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Efficacy of *Metarhizium anisopliae* var. *acridum* against *Acrida conica* (Orthoptera: Acrididae): Comparative application strategies and haemolymph biochemical disruptions

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Abstract
The spread of the slant-face grasshopper, known as *Acrida conica,* has recently caused substantial economic damage. *Metarhizium anisopliae* var. *acridum* is the most common entomopathogenic fungi which used as biological agents, they provide a safer and more efficient substitute for conventional insecticides. The study was conducted on *A. conica* nymphs in the Bahariya Oases, Egypt, during the summer season (2025) utilizing a sunflower oil based ultra-low volume (ULV) and water based low volume (LV) spray techniques. Mortality rates, median lethal time (LT_{50}), and haemolymph biochemical disruptions were studied.
The sunflower oil based ULV greatly increased fungal virulence, according to the results, which showed 96.4% nymphal mortality at 10 days post-treatment compared to 76% by water based LV application. It also achieved LT_{50} of 4.17 and slope 3.24, while water based LV had LT_{50} 6.12 and slope 2.18 indicating quicker fungal activity. Biochemical assessments performed five days after treating the fifth nymphal instar of *A. conica* with a sunflower oil based ULV application confirmed a severe metabolic collapse, reflected by a -63.9% decline in trehalase enzyme activity, indicating severe energy depletion. Lactate dehydrogenase (LDH), chitinase, and protease enzymes were all strongly activated, with increases of +237.5%, +413.1%, and +186.9% more than control, respectively.
These findings show that using *M. anisopliae* var. *acridum* in oil based formulation ULV protects fungal spores from desiccation and accelerating their penetration through the outer cuticle of *A. conica* nymphs leading to disrupting their critical functions. As a result, this technique is recommended as an effective and quick tool for the integrated management of *A. conica* controlling.

Keywords: *Acrida conica*, *Metarhizium anisopliae*, Trehalase, Protease, Chitinase, LDH

Introduction
Grasshoppers are extremely important economically. They are phytophagous insects, and their geographical distribution and excessive crop consumption pose a severe danger to global agricultural productivity, resulting in massive financial losses. (Riffat *et al*., 2017). Using chemical insecticides is the main method of controlling grasshopper outbreaks. But they pose a serious risk to human health and nontarget creature populations, which harms the environment, even though they are typically quick, cheap, and effective (Dakhel *et al*., 2020) [7], and public concern have driven comprehensive studies environmentally friendly methods for controlling pests, particularly biological pesticides.
Microbial agents such as bacteria, viruses, nematodes, protozoans, and pathogenic fungi are effective bio-control agents, they are considered bio-pesticides and are projected to have an increasing role in controlling many pests globally (Sree and Padmaja, 2008) [42]. Entomopathogenic fungi play very important role in integrated pest management, as they regulate insect populations (Mesquita *et al.* 2023) [27], unlike insecticides, these fungal pathogens cause their lethal effects by specifically targeting and infecting pest insects, they come into direct contact with an insect's cuticle. After adhering to an insect host, the conidia penetrates the cuticle via germ tube pressure and enzymatic destruction (Litwin *et al*., 2020) [22], additionally, spores of biological agent stay in soil and environment, in which other insects are susceptible to infestation (El-Gendy *et al*. 2022) [11].
The fungal pathogen *M. anisopliae* var. *acridum* is one of most potent biological tool for grasshoppers control (Bischoff *et al.*, 2009) [6]. Like other fungi, temperature and relative humidity have a big impact on its efficacy, so the investigation has focused on modifying formulations and utilization techniques to optimize fungal virility and persistency in the field (Inglis *et al.*, 2001) [14]. In grasshopper management, the most widely used technique is ULV spray. Due to the rapid evaporation of water, water based ultra-low volume droplets are not suitable for ULV spray techniques (Matthews *et al.,* 2014) [26]. Spores with oil formulations bypass the high humidity requirement during the infection phase and safeguard the conidia from soil irradiation (Kameel *et al*. 2025) [17].
Enzymes play crucial functions in various biological processes during an insect's life cycle; therefore the level of enzymatic activity indicates the insect's physiological activity. Numerous stimuli can induce disturbance in various enzymes activity, insects may respond drastically to challenges with different compounds, including the same enzymes.
The purpose of this study is to assess the toxic impact of the *M. anisopliae* var. *acridum* on nymphs of *A. conica* grasshopper, utilizing sunflower oil based ULV and water based LV sprayer approaches, as well as the effect on nymph haemolymph enzymes.

