.org/10.22268/AJPP-41.2.140145.

Şahin, F., & Yanar, Y. (2021). Pathogenicity of some local entomopathogenic fungus isolates on the cotton leafworm larvae, *Spodoptera littoralis* (Boisd.)(Lepidoptera: Noctuidae). *Egyptian journal of Biological pest control*, *31*, 1-6.

Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular biology and evolution*, *24*(8), 1596-1599. https://doi.org/10.1073/pnas.0404206101.

Tudi, M., Daniel Ruan, H., Wang, L., Lyu, J., Sadler, R., Connell, D., Chu, C., & Phung, D. T. (2021). Agriculture Development, Pesticide Application and Its Impact on the Environment. *International journal of environmental research and public health*, *18*(3), 1112. https://doi.org/10.3390/ijerph18031112.

Zhang, L., Yue, Q., Wang, C., Xu, Y., & Molnár, I. (2020). Secondary metabolites from hypocrealean entomopathogenic fungi: genomics as a tool to elucidate the encoded parvome. *Natural product reports*, *37*(9), 1164–1180. https://doi.org/10.1039/d0np00007h

Zimmermann, G. (2007). Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Science and Technology*, *17*(6), 553-596. https://doi.org/10.1080/09583150701309006.

Zohdy, N.; El-Moursy, A. A.; Kares, E. A.; Abdel-Rahman, A. M. and El- Mandarawy, M. B. (2000): Effect of delfin and baythroid on the total haemocyte counts (THCs) and haemocyte percentage in healthy and parasitized larvae of the cotton leafworm. Egyptian Journal of Agricultural Research. (4):1569-1586.