Asian Journal of Advances in Research

5(1): 537-543, 2022

LABORATORY COMPARISON BETWEEN TWO COMMERCIAL FUNGI COMPOUNDS AND TWO ISOLATES OF ENTOMOPATHOGENIC FUNGI, ON PUPAE OF THE COTTON LEAFWORM *Spodoptera littoralis* (BOISD.) (LEPIDOPTERA: NOCTUIDAE)

MARWA M. A. EL-SABAGH [a*], NASHWA S. AMIN [a] AND DALIA E. M. LOTFY [a]

[a] Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, P.O.Box 12611, Egypt.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Received: 05 January 2022
Accepted: 09 March 2022
Published: 14 March 2022

Original Research Article

ABSTRACT

The pupae of the cotton leafworm *Spodoptera littoralis* (Boise.) (Lepidoptera: Noctuidae), a pest affecting many economic crops were treated with two commercial fungi compounds and two isolates of entomopathogenic fungi, by applying the pupal dipping technique. Results showed that all of the tested entomopathogenic fungi adversely affected the adult emergence, Suspensions ($1x10^8$ spores /ml) of conidia harvested from *M. anisopliae* isolate resulted in the highest mortality (62%) followed by *B. bassiana* isolate (60%) than *M. anisopliae* commercial (51%) and the lowest mortality (49%) with *B. bassiana* commercial, the treatment also led to reducing the egg-laying capacity and egg hatchability. Treatment with these entomopathogenic fungi also caused several malformations in pupae and adults resulting from the treatment of pupae. Conclusion of our results that the two tested entomopathogenic fungi isolate *M. anisopliae* and *B. bassiana* was efficient and can be recommended as bio-insecticides to control the cotton leafworm *S. littoralis*, within the integrated pest management (IPM) program.

Keywords: Entomopathogenic fungi; *Beauveria bassiana*; *Metarhizium anisopliae*; Pupal dipping technique and *Spodoptera littoralis.*

1. INTRODUCTION

The cotton leafworm *Spodoptera littoralis* (Boise.) (Lepidoptera: Noctuidae), earlier known to be an Economic pest, This pest has been attack a wide range of crops (nearly 112 species of crops) in, Asia, Africa and Europe [1,2,3]. Because of their potential to inflict significant economic damage to crops, the pest has been treated to high dosages of insecticides. This widespread use of insecticides has resulted in the development of resistance in this pest to a variety of active ingredients of insecticides [4,5]. Microbial control agents are a key component of insect pest biological control [6].

Entomopathogenic fungi have a particular advantage over other microbial control agents in that they may attack all phases of insect development, including pupae [7]. The entomopathogenic fungi are the promising agents that are used against insect pests for

**Corresponding author: Email: marwa3m1980@gmail.com;*

several decades. These organisms include taxa of several fungal groups like Hypocreales of Ascomycota that *Beauveria bassiana* and *Metarhizium anisopliae* are the two most recognized species [8]. Besides entomopathogenic fungi cause natural mortality on insects, these agents are environmentally safe, so there is a worldwide interest of their using and improvement for biological control of insects **(Vincent *et al.,* 2007).** The present study aimed to investigation is to evaluate the pathogenicity of two commercial fungi compounds and two isolates of entomopathogenic fungi, on pupae of *S. littoralis* (Boisd.) in order to controlling the cotton leafworm *S. littoralis* (Boisd.) through sterilize in the adult.

2. MATERIALS AND METHODS

2.1 Rearing of *S. littoralis* (Boisd)

Susceptible strain of the cotton leafworm, *S. littoralis* (Boisd) obtained from the division of cotton leafworm of plant protection research Institute, Dokki, Egypt, formed the basis of the culture designed to supply insects used in the present study. At 27 ± 2° C and 75% R.H., all stages of *S. littoralis* were cultivated and tested. The larval stages were fed caster bean leaves on a regular basis. The pupae were collected and placed in clean jars with damp sawdust at the bottom to provide a pupation site. Adults were given a 10% sugar solution to drink [9].

2.2 The Tested Entomopathogenic Fungi

2.2.1 The commercial entomopathogenic fungi compounds

Two commercial biobased insecticides were used. They were as follows:

a. BIO-POWER® (*Beauveria bassiana* (Balsamo) Vuillemin) as 1.15% WP and were applied as 0.18 gm/ liter.
b. BIO-MAGIC® (*Metarhizium anisopliae* (Metchnikoff) Sorokin) as 1.75% WP and the applied as 0.06 gm/ liter.