Materials and Methods

1. Study site and insects

During July of 2025, an ecological survey was conducted to assess the principal insect pests of the Acrididae family that inhabited the Baharia oasis in Egypt's western desert. This location was discovered to be home to grasshoppers in which the slant-face grasshopper, *A. conica* was the most dominant. An alfalfa (*Medicago sativa*) field, highly infested with the 4th and 5th nymphal instars of *A. conica* grasshopper (more than 30 insects/m^2), was chosen to conduct the experiments.

2. Tested entomopathogenic fungus (TEPF)

Green Muscle®, a commercial mycopesticide derived from the strain IMI 330189 of *M. anisopliae* var. *acridum*, with a spore concentration of $2x10^{10}$ conidia/g.

3. Sprayer equipment used

The micron Ulva+ is a hand-held spinning disc sprayer for ultra-low volume (ULV) spray technique, calibrated 90 ml water/min.

Motorized knapsack mist blower with normal spray nozzle for low volume (LV) application, calibrated 800ml water/ min.

4. Formulations applied

Two distinct formulations were prepared

Oil based Formulation: 2.5 g of fungal powder was suspended in one liter of vegetable sunflower oil for ULV application**.**

Water-based Formulation: 2.5 g of fungal powder was suspended in one liter of distilled water supplemented with 0.1% Tween 80 as a surfactant for conventional application**.**

Both formulations were standardized to a final concentration of approximately $5x10^7$ to $5x10^8$ conidia/ml**.**

5. Experimental procedure:

The infested field was divided to plots of (15m×20m) = 300 m^2 each plot was isolated by a wide belt of (10m×20m) = 200 m^2. Five plots for each treatment (ULV and LV application), in addition to five Plots used as a control; the control was sprayed with water only. Each treatment and control was consisted of 5 replicates (cages) 0.5m × 0.5m. After treatment directly 90 insects (4th and 5th nymphal instar) are collected with sweep-net and put inside cages in shade area of treated plots. The insects fed with treated plants (alfalfa) from the same plot. Daily mortality and mean accumulative mortality were calculated for 10 days post treatment and corrected by Schneider-Orelli's formula (Püntener, 1981).

6. Spraying Conditions and Specifications

- **Spraying height:** 0.6 m above the plants.
- **Walking speed:** 3 km/hr.
- **Swath width:** 3 m.
- **Wind:** 5-6 m/sec.
- **Temperature:** 33 ± 2 ºC.
- **Spraying time:** between 7 and 9 A.M.

7. Biochemical Analysis

For biochemical study, the 5th nymphal instars of *A. conica* grasshopper were collected following from the fifth day of treatment with *M. anisopliae* var. *acridum* by sunflower oil based ULV spraying. To avoid melanization, haemolymph samples were retrieved by piercing a proleg and pulling the flowing haemolymph into a micropipette containing a phenylthiourea crystal. To obtain appropriate volume, samples were gathered together from many insects per repeat. The haemolymph underwent a centrifuge at 2000 [43] r.p.m. for 5 minutes, and only the supernatant fractions were utilized for the following enzymes activity:

7.1 Determination of trehalase activity

Trehalase was determined according to the method described by Ishaaya and Swiriski (1976) [15].

7.2 Determination of chitinase activity

Chitinase was determined according to the method described by Bade and Stinson (1981) [3].

7.3 Lactate Dehydrogenase (LDH) activity:

LDH was determined according to the method described by Nathan *et al*. (2005).

7.4 Determination of Protease activity:

Protease was determined according to the method described by Gatehouse *et al*. (1999) [13].

