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Field trials comparative between traditional and biorational insecticides for control grasshopper *Heteracris littoralis* (*Orthoptera*: *Acrididae*)

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Abstract

This study was conducted in Atmida region of Dakahlia Governorate, Egypt, during the summer of season 2023, for assessing and comparing the effectiveness of a conventional pyrethroid insecticide (Deltamethrin) with 3 biorational alternatives (Emamectin benzoate, Teflubenzuron, *Metarhizium anisoplia var. acridum)* against grasshopper nymphs of *Heteracris littoralis* (Rambur, 1838) (*Orthoptera*: *Acrididae*). Field results showed that the biorational insecticide emamectin benzoate was the most effective, with 85% and 99% mortality at 1 and 10 day post treatment respectively, compared to the traditional insecticide deltamethrin, which provided a rapid mortality (90% after 1 day) but slightly lower long-term effectiveness (95% after 10 days). Other biorational agents acted more slowly; teflubenzuron showed delayed activity, with toxicity increasing from 5% at 24 hours to 92% at 10 days, whereas *M. anisopliae* resulted in an 85% mortality rate after 10 days. All treatments significantly reduced total protein and lipid content; however, teflubenzuron caused the most severe reduction in total proteins (from 45.2 mg/g in control to 25.4 mg/g), whereas *M. anisopliae* and deltamethrin caused the greatest reductions in total lipids (14.1 mg/g and 15.2 mg/g, respectively, compared to 28.4 mg/g in control). Emamectin benzoate induced significantly increased on glutamic oxaloacetic (GOT) and glutamc pyruvic (GPT) transaminases activity (48.6, 65.4 U/L), followed by deltamethrin (35.2, 42.1 U/L) and *M. anisopliae* (28.9, 34.8 U/L) compared to control (12.5, 18.2 U/L) for GPT and GOT activity, respectively, while teflubenzuron showed the least biochemical disruption, with GPT and GOT levels. Thus, adding biorational agents into an integrated pest management (IPM) program could effectively control grasshoppers while reducing the harmful impacts of conventional pesticides.

Keywords: Grasshoppers, Deltamethrin, Emamectin benzoate, Teflubenzuron, *Metarhizium Anisoplia*

Introduction

Grasshoppers (*Orthoptera*: *Acrididae*) are among the most destructive herbivorous insects worldwide, posing a significant threat to agricultural production. Severe infestations can lead to complete defoliation and substantial economic losses for farmers (Zhang *et al*., 2019) [49]. For decades, the primary strategy for managing grasshopper populations has been the application of broad-spectrum synthetic insecticides, such as pyrethroids. Pyrethroids are classified into two groups I and II based on their toxicity and physical properties. Pyrethroid pesticides are neurotoxins aimed at the voltage-gated sodium channel receptor site (Valmorbida *et al*., 2022) [45]. They destroy insects by bonding to sodium channels, causing excitatory paralysis (Shafer *et al*., 2004[39]). Furthermore, they alter the membrane potential, resulting in an unusually stable condition of hyperexcitability in nerve cells. As a result of these changes, insects experience knockdown which is a sub-lethal incapacitating impact (Davies *et al*., 2007) [11]. Type II pyrethroids like deltamethrin also decrease the flux of chloride through the chloride channels. Additionally, relatively high concentrations can affect the receptors of γ-aminobutyric acid and cause cataleptic attacks (Shafer *et al*., 2008) [38], While effective in the short term, the reliance on conventional insecticides have several drawbacks. These include risks to human health, environmental contamination, detrimental effects on non-target organisms (including natural enemies and pollinators), and the increasing frequency of insecticide resistance (Bass and Jones, 2018) [7]. This has created an urgent need for sustainable pest management strategies that are both effective and environmentally benign, aligning with modern IPM principles (Dara, 2019) [10].