Both bioinsecticides were supplied from Gaara Establishment (Import and Export).

2.2.2 The isolates of entomopathogenic fungi

a. *B. bassiana* (Balsamo) AUMC NO (5133) and the applied as$1X10^8$ spores/ ml.
b. *M. anisopliae* (Metschnikoff) Sorokin AUMC NO. (5130) and the applied as $1X10^8$ spores/ ml.

Two tested isolates of entomopathogenic fungi were supplied from Assiutt University, Mycological Centre Faculty of Science.

2.3 Preparation of Commercial Entomopathogenic Fungi (EPFs)

The recommended dose for both commercial entomopathogenic fungi was used.

2.4 Preparation of Isolates Entomopathogenic Fungi (EPFs)

Isolates of *B. bassiana* (Balsamo) and *M. anisopliae* (Metchnikoff) Sorokin was cultivated on Sabouraud dextrose agar (SDA) Mould extract and 1000 ml. of water in flasks. Flasks were autoclaved at 21 °C for 15-20 min [10] The two fungoid sources were set as solution as follows:

Fungal cultures were full-grown on (SDA) medium g/l, [11] the mix was stimulated for ten min the hyphal debris was detached by sifting the mixture through fine mesh sieve. The conidial concentration of final suspension was resolute by direct count using Hemocytometer and Sequential thinning was ready in water with 0.1% Tween-80 and preserved at 5 ^{o}C till used [10].

2.5 Treatment of Pupae of *S. littoralis* (Boisd.)

To assess the entomopathogenic fungi for production of sterile adults of *S. littoralis*. The experiments were carried out by placing the pupae (one day old) in a small wire basket and immersed inside a beaker (100 ml.) containing the spores of tested entomopathogenic fungi for 20 min as dipping period with gentle shaking and left to dry, the treated pupae were transferred to 25 ml. glass jars [12]. All jars were lined with moistened 2 gm. artificial soil (Peat moss), which was also treated with the same concentrations of the fungi under study to maintain high humidity, it is also a simulation of nature. Twenty pupae were used for each test (ten males and ten females), three replicates were used for each concentration, left till adult emergence, while the untreated pupae was treated with distilled water containing Tween-80, 0.1% . Observations on the percentage of emerged adults were recorded both from treated and untreated individuals, furthermore, any induced malformations were recorded, either in pupae or emerged moths [12].

To evaluate the fecundity of adults and egg hatchability, treated pupae were collected; sexed and emerged moths were placed in pairs in breeding glass globes, supplied with leaves of tafla, *Nerium oleander*

(L.) as an oviposition site. Each globe was provided with 10 % solution of honey saturated in cotton wool which was placed in small cup in glass globes for moths feeding. The solution of honey was renewed daily to avoid fermentation and growth of microorganisms. Furthermore, any induced malformations were recorded, either in pupae or emerged moths.

2.6 Statistical Analysis

a. Egg laying (total number of eggs per female) calculated from daily counts of deposited eggs on tafla, *N. oleander*. Egg hatchability percentage calculated according to Zidan and Abdel-Megeed [13] as follows:

% Egg hatchability = (No. hatched eggs / No. deposited eggs) X 100

b. Sterility observed percentage calculated according to Zidan and Abdel-Megeed [13] as follows:

% Sterility observed = 100 – Egg hatchability percentage

c. Abbott's formula Abbott [14] was used to do statistical analysis of mortality data. Duncan, [15], Duncan's Test (DMRT), the probability level was determined to compare the differences among some parameter means ($P<0.05$) by the Costat system for Windows, Version 6.311, Berkeley, CA, USA, Costat application (2006).

3. RESULTS AND DISCUSSION

Results for assessing of two commercial fungi compounds and two isolates of entomopathogenic fungi were listed in Table 1. Results showed that the pupal duration of treated pupae with all tested entomopathogenic fungi was decreased compared to untreated group. In addition, results revealed that isolates of entomopathogenic fungi significantly decreased the pupal duration compared to commercial entomopathogenic fungi and untreated group. These results agreed with [16,17,18,19] when treated different insects with entomopathogenic fungi.