8. Statistical Analysis

Data were analyzed using ANOVA via SAS software (SAS 1995). according to Tukey's Honestly Significant Difference (HSD) test at a significance level of ($P < 0.05$). LdP Line software was used to do probit analysis to estimate the LT_{50} (Median Lethal Time), 95% Confidence Limits, Slope and Chi-square (χ^2).

Results

Comparison of mortality rates between ULV and LV spray techniques

The obtained result in Table (1) illustrated the toxicity of *M. anisopliae* var. *acridum* on the 4th and 5th nymphal instars of *A. conica* during oil based ULV and water based LV application strategies under field condition. In general, the corrected mean cumulative mortality percentages of infected grasshopper nymphs have positive correlation with time during the 10 observation days. Statistical analysis revealed significant differences between the two application from the second day to tenth day post treatment, where ULV treatment was the most effective within treatment days and recorded mortality percentages ranged from 11.1 % to 96.4% comparing with 6.7% to 76% in LV treatment at the 2nd to 10th day post treatment respectively.

Table 1: Corrected mean cumulative mortality (%) of infected grasshopper *A. conica* nymphs with *M. anisopliae* var. *acridum* under field condition

Days	Control Mortality (%)	(oil based) ULV corrected mortality (%)	(water based) LV corrected mortality (%)
1	0.0±0.0	0.0^a±0.0	0.0^a±0.0
2	0.0±0.0	11.1^a±1.2	6.7^b±0.8
3	1.1±0.2	22.4^a±1.8	12.3^b±1.1
4	1.1±0.2	42.7^a±3.1	24.8^b±2.4

5	3.2±0.4	59.8^{a}±4.2	35.7^{b}±3.5
6	3.2±0.5	74.8^{a}±3.6	50.6^{b}±4.1
7	5.1±0.7	88.3^{a}±2.1	63.8^{b}±3.8
8	5.1±0.8	91.8^{a}±1.5	70.7^{b}±2.9
9	5.1±0.6	94.1^{a}±0.9	74.3^{b}±2.2
10	7.4±0.9	96.4^{a}±0.7	76.0^{b}±1.9

Values are expressed as Mean ±Standard Error (SE). Significance: Means within the same row followed by different superscript letters (a, b) are significantly different ($P < 0.05$).

Probit analysis demonstrated that the oil based ULV spraying has the highest lethal efficacy, with LT_{50} of 4.17 days and high slope value (3.24). In contrast, the water based LV spraying showed a delayed response, with LT_{50} of 6.12 days and slope 2.18. Also two applications have low value of X^2 (1.45 & 2.12) and nonoverlaping 95% confidence limits were 3.82 – 4.51 and 5.68 – 6.64 for ULV and LV spraing respectively (Table 2).

Table 2: Probit analysis parameters for the lethal activity of *M. anisopliae* var. *acridum* against *A. conica* nymphs under field conditions.

Application Method	LT_{50} (Days)	95% Confidence Limits (LCL – UCL)	Slope ± SE	Chi-square (χ^2)
ULV (Oil-based)	4.17	3.82 – 4.51	3.24 ± 0.28	1.45
LV (Water-based)	6.12	5.68 – 6.64	2.18 ± 0.19	2.12

LT_{50}: Lethal time required to kill 50% of the population; LCL: Lower confidence limit;
UCL: Upper confidence limit; SE: Standard error; χ^2: Chi-square value.

Infection impact on enzymes activity:

Table (3) indicate that, enzymes activity of the haemolymph of infected fifth nymphal instars of *A. conica* grasshopper with (TEPF) by ULV application after 5 days resulted in considerable metabolic alterations ($P < 0.001$ and $P < 0.0001$), the activity of trehalase enzyme decreased than control recorded 48.7 U/mg compared with 135.2 U/mg for control meaning that the fungal infection led to reduction reached -63.9% than in control. In contrast, highly significant increases in chitinase, protease and (LDH) enzymes activity were recorded +413.1%, +186.9% and +237.5% respectively more than control.

Table 3: Impact of *M. anisopliae* var. *acridum* infection on haemolymph enzymes activity of the 5th nymphal instars of *A. conica* grasshopper (5 days post-treatment).