Biorational insecticides, also known as reduced-risk or "environmentally friendly" pesticides, include several classes of compounds that target pest-specific biochemical sites while minimizing harm to non-target organisms and the environment (Matyjaszczyk, (2018) [28]. These include: Avermectins (e.g., Emamectin benzoate) are nerve poisons derived from *Streptomyces avermitilis* fermentation products. They stimulate the gama-aminobutyric acid (GABA) system, a chemical transmitter produced at nerve endings (Fritz *et al*., 1979) [16], blocking the post-synaptic potential of neuromuscular cells, junction leading to paralysis and eventually to death, their efficacy at low application rates, and their favorable environmental profile with rapid degradation and limited bioaccumulation (Rosell, *et al*., 2008) [33]. Avermectin compounds are safe for mammals, including humans, due to their restricted capacity to penetrate the blood-brain barrier, the absence of glutamate-gated chloride channels in mammals, and avermectins' reduced affinity for other ligand-gated chloride channels in mammals (Rohrer and Arena, 1995) [32], recent regulatory frameworks in the Environmental Protection Agency (EPA) classify emamectin as a reduced-risk insecticide for many agricultural uses. Also insect growth regulators (IGRs) as chitin synthesis inhibitours (e.g., Teflubenzuron) disrupt normal growth and development by interfering with chitin synthesis during molting, leading to death with minimal impact on non-target organisms (Ganguly *et al*., 2020) [17]. Additionally, entomopathogenic fungi, such as *M. anisopliae*, are biological control agents that infect insects through cuticle penetration and proliferation within the hemocoel, providing highly selective control (Litwin *et al*., 2020) [27]. According to EPA standards, *M. anisopliae* has no effect on humans or other mammals in the field (Ahirwar and Singh, 2023) [5]. Incorporating biorational pesticides into IPM systems reduces reliance on hazardous substances and offers long-term advantages for managing resistance and safeguarding the environment (Yadav *et al*., 2025) [46].

This study was designed to compare between the efficacy of one conventional insecticide (Deltamethrin) and three biorational alternatives (Emamectin benzoate, Teflubenzuron, and *M. anisopliae var. acridum*) against grasshoppers.

Materials and Methods

Study Site and Insect Population

The field experiment was conducted in the Atmeda village, Dakahlia Governorate, Egypt, during summer of 2023 growing season where a cultivated area with *Zea mays*, and heavily infested with many species of grasshoppers *Eyprepocnemis plorans* (Charpentier), *Aiolopus strepens* (Latreille) and *Acrotylus insubricus* (Scopoli), ranged between (20-25 insects/m2) but *Heteracris littoralis* (Rambur), was the most prevalent.

Tested compounds

In this investigation, four water-based pesticides were categorized into conventional insecticide and biorational alternatives were used as following active ingredients, trade names, insecticide groups and rates of application

1. Deltamethrin (Kafrothrin 2.5% EC) a conventional pyrethroid insecticide applied at 250 cm³/feddan.
2. Emamectin Benzoate (Excellent 1.9% EC) a microbial biorational insecticide applied at 250 cm³/feddan.
3. Teflubenzuron (Kafrosil 5% EC) an insect growth regulator biorational insecticide applied at 160 cm³/feddan.
4. *M. anisopliae* var. acridum isolate IMI330189 (Green Muscle®) a microbial biorational insecticide applied at 250 g/feddan.

Experimental design and mortality assessment

The selected area was split into 5 plots including control plot, each plot was about 1050 m² (35×30m), the plots were aligned in parallel direction to the prevailing wind and isolated by wide area (10×25m) to inhibit pollution of spray drift from one plot to the other during spraying. Control plot was put upwind in relation to the others and sprayed with water only. Spraying was executed in the early morning where suitable conditions for spraying (wind speed: 4 m/sec., temperature: 30±2°C., and relative humidity: 62%. Each plot was of a standardized size. Pre-treatment counts of the last grasshopper *H. littoralis* nymphs were taken. All treatments were applied at their recommended field doses using a Motorized knapsack (Solo) with flow scale graded from 1 to 5; the scale no 3 was selected. Post-treatment to define the number of grasshopper nymphs, each treatment was represented by 5 replicate cages (0.5m× 0.5m), the insects were collected randomly from the same treatment after application directly by using sweep net and placed 50 insects in each cage, the cages were kept and fed with treated plants to the insects treated, by routine daily work includes removing the previous uneaten food, feaces and dead insects and counting the living insects before introducing the fresh food. Mortality was assessed by counting live and dead last nymphal instar of grasshopper *H. littoralis* at 1, 3, 7, and 10 days after treatment and corrected mortality was calculated by Schneider-Orelli's formula (Püntener, 1981).