Results also showed that the highest mortality rate was obtained when treating pupae with *M. anisopliae* isolate (62%), while the lowest mortality rate was obtained due to treatment with the commercial formula of *B. bassiana* (49%) (Table 1 and Fig. 1). High reduction in adult emergence were recorded after treatment of pupae with tested entomopathogenic fungi (commercial and isolates). Deformed adults were observed with decreased wings or reduced body size. They couldn't fly and died without mating. It has been investigated that the pupae treated with entomopathogenic fungi often result reduction in the adult emergence [20], increase in pupal duration and deformed adults [21]. These results also agreed with Nguyen et al. [22] who found that *B. bassiana* showed a high reduction in adult emergence from pupae of *Helicoverpa armigera* using the soil treatment method and the pupal dipping technique. Our results also agree with El-Banna et al. [19] who recorded significantly decreased in adult emergence percentage when they treated the pupae of the cotton leafworm *S. littoralis* with two commercial biopesticides and two IGRs compounds, under laboratory conditions as a latent effect of the sub-lethal doses, and Abd El-Kareem [23] who recorded decrease in the adult emergence percentage and mean adult life span of treated larvae of *S. littoralis* with commercial entomopathogenic fungi (Bioranza).

Further, the latent effect of tested entomopathogenic fungi on fecundity and fertility of laid eggs obtained from adults treated as pupae presented in Table 2. Results revealed that treatment with all entomopathogenic fungi minimized the produced eggs/female compared to untreated group. Results also showed that the least mean number of produced eggs/a female was observed in *M. anisopliae* and *B. bassiana* isolate, then it is followed by the effect the commercial entomopathogenic fungi treatments compared to untreated group. Furthermore, results showed high decrease in the mean number of hatched eggs due to treatment with all tested entomopathogenic fungi. In addition, *M. anisopliae* and *B. bassiana* isolate caused the least number of hatched eggs compared to untreated group. Also it is apparent from the results that all entomopathogenic fungi induced degrees of sterility ranging from 7.029 to 15.5%. Our results were in accordance with Ullah et al., [24] and Garrido- Jurado et al., [18].

In addition, in our results that treatment of pupae with entomopathogenic fungi cause the formation of pupal-adult intermediate or malformed adults Fig. 2: (A1), the deformations made moths incapable to have typical flight conduct because many moths failed to spread their poorly developed wings Fig. 2: (B1), while others failed to shed their puparium Fig. 2: (B2) this results agree with [16,17,25,18].

Table 1. Effect of dipping on pupae of the cotton leafworm *S. littoralis* (Boisd.) in tested entomopathogenic fungi on the pupal duration, Mortality% and adult emergence %

Tested entomopathogenic fungi	Concentration %	Pupal stage duration (Days ±S.E.)	Mortality %	Adult emergence %
B. bassiana commercial	0.18 gm/ liter	10.3±0.3 [b]	49 [b]	51[b]
M. anisopliae commercial	0.06 gm/ liter	10 ± 0.5 [b]	51 [b]	49 [b]
B. bassiana isolate	$1X10^8$ spores/ ml.	9.6±0.1[b]	60 [a]	40 [c]
M. anisopliae isolate	$1X10^8$ spores/ ml.	8.3±0.5 [c]	62 [a]	38 [c]
Control	-	11.3±0.3 [a]	0 [c]	100 [a]

Means followed by the same small letter in a column are not significantly different at the 5% level of probability (Duncan's Multiple Range Test)