Enzymes	control	Infected nymph	% change to control	t-test (P)
Trehalase U/mg)	135.2±6.4	48.7±3.2	- 63.9	0.002**
Chitinase (U/mg)	3.8±0.4	19.5±1.1	+ 413.1	0.0001***
Protease (U/mg)	9.2±0.7	26.4±1.9	+186.9	0.004**
LDH (U/L)	18.4±1.5	62.1±4.8	+ 237.5	0.001**

Significant differences are indicated by asterisks: (*) $P < 0.05$, (**) $P < 0.001$ and (***) $P < 0.0001$.

Discussion

The investigation's findings provide compelling evidence that *M. anisopliae* var. *acridum* is an effective biocontrol agent against *A. conica* nymphs. Numerous studies have shown its effective against a range of Orthoptera pests; for instance, this results parallel with (El-Dydamony, 2011) [10] reported the mortality of fifth nymphal instar of *S. gregaria* reached to 88% after 9 days from treatment also Kassa *et al.* (2004) [20] demonstrated the effectiveness of green muscle against *Locusta migratoria*, in addition to Fathy *et al.* (2025) [12] observed similar efficacy on nymphs of *L. migratoria*. Furthermore, the greater mortality rates observed in treated plots illustrate the effectiveness of *Metarhizium* spp. against numerous orthopteran pests (Yasin *et al.* 2024) [44].

The use of oil based formulations has increased the virulence of fungal conidia and produced extremely promising control of Acrididae species (Bateman, 1997) [5]. Oil formulation based could promote fungal conidia adherence and spore maturation at low relative humidity and maintain spore vitality, even at extreme temperatures (Muniz *et al.* 2020) [32]. This is consistent with the current investigation, which showed that TEPF is more successful when sunflower oil based ULV spraying is used, as evidenced by the observed reduction in the median lethal time (LT_{50}) value and the higher rates of accumulative mortality in oil based ULV treatments compared to water based LV treatments. Furthermore, the high slope of the (ULV) application indicates a rapid and uniform mortality response within the *A. conica* nymph population under field conditions, with no overlap in the 95 % confidence limits for water based spraying, this is conclusive evidence that oil based ULV spraying is indeed superior. So the formulation and spray technique are important in determining the total bioinsecticidal action.

Bateman *et al.* (1993) [4] stated similar results, demonstrating that cotton seed oil formulations including *M. flavoviride* had better insect infectivity against *Schistocerca gregaria*. Also, Ali *et al.* (2019) [1] revealed additive activity after 15 days of treatment and decline in LT_{50} when the nymphs of *Chrotogonus homalodemus* grasshoppers were subjected to a mixture of *M. anisopliae* and essential oils. In addition, Kameel *et al.* (2025) [17] found that utilizing sunflower oil and essential oils in varied amounts to battle *Heteracris littoralis* grasshoppers resulted in higher insect infectivity because the oil shielded fungal conidia from UV rays and increased their attached to the host.

Enzymes play crucial functions in various biological processes of an insect's life; so the enzymatic activity level indicates the insect's physiological activity. Numerous stimuli can induce disturbance in various enzymes activity, insects may respond drastically to challenges with different compounds, including the same enzymes (Li *et al.*, 2017). The results of this study showed highly significant increasing in chitinase, protease and (LDH) enzymes activity and significant decreasing in trehalase enzyme activity after 5 days of TEPF's infection with oil based formulation ULV application in field compared with control.

Trehalase activity functions as a reliable biomarker for determining nutritional (Ishaaya and Swirkski 1976) [15]; there are multiple possible explanations for the observed decrease in trehalase activity in the nymphs treated with the fungus. *M. anisopliae* may produce their own trehalase enzyme to hydrolyze the host's trehalose and use the resulting glucose as an energy source for their growth and spared within the insect (Peng *et al.* 2015) [34]. The total estimated trehalase activity may drop as a result of the fungi's intake of host trehalose. Another hypothesis is that the locust's energy reserves are depleted by the toxicity stress of the fungal infection and the metabolic needs of mounting an immunological response, which reduces the need for trehalose hydrolysis into glucosemay (Rashwan 2013). According to Maha (2015) [24, 37] study, a fungal infection may actually interfere with the host's ability to metabolize carbohydrates.