Hemolymph Collection and Biochemical Analysis

For biochemical analysis, live last nymphal instars of *H. littoralis* grasshopper were collected after the third day of each treatment group. Hemolymph samples were extracted by cutting a proleg and drawing the flowing hemolymph into a micropipette containing a crystal of phenylthiourea to prevent melanization. Samples were pooled from multiple insects per replicate to obtain sufficient volume, the hemolymph was centrifuged at 2000 r.p.m. for 5 min, and only the supernatant fractions were used for assay directly for the following assays**:**

- Total protein (mg/g) Determined using bovine serum albumin as a standard, following the Bradford method (Bradford, 1976) [8].
- Total lipids (mg/g) Determined with phosphovanillin reagent and a standard curve, as outlined by Knight *et al*. (1972) [25].
- The activity of GPT (glutamic pyruvic transaminase) and GOT (glutamic oxaloacetic transaminase) enzymes: was measured according to the method of (Harold, 1975) [18] using a kit of Bioadwic. The enzyme activity was measured calorimetrically at 546 nm by spectrophotometer.

Statistical analysis

All statistical analyses were carried out using SAS (1998) software.

Results and Discussion

Mortality Rates

Table (1) shows the efficacy of used insecticides: deltamethrin, emamectin benzoate, teflubenzuron, and *M. anisoplia*, on the percentage accumulative mortality of *H. littoralis* in the field, at 1, 3, 7, 10 days post-treatment. It was evident that the duration of time following therapy was positively correlated with mortality.

At 1-day Post-Treatment a clear differentiation in insecticidal activity was already evident. Deltamethin and emamectin benzoate demonstrated superior and comparable efficacy, with average mortality rates of 90% and 85%, respectively, while teflubenzuron produced negligible mortality which was 5% and *M. anisopliae* not recorded any mortality

At 3 days Post-Treatment, emamectin benzoate was the highest efficacy, inducing accumulative mortality of 95%. Followed by deltamethrin with 92%. However, the mortality rate remains very low with teflubenzuron and *M. anisopliae.*

At 7 days Post-Treatment, emamectin benzoate still has the highest impact followed by deltamethrin, where the cumulative mortality reached 98% and 94% respectively. Teflubenzuron provided intermediate efficacy with 75% mortality, while *M. anisopliae* remained the least effective treatment, achieving 60% mortality.

At 10 days Post-Treatment, All the pesticides used achieved high efficiency in producing accumulative mortality reached to 99%, 95%, 92%, 85% with emamectin benzoate, deltamethrin, teflubenzuron, and *M. anisoplia*, respectively.

Table 1: The accumulative mortality percentages of the 5th nymphal instar of *H. littoralis* treated by certain insecticides under field condition.

Pesticidal used	**% Mortality after**			
	1 day	**3 days**	**7 days**	**10 days**
Deltamethrin	90	92	94	95
Emamectin benzoate	85	95	98	99
Teflubenzuron	5	20	75	92
M. anisopliae	0	15	60	85

