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Original Research Paper

Evaluation of Neem-Based Insecticides Against Resistant Strains of *Helicoverpa armigera*

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Key Words

Helicoverpa armigera, Neem insecticides, Resistance management, Bioefficacy, Integrated pest management.

Abstract

The increasing resistance of *Helicoverpa armigera* (Hubner) to synthetic pyrethroids necessitates the exploration of environmentally sustainable alternatives for effective pest management. This study evaluated the bioefficacy and ecological relevance of three neem-derived formulations azadirachtin 0.15% EC, neem oil 0.03% EC, and neem seed kernel extract (NSKE 5%) against cypermethrin-resistant and susceptible strains of *H. armigera* under laboratory and field conditions during 2022–2023. Leaf dip bioassays revealed a high resistance level to cypermethrin in the resistant strain (24.6-fold; LC_{50} = 12.84 ppm) compared to the susceptible strain (LC_{50} = 0.52 ppm). In contrast, neem-based formulations exhibited significantly lower resistance ratios, with azadirachtin (2.3-fold), neem oil (2.1-fold), and NSKE (2.5-fold), indicating minimal cross-resistance. Among the treatments, azadirachtin demonstrated the highest larvicidal activity, with LC_{50} values of 5.21 ppm and 11.89 ppm against susceptible and resistant strains, respectively. Field evaluations in cotton agroecosystems showed that azadirachtin 0.15% EC at 2.0 L ha^{-1} achieved superior pest suppression (68.3%) and reduced fruiting body damage (61.5%) compared to cypermethrin (42.7% and 38.9%). Additionally, oviposition deterrence assays indicated a significant reduction (74.6%) in egg laying on treated plants. The findings highlight the potential of neem-based insecticides as eco-friendly alternatives that not only effectively manage resistant pest populations but also reduce selection pressure and environmental risks associated with synthetic chemicals. Integration of such botanicals into resistance management programs can contribute to sustainable pest control and improved agroecosystem health.

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Introduction

Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) is one of the most devastating polyphageous pests that attack cotton, pigeonpea, chickpea, tomato and other economically important crops in Asia, Africa and the Mediterranean region (Kranthi et al., 2002). Annually, this pest costs the Indian population more than US 500 million in losses of crops despite the intensive use of insecticides (Sharma, 2020). Unregulated application of synthetic pyrethroids and organophosphates since the 80s has led to extreme cases in building resistance, and field populations have developed resistance ratios of over 100-fold to cypermethrin and other pyrethroids (Armes et al., 1996). Mechanisms that mediate this resistance are an increase in metabolic detoxification, insensitivity to the target site, and decrease in cuticular penetration (Ahmad et al., 1995).

The ineffectiveness of traditional insecticides has led to the need to urgently look into the possibilities of alternative ways of managing pests without involving the current resistance mechanisms. The Azadirachta indica A. Juss (Meliaceae) which is the source of botanical insecticides provide promising alternatives because of their unique modes of action, environmental safety, and low potential harm to non-target organisms (Isman, 2006). The active constituent of neem, Azadirachtin, acts as an ecdysteroid antagonist, feeding repellent, growth controller and oviposition suppressor (Mordue & Nisbet, 2000). Other previous researchers reported moderate levels of efficacy of neem formulations against susceptible strains of *H. armigera*, where the LC_{50} of azadirachtin varied between 3-8 ppm (Boeke et al., 2004). But there is a dearth of information in their respect to their performance with known resistant strains especially those that are pyrethroid resistant. Understanding pest population dynamics is essential for designing effective control strategies, as fluctuations in population density directly influence the success of pest management interventions (Ghulam & Farman, 2025).

Overuse of synthetic insecticides has led to an increase in target pest population resistance, as well as, dramatic ecological imbalances in agroecosystems (Panwar et al., 2025). The constant use of pesticides has a serious negative impact on the soil health by increasing microbial diversity and affecting soil enzyme activity and hence reduces nutrient cycling and the long-term soil fertility. Moreover, the non-target organisms, such as, pollinating insects, parasitoids, and the predatory arthropods are very sensitive to the chemicals and their populations decline and the normal biological control systems are interfered with. These disequilities tend to cause second-order pest infestations and contribute to the escalation of chemical addiction. In addition, the residues of pesticides may remain in the environment and pollute water bodies and food chains, which also is harmful to the ecology and health on a wider scale (Harinathan et al., 2025). The cumulative impacts are endangering both stability of the ecosystems and the survival of biodiversity, and environmentally friendly methods of controlling pests are urgently required. In that regard, botanical insecticides like neem-based preparations can be a good alternative because they are biodegradable, specific and they have low ecological footprint.