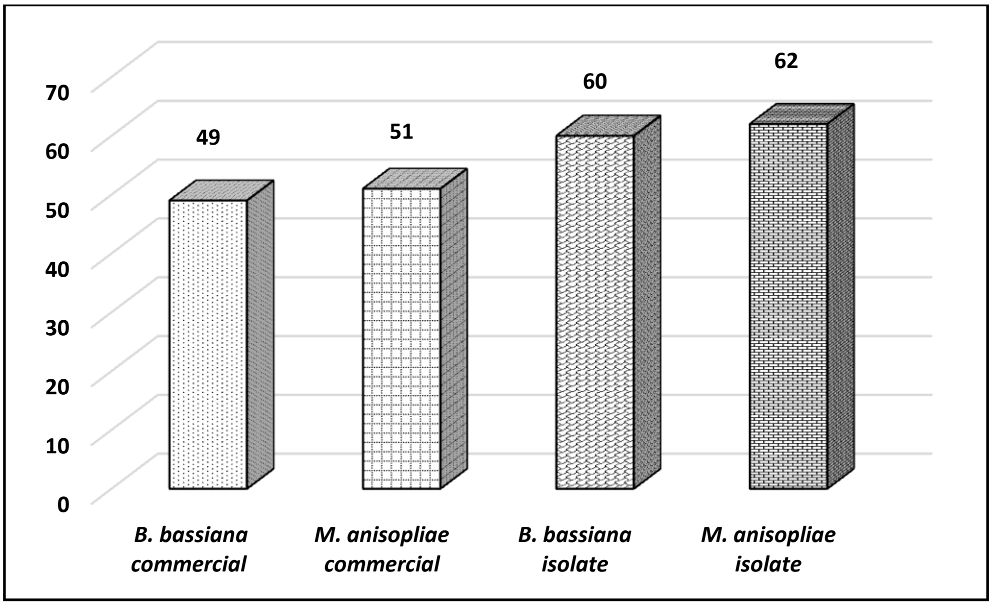

Fig. 1. Mortality % of dipping the pupae of the cotton leafworm *S. littoralis* (Boisd.) in tested entomopathogenic fungi

Table 2. Effect of dipping on pupae of the cotton leafworm *S. littoralis* (Boisd.) in tested entomopathogenic fungi on adult fecundity and sterility

Tested entomopathogenic fungi	Mean no. of eggs/female ± S.E.	Mean no. hatched eggs/female ± S.E.	Eggs hatchability %	Sterility %
B. bassiana commercial	530.6 ± 4.6 [d]	493.3 ± 4.2 [c]	92.9 [b]	7.029
M. anisopliae commercial	761 ± 1.2 [b]	642.6 ± 1.7 [b]	84.4 [e]	15.5
B. bassiana isolate	551.6 ± 2.4 [c]	482 ± 2.9 [d]	87.38 [c]	12.6
M. anisopliae isolate	410.6± 4.3 [e]	350.6 ± 1.2 [e]	85.3 [d]	14.6
Control	2557 ±1.1 [a]	2498.3±2.6 [a]	97.7 [a]	2.3

Means followed by the same small letter in a column are not significantly different at the 5% level of probability (Duncan's Multiple Range Test)