In line with previous research on sustainable pest management, the field study's findings unequivocally showed the different mechanisms of action and durations of efficacy between traditional synthetic pesticides and biorational alternatives for grasshopper control. There was a noticeable difference between the "slow-acting" biorational agents and the "fast-acting" conventional pesticides when *H. littoralis* nymphs were treated with deltamethrin and biorational substitutes. Since agricultural damage must be stopped right once during acute outbreaks, the pyrethroid neurotoxin deltamethrin provides the quick suppression required. (Johnson *et al*., 1986) [22]. Deltamethrin's 90% 24-hour mortality rate is in line with the quick knockdown characteristic of pyrethroids, which exhibit exceptional effectiveness against acridid pests in similar agroecological situations (Davies *et al*., 2007) [11]. However, its great initial efficiency (90% in 24 hours) is known to cause environmental problems, including toxicity to aquatic animals and beneficial arthropods (Sparks and Nauen, 2015) [42]. Emamectin benzoate, on the other hand, demonstrated 85% and 99% mortality by day 1 and 10, respectively, making it the most effective of the biorational insecticides and a potent competitor to deltamethrin. This high potency is supported by recent studies showing its effectiveness against a range of *Orthoptera*n and lepidopteran pests (Ishaaya *et al*., 2002) [20], due to its unique mechanism of action on glutamate-gated chloride channels (specific to invertebrates). The other biorational alternatives, teflubenzuron and *M. anisopliae*, demonstrated delayed but ultimately high control levels. Teflubenzuron's gradual action is typical of benzoylurea IGRs, which do not immediately cause death but instead prevent the growth of new exoskeletons during moulting by inhibiting chitin synthesis (Doucet and Retnakaran 2012) [12]. Its high efficacy (92%) by day 10 indicates that it effectively targets grasshopper nymphal stages. The gradual demise of *M. anisopliae* also reflects the time required for the fungal infection stages of conidial adhesion, cuticle penetration, hemocoel colonization, and mycosis (Litwin *et al*., 2020) [27].

The results of this study are consistent with several earlier studies, such as Said *et al*. (2026) [3], who found that after 24 and 48 hours of deltamethrin treatment, *Acrotylus insubricus* nymphs infesting maize in the Bahariya Oasis, Egypt, had high mortality rates of 98.3% and 99.7%. In addition to 96.2%, the general mean mortality percentage for *H. annulosa* fourth and fifth nymphs under field conditions was 88% following two days of treatment with deltamethrin and emamectin benzoate, respectively (Said and Dar, 2023) [35]. After seven days of treatment, emamectin benzoate caused an 85.31% decrease in grasshopper populations, according to Abd El-Wahed *et al*. (2026) [3].

Additionally, the results are in line with Krokene's (1993) [26] findings that grasshoppers were significantly impacted by the chitin synthesis inhibitor teflubenzuron at doses below suggested levels. This study supports the effectiveness of *M. anisopliae* as a biocontrol agent for *H. littoralis* and this agree with Kameel *et al*. (2025) [15] who stated that *M. anisopliae* effectively controlled *H. littoralis.* While Fathy *et al*. (2025) [15] reported the similar effectiveness on *locusta migratoria* nymphs. Further, *Metarhizium spp*. have been shown to be effective against many *Orthoptera*n pests, as evidenced by the higher mortality rates seen in treated plots (Yasin *et al*., 2024) [47]. In Africa, Asia, and Australia, the entomopathogenic fungus *M. acridum* has been effectively employed as a fungal insecticide to manage locusts and other acridids (Hunter *et al*. 2001) [19].

Biochemical Impact

The biochemical parameters measured in *H. littoralis* nymph's hemolymph after 3 days of treatment are shown in Table (2 & 3). All insecticide treatments led to significant ($P < 0.05$) alterations in the biochemical profile of the grasshopper nymphs compared to the control group with varying degrees of physiological disruption.

Total Proteins and Lipids

According to the data assorted in Table (2) a significant decrease in total proteins and lipids content was observed in all treatments.

In total proteins content, teflubenzuron caused the most drastic reduction in protein level (from 45.2 mg/g in control to 25.4 mg/g), followed by *M. anisopli*a, emamectin benzoate, and deltamethrin, have protein content reduction reached to 30.8, 32.1, 38.5 mg/g respectively, compared to 45.2 mg/g in control.

In total lipids content, *M. anisopliae* and deltamethrin induced the greatest reduction in total lipids (14.1 mg/g and 15.2 mg/g respectively, compared to 28.4 mg/g in control), while emamectin benzoate and teflubenzuron caused a similar significant reduction in lipids content was 22.3 and 20.8 mg/g respectively.