The current research was designed to address this knowledge gap that is of great importance by comparatively assessing three of the commercially available insecticides made using neem against a characterized cypermethin-resistant population of *H. armigera*. The aim was as follows: (i) to identify the toxicity of various neem formulations against the susceptible and resistant strains using laboratory bioassays, (ii) to identify oviposition deterring effects on the resistant moths, (iii), to determine field efficacy against the resistant populations in cotton agroecosystems, and (iv) to calculate the ratio of resistance and determine the potential of cross resistance. The study gives empirical data on the applicability of neem-based insecticides in the resistance management program to control *H. armigera* in a sustainable manner.

Environmental Relevance

The insecticides that are made using neem are a possible alternative to the traditional synthetic pesticides since they are highly biodegradable material, have low persistence, and also less toxic to non-target organisms (Wheeler & Isman, 2001). Compounds like azadirachtin break down easily in the environmental conditions and hence the pueblo of the compound in the soil and water systems gets minimal hence reduces the chances of the contamination of the ecology. In contrast to general purpose synthetic insecticides, neem combinations have selective toxicity, and in most cases, when used, herbivorous pests are targeted, but normal arthropods, such as pollinators, parasitoids, and predators, which are critical to maintaining ecological balance, are not affected. The result is natural biological control and minimizes the tendency to occur secondary pest outbreaks. Moreover, the combinatorial action of neem-based compounds is more complex and thus this also results in reduced chances of resistance development, and thus enhances sustainability of pest management programmes in the long term. As such, assessing the effectiveness of neem-based insecticides on the resistant pest populations is not only pertinent as far as the control is concerned but also is fundamental to the development of environmentally-conscienceable and ecosystem-friendly agricultural activities.

Materials and Methods

Insect Culture Maintenance

Experiments were conducted during the cropping seasons of 2022 and 2023 at the Entomology Research Farm, Indian Agricultural Research Institute, New Delhi. A susceptible strain of *H. armigera* was obtained from the National Centre for Integrated Pest Management, New Delhi, and maintained in the laboratory without insecticide exposure for >30 generations (Kambrekar, 2016). A cypermethrin-resistant strain was established from field collections in cotton-growing regions of Haryana, India, where control failures with pyrethroids were reported. The resistant strain was selected with cypermethrin for 15 consecutive generations to achieve a stable resistance ratio >20-fold. Both strains were reared on artificial diet (Armes et al., 1996) at 27 ± 2°C, 65 ± 5% relative humidity, and 14:10 L:D photoperiod.

Insecticidal Formulations

Three neem-based insecticides were evaluated: (i) azadirachtin 0.15% EC (Azatin®), (ii) neem oil 0.03% EC (Neemark®), and (iii) neem seed kernel extract (NSKE 5% w/v) prepared following the method of Singh and Singh (2000). Cypermethrin 10% EC served as a synthetic check and untreated control received distilled water with 0.05% Tween-80.