Chitin-degrading enzymes play an important role in the life of insects, especially during nymph molting, where epidermal cells synthesize chitinases and proteases, which accumulate in the molting fluid (Reynolds and Samuels, 1996) [36]. Most of the digestion products are transported via the molting fluid to the mouth and anal openings, and are subsequently accumulated in the midgut (Yarema *et al*., 2000) [43]. Increased protease and chitinase activity in treated nymphs is likely due to a combination of variables, including fungal infection and host reaction. where *Metarizium* fungi secret extracellular enzymes, such as proteases and chitinases, to dissolve the insect cuticle, allowing them to enter the host's hemocoel and infect it (Segura-Vega *et al.*, 2024) [41], and this may lead to increase of the detected protease and chitinase levels in the treated nymphs. Furthermore, the insect may increase its own protease and chitinase enzymes activity as a defense strategy in response to the fungal infection (Jiang *et al.*, 2019) [16]. This outcome is consistent with (Muhammad 2025) [12], who showed that treatment with *M. anisopliae* (AUCM5130) under semi-field circumstances resulted in changed enzyme activity in *L. migratoria* nymphs, including enhanced protease and chitinase and decreased trehalase.

LDH is a very important enzyme in which we can detect the alterations in insect activity during the course of development. LDH is an important glycolytic tools that is present in virtually all tissues (Kaplan and Pesce, 1996) [19]. The relative activities of the insect dehydrogenases may be related to the tissue functions and energy requirements (Dickinson and Sullivan, 1975) [8]. LDH metabolism is commonly viewed as a stress indicator (Mojarab-Mahboubkar *et al.*, 2022) [30]. In this work, LDH activity increased compared to the control, which differs with (Mohamed 2009) [29], who discovered lower LDH enzyme activity when *S. gregaria* was treated with *M. anisopliae*. While this is consistent with the findings of other orders, Mirhaghparast *et al.* (2013) [28] found an increase in *Spodoptera littoralis* nymph LDH enzyme activity following *M. anisopliae* infection.

Conclution

In this regard, the findings of this study are intriguing, indicating that entomopathogenic fungus *M. anisopliae* var. *acridum* is a non-toxic, ecologically friendly replacement to chemical based insecticides and their detrimental effects leading environmental imbalance, so it can play a vital role in integrated pest management. In addation to, oil based formulation ULV spray technique being more efficient than water based LV application in managing *A. conica* nymphs.