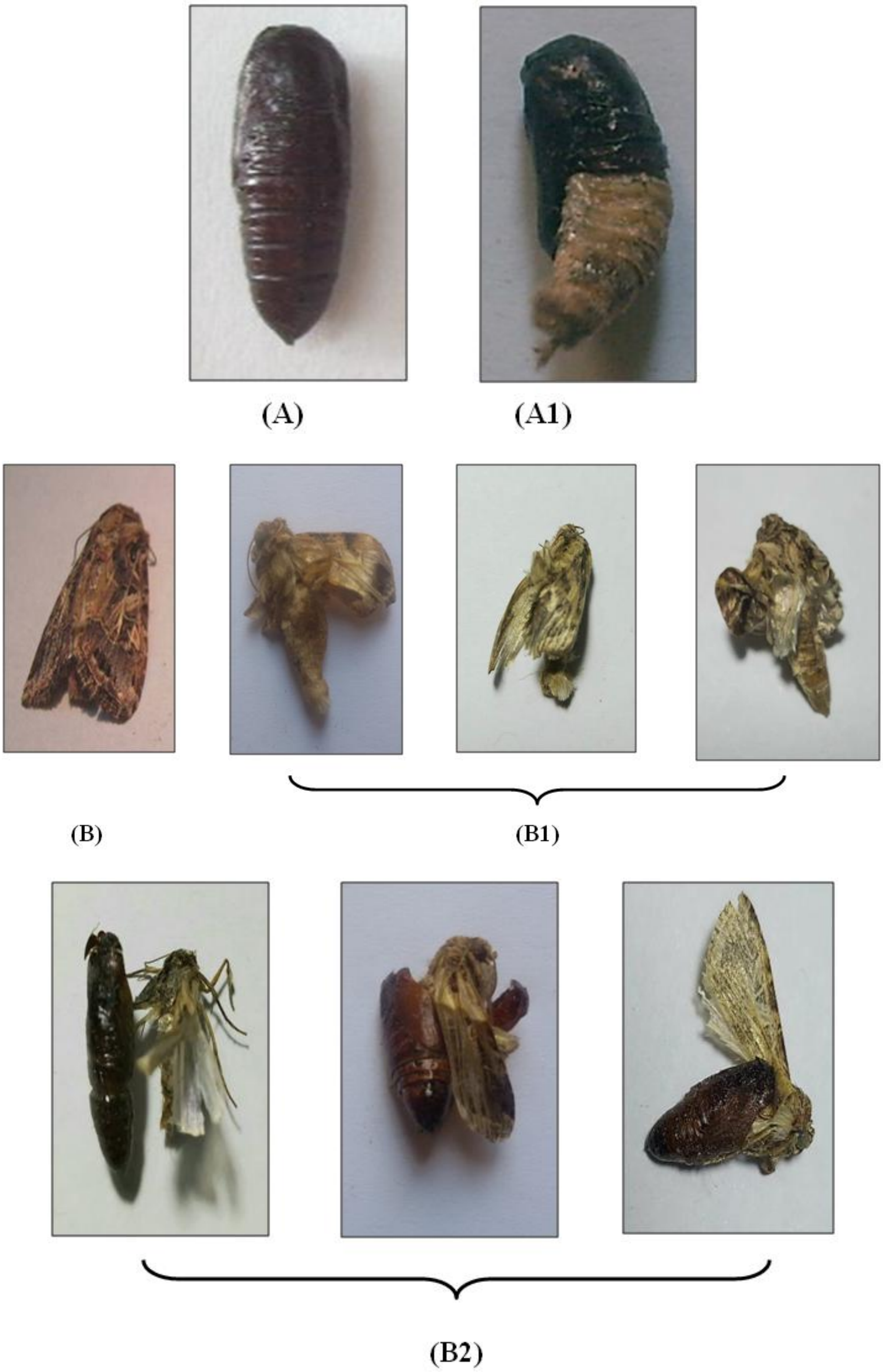

Fig. . Morphological malformation in pupae and adults of *S. littoralis* as induced by treatment pupa with entomopathogenic fungi
(A) Normal pupa. (A1) pupal-adult intermediates
(B) Normal *S. littoralis* moth. (B1) Adults with poorly developed wings
(B2) Adults failed to shed their puparium