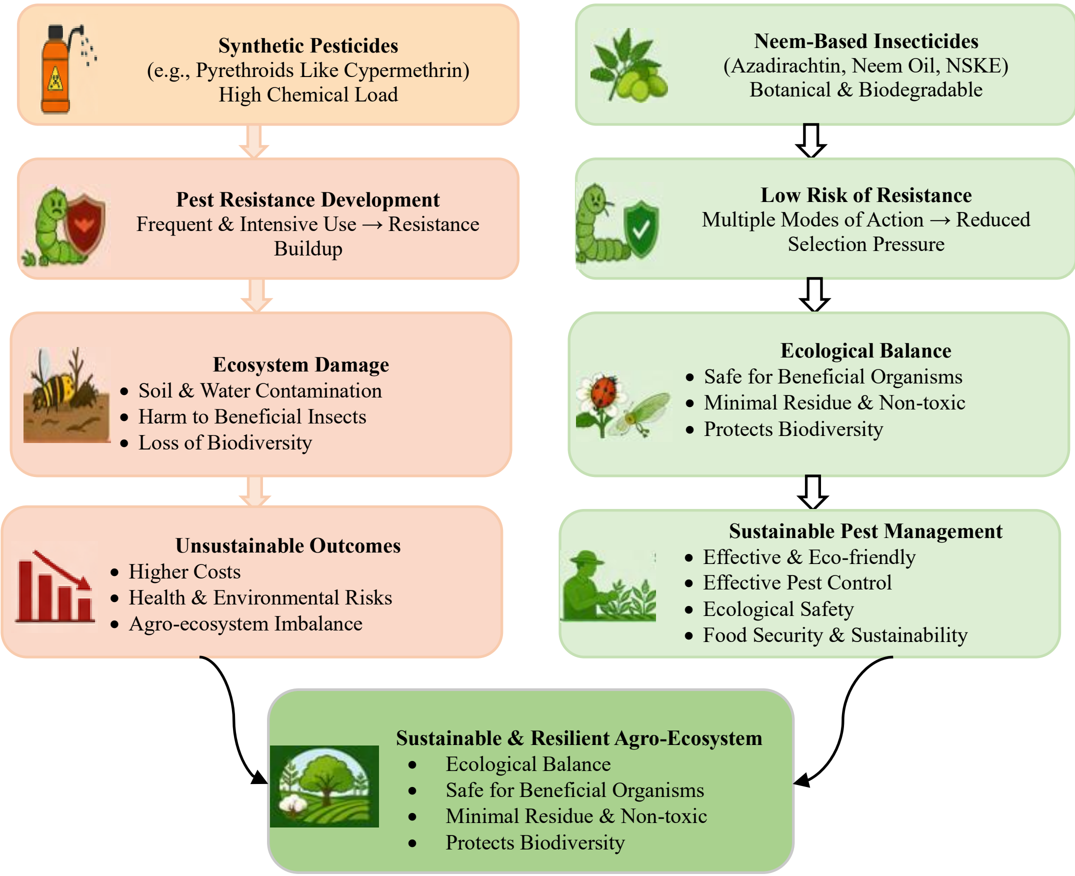

Figure 1: Agro-Ecological Framework for Neem-Based Pest Management

Figure 1 shows the agro-ecological model of neem-based pest management, whereby the agro-ecosystem would shift away and stop the use of conventional synthetic pesticides that create resistance and degrade the environment, in favor of neem-based interventions that facilitate sustainable pest control. The model shows the role of synthetic pesticides in escalating resistance, harming the ecosystem, and neem-based formulations in reducing the selection pressure, neem-based pesticides would guarantee the safety of the non-target organisms and maintain ecological stability. The model, as a whole, focuses on better agroecosystem stability, maintenance of beneficial organisms and long run sustainability of cotton pest management systems.

Laboratory Bioassays

Leaf dip method: Fresh cotton leaves (variety Bt F-1861) were dipped in aqueous dilutions of test insecticides for 20 seconds, air-dried, and placed in Petri dishes (9 cm diameter) lined with moist filter paper. Second instar larvae (F1 generation, 10 replicates of

10 larvae each) were introduced onto treated leaves. Mortality was recorded at 24, 48, and 72 hours post-treatment. Larvae failing to respond to gentle prodding were considered dead.

Diet incorporation method: Test solutions were mixed with warm artificial diet at concentrations of 1.0, 2.5, 5.0, 10.0, and 20.0 ppm for neem formulations and 0.1, 0.5, 1.0, 5.0, and 10.0 ppm for cypermethrin. Two hundred microliters of diet were dispensed into each well of 24-well plates. A single neonate larva was released per well (three replicates of 24 larvae each). Observations on mortality, larval weight, pupation, and adult emergence were recorded.

Oviposition deterrence assay: Treated and untreated cotton twigs were placed in oviposition cages (30×30×30 cm) containing 10 pairs of freshly emerged resistant moths. Eggs laid on each twig were counted after 48 hours. Percentage deterrence was calculated using the formula:

$$Detterrence(\%) = \left[\frac{(C - T)}{C}\right] \times 100$$

Where C = eggs on control and T = eggs on treatment.