Table 2: Total protein and lipid contents of the 5th nymphal instar of *H. littoralis* after 3 days of treatment with deltamethrin, emamectin benzoate, and teflubenzuron and *M. anisopliae* under field condition

Insecticides	**Tota Proteins mg/g Mean ± SE**	**Total lipids mg/g Mean ± SE**
Control	45.2 ± 1.5^{a}	28.4 ± 0.8^{a}
Deltamethrin	38.5 ± 1.8^{b}	15.2 ± 1.1^{c}
Emamectin benzoate	32.1 ± 2.1^{c}	22.3 ± 1.4^{b}
Teflubenzuron	25.4 ± 1.2^{d}	20.8 ± 0.9^{b}
M. anisopliae	30.8 ± 2.5^{e}	14.1 ± 1.3^{c}

Values followed by the same small letter are not significantly different at ($P < 0.05$).

Results are presented as mean ± standard error.

Proteins and lipids are crucial energy sources in a variety of insect physiological processes. The current study found lower levels of proteins and lipids in all insecticidal treatments. This could be because of cell death or necrosis, insufficient amino acid incorporation into polypeptide chains, impaired protein synthesis machinery by its tool (amino acids) of the detoxification mechanism, where the protein aids in the synthesis of microsomal detoxifying enzymes, helping to detoxify the toxicants that invaded the insect body, or an imbalance between the rate of protein synthesis and the rate of biodegradation (Ismail, 2020) [21].

Teflubenzuron's high protein depletion was unexpected and may imply that it selectively inhibits the protein synthesis pathways involved in the formation of chitin. This corresponds to Teflubenzuron exposure has been found in female migrating locusts to diminish hemolymph proteins, impairing development, oviposition, and molting (Acheuk *et al*., 2011) [4].

The notable decreases in impact on total lipids with deltamethrin and *M. anisopliae* indicate that lipid reserves are quickly mobilized and used for energy to fight toxic stress, which is in line with earlier studies on pyrethroid induced cellular metabolic disorders (Soderlund, 2012) [40] and fungal infection mechanisms (Litwin *et al*., 2020) [27], or to make up for the incapacity to feed normally during the onset of intoxication (Tony *et al*., 2023) [44]. Because emamectin benzoate and teflubenzuron have more focused modes of action, their minimal lipid drop may indicate a different metabolic disruption pattern. Our study agreed with others on other insects such as the Assar *et al*. (2016) [6], which stated that teflubenzuron dramatically reduced total proteins and lipids in *Spodoptera littoralis* larvae in their fourth instar. As well as Rashad *et al*. (2015) [31] who reported that a significant reduction in protein and lipid levels of *Pectinophora gossypiella* (Saunders) treated with teflubenzuron.

Transaminase enzymes (GPT and GOT) activity

A predominant enhancing effect was exhibited by all tested compounds on the GOT and GPT activity in hemolymph of the last nymphal instar of *H. littoralis* except teflubenzuron, which recorded (15.4, 22.6 U/L), was not significantly different from the control (12.5, 18.2 U/L) for GPT and GOT enzyms activity respectively (Table. 3), while the emamectin benzoate treatment resulted in the highest significantly increased GPT and GOT activity were (48.6, 65.4 U/L) respectively, followed by deltamethrin (35.2, 42.1 U/L), while *M. anisopliae* showed intermediate elevations (28.9, 34.8 U/L) compared to control (12.5, 18.2 U/L) for GPT and GOT activity respectively.

Table 3: The effect of deltamethrin, emamectin benzoate, teflubenzuron and *M. anisopliae* on activity of GPT and GOT enzyms (U/L) of the 5th nymphal instar of *H. littoralis* after 3 days of experimental field

Insecticides	GPT (U/L) Mean ± SE	GOT (U/L) Mean ± SE
Control	12.5±1.2^{d}	18.2±0.9^{d}
Deltamethrin	35.2±2.4^{b}	42.1±3.1^{b}
Emamectin benzoate	48.6±3.5^{a}	65.4±4.2^{a}
Teflubenzuron	15.4±1.0 ^{cd}	22.6±1.5^{d}
M. anisopliae	28.9±2.1^{c}	34.8±2.8c

Values followed by the same small letter are not significantly different at ($P < 0.05$).