References

1. Ali SS, Said SM, Ibrahum AA, El-Dydamony MK. Integrated action between entomopathogenic fungi and some essential oils on the grasshopper, *Chrotogonus homalodemus* (Blanchard) (Orthoptera: Pyrgomorphidae). Academic Journal of Entomology,2019:12(3):88-93.
2. Alves RT, Bateman RP, Prior C, Leather SR. Effects of simulated solar radiation on conidial germination of *Metarhizium anisopliae* in different formulation. Crop Protection,1999:17(8):675-679.
3. Bade ML, Stinson A. Biochemistry of insect differentiation. A system for studying the mechanism of chitinase activity *in vitro*. Archives of Biochemistry and Biophysics,1981:206(1):213-221.
4. Bateman RP, Carey M, Moore D, Prior C. The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. Annals of Applied Biology,1993:122(1):145-152.
5. Bateman RP. Methods of application of microbial pesticide formulations for the control of locusts and grasshoppers. Memoires Entomology Society Canada,1997:129(S171):69-81.
6. Bischoff JF, Rehner SA, Humber RA. A multilocus phylogeny of the *Metarhizium anisopliae* lineage. Mycologia,2009:101(4):512-530.
7. Dakhel WH, Jaronski ST, Schell S. Control of pest grasshoppers in North America. Insects,2020:11(9):566.
8. Dickinson WJ, Sullivan DT. Gene-Enzyme Systems in *Drosophila*. Springer, Berlin, 1975, 130-136.
9. Duraimurugan P, Bharathi E, Dharavath NR, Selvam H. Pathogenicity of native strains of *Bacillus thuringiensis*, *Beauveria bassiana* and *Metarhizium rileyi* as entomopathogens against the polyphagous borer, *Conogethes punctiferalis* (Guenée) (Crambidae: Lepidoptera). Egyptian Journal of Biological Pest Control,2024:34(1):50.
10. El-Dydamony MK. Studies on the impact combined role of growth inhibitor and fungi on the desert locust. M.Sc. Thesis. Faculty of Science (Boys), Al-Azhar University, 2011.
11. El-Gendy IR, Zawrah MFM, El-Banobi MI. Virulence effect of *Metarhizium anisopliae* (Met.) and *Beauveria bassiana* (Bals.) fungi against the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). Egyptian Journal of Biological Pest Control,2022:32:43.
12. Fathy Z, Muhammad J, Ali SS. The pathology of native entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae), on biological processes and ovarian structure of the African migratory locust, *Locusta migratoria migratorioides* (Orthoptera: Acrididae). Egyptian Journal of Biological Pest Control,2025:35(1):6.
13. Gatehouse AMR, Norton E, Davison GM, Babble SM, Newell CA, Gatehouse JA. Digestive proteolytic activity in larvae of tomato moth, *Lacanobia oleracea*; effects of plant protease inhibitors *in vitro* and *in vivo*. Journal of Insect Physiology,1999:45:545-558.
14. Inglis GD, Goettel MS, Butt TM, Strasser H. Use of hyphomycetous fungi for managing insect pests. In:

Fungi as Biocontrol Agents. CABI Publishing, 2001, 23-69.
15. Ishaaya I, Swiryski E. Trehalase invertase and amylase activities in the black scale *Seisstiae oleae*, and their relation to host adaptability. Journal of Insect Physiology,1976:16:1025-1029.
16. Jiang W, Peng Y, Ye J, Wen Y, Liu G, Xie J, *et al*. Effects of the entomopathogenic fungus *Metarhizium anisopliae* on the mortality and immune response of *Locusta migratoria*. Insects,2019:11(1):36.
17. Kameel E, Said SM, Hekal SHA, Mikhail WZ, Sobhy HME. Efficacy of the entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* in controlling *Heteracris littoralis* (Rambur, 1838) using plant oil carrier substance combined with essential oils. Journal of Harbin Engineering University,2025:46(5):259-268.
18. Kaplan LA, Pesce AJ. Clinical Chemistry-Theory Analysis and Correlation. Mosby-Year Book, MO, 1996, 609-610.
19. Kassa A, Stephan D, Vidal S, Zimmermann G. Laboratory and field evaluation of different formulations of *Metarhizium anisopliae* var. *acridum* submerged spores and aerial conidia for the control of locusts and grasshoppers. BioControl,2004:49:63-81.
20. Li Z, Li M, He J, Zhao X, Chaimanee V, Huang WF, *et al*. Differential physiological effects of neonicotinoid insecticides on honey bees: A comparison between *Apis mellifera* and *Apis cerana*. Pesticide Biochemistry and Physiology,2017:140:1-8.
21. Litwin A, Nowak M, Różalska S. Entomopathogenic fungi: unconventional applications. Reviews in Environmental Science and Bio/Technology,2020:19(1):23-42.
22. Lomer CJ, Prior C, Kooyman C. Development of *Metarhizium* spp. for the control of grasshoppers and locusts. Memoirs of the Entomological Society of Canada,1997:171:265-286.
23. Maha SN. Response of green stinkbug *Nezara viridula* (Linnaeus), to the activity of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*. Journal of Plant Protection and Pathology, Mansoura University,2015:6(12):1633-1644.
24. Magalhaes BP, Leco QM, Faria MR, Schmidt FGV, Guerra WD. Field trial with the entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* against bands of the grasshopper *Rhammatocerus schistocercoides* in Brazil. Biocontrol Science and Technology,2000:10:427-441.
25. Matthews G, Bateman R, Miller P. Pesticide application methods. 4th ed. Wiley-Blackwell, 2014, 536.
26. Mesquita E, Hu S, Lima TB, Golo PS, Bidochka MJ. Utilization of *Metarhizium* as an insect biocontrol agent and a plant bioinoculant with special reference to Brazil. Frontiers in Fungal Biology,2023:4.
27. Mirhaghparast SK, Zibaee A, Hajizadeh J. Effects of *Beauveria bassiana* and *Metarhizium anisopliae* on cellular immunity and intermediary metabolism of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae). Invertebrate Survival Journal,2013:10(1):110-119.
28. Mohamed ES. The effect of entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* on flight muscle in the desert locust *Schistocerca gregaria* (Forskal). M.Sc. Thesis. Cairo University, 2009.
29. Mojarab-Mahboubkar M, Sendi JJ, Mahmoodi N. The sweet wormwood essential oil and its two major constituents are promising for a safe control measure against fall webworm. Pesticide Biochemistry and Physiology,2022:184:105124.
30. Muhammad J. Biochemical and Molecular Profile Alterations in *Locusta migratoria migratorioides* (Orthoptera: Acrididae) Induced by the Entomopathogenic Fungi. Journal of Natural Pesticide Research,2025:14:100154.
31. Muniz ER, Paixão FR, Barreto LP, Luz C, Arruda W, Angelo IC, *et al*. Efficacy of *Metarhizium anisopliae* conidia in oil-in-water emulsion against the tick *Rhipicephalus microplus* under heat and dry conditions. Biocontrol,2020:65(3):339-351.
32. Nathan SS, Chung KN, Murugan K. Effect of botanical insecticides and bacterial toxins on the gut enzyme of the rice leaf folder *Cnaphalocrocis medinalis*. Pesticide Biochemistry and Physiology,2005:82(3):193-208.
33. Peng G, Jin K, Liu Y, Xia Y. Enhancing the utilization of host trehalose by fungal trehalase improves the virulence of fungal insecticide. Applied Microbiology and Biotechnology,2015:99(20):8611-8618.
34. Püntener W. Manual for field trials in plant protection. 2nd ed. Agricultural Division, Ciba-Geigy Limited, 1981.
35. Reynolds SE, Samuels RI. Physiology and biochemistry of insect moulting fluid. Advances in Insect Physiology,1996:26:157-232.
36. Rashwan MH. Biochemical impacts of Rynaxypyr (Coragen) and Spinetoram (Radiant) on *Spodoptera littoralis* (Boisd.). Nature and Science,2013:11(8).
37. Riffat S, Santosh K, Durdane Y. Application of entomopathogenic fungi for insect pests control. Journal of Entomology and Zoology Studies,2017:5(6):7-13.
38. Rondelli VM, de Oliveira JL, Fraceto LF. Carrier systems for entomopathogenic fungi: A review. Biocontrol Science and Technology,2020:30(9):898-915.
39. SAS. Statistical Analysis System Stat User's Guide, Release 6.03. SAS Institute Inc., Cary, NC, USA, 1995, 125-154.
40. Segura-Vega J, González-Herrera A, Molina-Bravo R, Solano-González S. Computational identification and characterization of chitinase 1 and chitinase 2 from neotropical isolates of *Beauveria bassiana*. Frontiers in Bioinformatics,2024:4:1434442.
41. Sree KS, Padmaja V. Oxidative stress induced by destruxin from *Metarhizium anisopliae* (Metch.) involves changes in glutathione and ascorbate metabolism and instigates ultrastructural changes in the salivary glands of *Spodoptera litura* (Fab.) larvae. Toxicon,2008:51:1140-1150.
42. Yarema C, McLean H, Caveney S. L-Glutamate retrieved with the moulting fluid is processed by a glutamine synthetase in the pupal midgut of *Calpodes ethlius*. Journal of Insect Physiology,2000:46:1497-1507.
43. Yasin M, Khan A, Qayyum MA, Yousuf HMB, Mehfooz A, Hunter D. Biological control of locusts and grasshoppers. Journal of Orthoptera Research,2024:33(2):289-304.