Field Experiments

Field trials were conducted in cotton (variety Bt F-1861) during kharif 2022 and 2023 in a randomized block design with five replications. Plot size was 5×4 m with row spacing of 67.5 cm. Treatments comprised azadirachtin 0.15% EC at 1.0 and 2.0 L ha^{-1}, neem oil 0.03% EC at 2.5 L ha^{-1}, NSKE 5% at 5.0 L ha^{-1}, cypermethrin 10% EC at 500 ml ha^{-1}, and untreated control. Applications were initiated at flower bud formation using a knapsack sprayer (500 L ha^{-1} spray volume) and repeated at 15-day intervals. Observations recorded included larval population (per 5 plants), fruiting body damage (%), and seed cotton yield (kg ha^{-1}).

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using SPSS version 26.0. Probit analysis (Finney, 1971) determined lethal concentration values (LC50) with 95% confidence limits. Resistance ratios were calculated as the LC_{50} of resistant strain divided by the LC_{50} of susceptible strain. Treatment means were compared using Tukey's HSD test at $p<0.05$.

Results and Discussion

The bioassays of different strains in the laboratory showed a high level of difference in susceptibility (Table 1). On the susceptible strain, cypermethrin showed the greatest toxicity with LC_{50} value of 0.52 ppm (95% FL: 0.44-0.61 ppm), then azadirachtin, neem oil and NSKE. Conversely, the resistant one was 24.6-fold resistant to cypermethrin (LC_{50} = 12.84 ppm), which is a confirmation of the high degree of pyrethroid resistance. Nonetheless, the neem preparations showed significantly reduced ratio of resistance: azadirachtin (2.3-fold), neem oil (2.1-fold), and NSKE (2.5-fold), which means that there is little cross-resistance.

Azo-adadirachtin against the resistant strain (11.89 ppm) had a LC_{50} value that was within the effective range indicating that it can be used in the field. This is consistent with that of Koul et al., (2004) who expressed ratios of resistance against pyrethroid-resistant Plutella xylostella to azadirachtin as less than 3.0. The low cross-resistance identified is probably because of the multiple modes of action of neem, which is not similar to the sodium channel modulation of pyrethroids. The main antagonist of Azadirachtin is ecdysteroid receptors and molting hormone regulation which pyrethroid resistance mechanisms do not counteract (Mordue & Nisbet, 2000).

The effect of exposure time on the mortality was progressive across all neem formulations (Figure 1). At 10 ppm, Azadirachtin led to 72.4 mortality in resistant larvae after 72 hours as opposed to 28.6 mortality in resistant larvae after 24 hours indicating slow but prolonged activity of insect growth regulators. This time lag indicates the time needed by azadirachtin to influence the ecdysteroid metabolism and molting processes. The same was replicated on neem oil and NSKE and the highest rates of mortality were found after 72 hours of the treatment. Cypermethrin demonstrated high knock down effect on the susceptible larvae (91.2% mortality at 24 hours) and low efficacy against resistant larvae (34.8% mortality at 72 hours at 10 ppm concentration).

Formulations of Neem showed good deterrents to resistant moths (Table 2). Equal 2.0 L ha-1 concentration of azadirachtin 0.15% EC led to 74.6% egg laying reduction, then neem oil (63.4%), and NSKE (58.9%). This anti-oviposition action is complementary to larvicidal effect, and this lowers successional generation pressure. The mechanism of deterrence is that of sensory disruption by acting of azadirachtin on the chemoreceptors such that treated surfaces become invisible or intolerable to oviposition (Schmutterer, 1990). This behavioral impact is especially useful in fighting resistant populations that survive lethal doses as it will avoid their reproduction and the accumulation of resistance.

Two years of field assessment gave pooled data that showed there was a large difference in the efficacy of the treatment (Table 3). About resistant populations, azadirachtin 2.0 L ha^{-1} was used with the lowest larval population (3.2 larvae per 5 plants) and fruiting body injury (12.4% damage) after 15 days of second spray, which was significantly better than cypermethrin (6.8 larvae, 28.7% damage). Neem oil and NSKE also gave moderate control compromising larval populations by 52.3 and 48.7% respectively relative to untreated control (11.4 larvae per 5 plants). The seed cotton yield was the greatest in azadirachtin 2.0 L ha^{-1} (1825 kg ha^{-1}), which is 41.6% and 23.4% higher as compared to control and cypermethrin application respectively.

The laboratory results are confirmed by the better field performance of azadirachtin over resistant strains and the practical use of the compound is proved. Synthetic pyrethroids did not offer economic control since the resistance levels were very high, but the neem formulations did not experience such changes (Reddy et al., 2025). This resistance to resistant population is due to their complicated chemistry which has several bioactive limonoids which have synergistic activity and their development is not an easy task due to their evolutionary complexity (Isman, 2006). Also, sublethal effects of neem such as decreased feeding, retarded growth, and morphological imperfections of the survival larvae are also a cause of the overall population suppression.