Results are presented as mean ± standard error

Two vital, crucial enzymes in biological processes are (GOT) and (GPT). They are involved in biosynthesis and amino acid catabolism. Because transaminases are involved in detoxification and metabolism, their disruption from the usual value brought on by the toxic stress of pesticides indicates a biochemical imbalance in the tissues and cellular function (Enan and Berberian, 1986) [13]. Furthermore, the metabolism of carbohydrates is catalyzed by transaminases, particularly GPT (Katumuma *et al*., 1968) [24]. In general, it is considered that transaminase activity in the insect body is controlled by secondary feedback or homeostatic systems that respond to spontaneous or hormonally caused changes (Nohel and Salama, 1972) [29].

H. littoralis nymphs exhibited elevated transaminase enzyme activity (GOT and GPT) in response to cytotoxic and physiological stress caused by traditional and biorational substances tested. And this an indicator of cellular damage and metabolic dysfunction in insects, and they are expected to rise in response to toxin-induced tissue damage and oxidative stress by elongated time.

The considerable increase in both enzymes with emamectin benzoate indicates that it is a strong bioactive substance that causes significant physiological stress in target insects notwithstanding its biorational classification followed by deltamethrin where excessive exposure to pyrethroids can lead to alterations in biochemical and physiological processes, as well as clinical effects. Features (Sakr, 2003) [36].

The results corroborate with other insects as those of Soliman (2025) [15], who found that emamectin benzoate treatment significantly increased GOT and GPT activity in *Spodoptera frugiperda*. while, emamectin benzoate caused a significant increase only on *S. littoralis* GPT (Abd-EL-Aziz, 2014) [1].

Also, transaminase activity increased in *Tribolium castaneum* of Nazimabad collection after deltamethrin treatment (Tabassum *et al*., 2014) [43]. In *Culex pipiens* larvae, deltamethrin caused stimulatory effects on GOT activity (Youssef, 2017) [48].

Teflubenzuron's classification as a safer biorational option with greater selectivity is supported by the small, non-significant increase in GOT and GPT and this in agreement with Rashad *et al*. (2015) [31] for *Pectinophora gossypiella* (Saunders), treated with teflubenzuron. *M. anisopliae* caused mild increases in enzyme levels, which are indicative of the physiological strain brought on by fungal growth in the hemocoel. The obtained results are in harmony with that recorded by Abdel-Salam, (2001) [2]) stated that GOT and GPT activities in *Pterochloroides persicae* larvae were significantly increased after treatment with *M. anisopliae*, also similar results were obtained by Butt *et al*. (1995) [9], who used the *M. anisopliae* to control *A. gossypii*.

In the current study, the disruption of transaminase activity by studied compounds could be attributed to the fact that the relationships between protein synthesis and transaminase levels were influenced by hormonal control of protein synthesis and neurosecretory hormones involved in the regulation of transaminase levels, thereby disrupting various physiological activities and eventually leading to death (Ezz and Fahmy, 2009) [14].

Conclusion

This comparison study demonstrates that the three biorational alternatives tested, emamectin benzoate, teflubenzuron, and *M. anisopliae var. acridum*, are extremely effective tools for managing grasshopper *H. littoralis* in modern IPM systems. Emamectin benzoate combines great efficacy (99% death) with the environmentally friendly properties of biorational insecticides, such as low application rates, a unique mechanism of action that targets invertebrate-specific receptors, and rapid environmental degradation. This contrasts with the standard pesticide deltamethrin, which provides quick control but has well-documented environmental concerns. Furthermore, teflubenzuron and *M. anisopliae var. acridum*, particularly teflubenzuron, demonstrated good long-term efficacy with minimal physiological disruption, enabling environmentally acceptable and sustainable approaches in accordance with current IPM criteria.

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