4. CONCLUSION

From previously discussed results, results proved the efficiency of *M. anisopliae* and *B. bassiana* isolate that caused high percentage of mortality in the pupae and morphological malformations in both of pupae and adults resulting from the treatment of pupae. So we recommend using these two fungal isolates by made a special formula to dominate the cotton leafworm, *S. littoralis*, as a new approach in integrated pest management (IPM) program against *S. littoralis* by applying this two fungal isolates on the pupae where they are in the partially moist places in the field.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pineda S, Schneider MI, Smagghe G, Martínez AM, Del Estal P, Viñuela E, Valle J, Budia F. Lethal and Sublethal Effects of Methoxyfenozide and Spinosad on *Spodoptera littoralis* (Lepidoptera: Noctuidae). Journal of Economic Entomology. 2007;100 (3):773–780. Available:https://doi.org/10.1093/jee/100.3.773
2. El-Sheikh ESA. Comparative toxicity and sublethal effects of emamectin benzoate, lufenuron and spinosad on *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae). Crop Protection. 2015;67:228–234. Available:https://doi.org/10.1016/j.cropro.2014.10.022
3. Barrania AA. Effects of some Insecticides on some Biological Parameters of Cotton Leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae). Alexandria Science Exchange Journal, 40(APRIL-JUNE). 2019;307–313. Available:https://doi.org/10.21608/asejaiqjsae.2019.34182
4. Zedan SA, Shoulkamy MA. Use of entomopathogenic fungi for control of the Egyptian cotton leafworm, *Spodoptera littoralis*. J Nutr Food Sci. 2013;3:4. Available:https://doi.org/10.4172/2155-9600.S1.010, 01
5. Hawkins NJ, Bass C, Dixon A, Neve P. The evolutionary origins of pesticide resistance. Biological Reviews. 2019;94(1): 135–155. Available:https://doi.org/10.1111/brv.12440
6. Dent D. Biological control. In: Insect Pest Management. 2nd edition. London. CABI publishing. Ch. 2000;6:180-234.
7. Ferron P. Biological control of insect pests by entomopathogenic fungi. Annu Rev Entomol. 1978;23:409–442.
8. Vincent C, Goettel MS, Lazarovits G. Biological control, a global perspective. CABI publishing. Oxfordshire, United Kingdom; 2007.
9. Eldefrawi ME, Toppozada A, Mansour N, Zeid M. Toxicological Studies on the Egyptian Cotton Leafworm, *Prodenia litura.* I. Susceptibility of Different Larval Instars of *Prodenia* to Insecticides1. Journal of Economic Entomology. 1964;57(4):591–593. Available:https://doi.org/10.1093/jee/57.4.591
10. Hicks BJ, Watt AD, Cosens D. The potential of *Beauveria bassiana* (Hyphomycetes: Moniliales) as a biological control agent against the pine beauty moth, *Panolis ammea* (Lepidoptera: Noctuidae). Forest Ecol. & Management. 2001;149:275-281.
11. Sabouraud R. Contribution a l'etude de la trichophytie humaine. Etude clinique, microscopique et bacterioloqique sur la pluralité des trichophytons de l'homme. Annales de Dermatologie Syphilol. 1892;3: 1061–108.
12. Anand R, Birendra P, Bhupendra NT. Relative susceptibility of *Spodoptera litura* pupae to selected entomopathogenic fungi. International Organization for Biological Control (IOBC). 2009;54:85–92.
13. Zidan H, Abdel-Megeed MI. New Trends in Pesticides and Pest Control-Part II Al-Dar Al-Arabia for Publishing and Distribution, Cairo, Egypt; 1987.
14. Abbott WS. A method of computing the effectiveness of an insecticide. J Econ. Entomol. 1925;18:265-267.
15. Duncan BD. Multiple range and multiple F tests. Biometrics. 1955;11(1):1-42.
16. Amer MM, El-Sayed TI, Bakheit, HK, Moustafa SA, El-Sayed YA. Pathogenicity and genetic variability of five entomopathogenic fungi against *Spodoptera littoralis*. Research Journal of Agriculture and Biological Sciences. 2008;4(5):354-67.
17. Hussein HM, Zemek R, Habuštová SO, Prenerová E, Adel MM. Laboratory evaluation of a new strain CCM 8367 of Isaria fumosorosea (syn. Paecilomyces fumosoroseus) on *Spodoptera littoralis* (Boisd.). Archives of Phytopathology and Plant Protection. 2013;46(11):1307-19.
18. Garrido-Jurado I, Resquín-Romero G, Yousef-Naef M, Ríos-Moreno A, Quesada-Moraga E. Soil drenching with entomopathogenic fungi for control of the soil-dwelling life stages and

adults of the same generation of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Bulletin of Entomological Research. 2020; 110(2):242-8.

19. El-Banna, Heba MS, El-Sabagh Marwa MMA, Abd El-Kareem, Sara MI, Ibrahim Suzan A. Susceptibility of different stages of a field strain of the cotton leaf worm *Spodoptera littoralis* (Boisd.) to two bioinsecticides and two insect growth regulator compounds under laboratory conditions. Uttar Pradesh Journal of Zoology. 2020;41(12):20-27.
20. Ekesi S, Maniania NK, Lux SA. Mortality in three African tephritid fly puparia and pupa caused by entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. Biocontrol Sci. Technol. 2002;12(1):7-17.
21. Hafez M, Zaki FN, Moursy A, Sabbour M. Biological effects of the entomopathogenic fungus, Beauveria bassiana on the potato tuber moth, Phthorimaea operculella (Seller). J. Pest. Sci. 1997;70(8):158-159.
22. Nguyen NC, Borgemeister HP, Zimmermannn G. Laboratory investigations on the potential of entomopathogenic fungi for biocontrol of *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae and pupae. Biocon Sci Tec. 2007;17(12): 853-864. Available:https://doi.org/10.1080/09583150701546375
23. Abd El-Kareem SMI. Effect of some entomopathogens and insecticides against the cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Ph. D. thesis, Fac. of Science, Ain Shams University, Cairo, Egypt. 2012; 185.
24. Ullah MI, Altaf N, Afzal M, Arshad M, Mehmood N, Riaz M. Effects of entomopathogenic fungi on the biology of *Spodoptera litura* (Lepidoptera: Noctuidae) and its reduviid predator, *Rhynocoris marginatus* (Heteroptera: Reduviidae). International Journal of Insect Science. 2019;11:1179543319867116.
25. Doucet D, Retnakaran A. Targeting cuticular components for pest management. Extracellular Composite Matricesin Arthropods: Springer. 2016;369-407.