The ratios of the differential resistance measures of cypermethrin and neem formulations have far reached implications in terms of insecticide resistance management (IRM) (Das et al., 2024). Resistance ratio of cypermethrin (24.6-fold) is very high, hence indicates that cypermethrin possess severe control failures in fields whereas low ratios of neem products (less than 3.0-fold) point to neem products being effective tools. Neem-based insecticides may be silenced by synthetic chemicals, thus restoring their susceptibility. Strategic rotation using botanicals in turn has delayed the evolution of resistance in Australian populations of *H. armigera* (Kranthi et al., 2002).

Neem formulations need to be economically viable. Whereas the infected cost of azadirachtin 2.0 L ha-1 is estimated to be 15 % higher than that of cypermethrin, the yield of 23.4 % and less frequency of repetitive applications counterbalance this increase in costs. In addition, the fact that neem does not harm the insects such as *Cotesia plutellae* and *Chrysoperla carnea* maintains natural biological control which makes it cost-effective in the long run in terms of pest management (Schmutterer, 1990).

Ecological and Environmental Implications

Inclusion of neem-based insecticides in pest management programs is very effective in reducing the total chemical burden of agroecosystems and thus environmental pollution and corresponding ecological hazards. Because of their selective mode of action, the neem formulations do not harm the good organisms, such as predators and parasitoids, which are important in the natural control of the biology and stability of the ecosystem. This natural enemy preservation minimises chances of the pest resurgence and secondary pest outbreak. Also, the multi-targeted biochemical pathways of the neem compounds also lead to slower pest population developing resistance and thus increases the sustainability of pest control programs. Increasing the reliance on synthetic insecticides and neem-based interventions fosters a more ecologically balanced agroecosystem by enhancing agroecosystem resilience and health.

Conclusion

The current research paper has shown that insecticides obtained using neem, especially azadirachtin 0.15% EC, are effective in the laboratory and field environment in treating cypermethrin-resistant strains of *Helicoverpa armigera*. The population that was resistant displayed a strong resistance ratio of cypermethrin (24.6-fold), which proved that synthetic pyrethroids face severe restrictions in the existing pest management systems. Neem-based preparations, by comparison, had much lower resistance ratios (<3-fold) and there were little cross-resistance and a maintained efficacy against resistant populations. Azadirachtin was the most effective larvicidal treatment as well as a powerful oviposition repellent, which led to a decrease in pests' multiplication and growth. Field tests also confirmed these results and the use of azadirachtin resulted in significant reductions in the density of larvae and the damage of fruiting bodies as well as significant increase in seed cotton production compared to the use of conventional insecticides. In addition to direct pest control, the findings highlight the environmental benefits of the neem-based insecticides such as less chemical burden, less environmental retention, and greater safety to the non-target organisms. These features help in maintaining natural enemies and stability of the agro eco system. Neem-based products should be part of pest management initiatives as it presents a potential solution to having sustainable agriculture through decreased use of synthetic pesticides and resistance development reduction. Such results justify the use of botanical insecticides in the programs of Integrated Pest Management (IPM) to attain long-term controls on pests at the same time ensuring that there is environmental integrity (Nalini et al., 2025). Additional studies are required in future to be done on the multi-season field validation, interactions with useful arthropods, and large-scale applications to further support the role of neem-based solutions in the environmentally responsible crop protection systems.

Table 1: Toxicity of Insecticides Against Susceptible and Cypermethrin-Resistant Strains of *Helicoverpa armigera* Using Leaf Dip Method (72 Hours Post-Treatment)

Insecticide	**Susceptible Strain**		**Resistant Strain**		**Resistance Ratio**
	LC50 (ppm)	95% FL	LC50 (ppm)	95% FL	
Cypermethrin 10% EC	0.52	0.44-0.61	12.84	11.23-14.67	24.6
Azadirachtin 0.15% EC	5.21	4.67-5.82	11.89	10.45-13.52	2.3
Neem oil 0.03% EC	8.34	7.41-9.39	17.64	15.78-19.71	2.1
NSKE 5%	10.47	9.23-11.87	26.34	23.45-29.58	2.5

FL = Fiducial limits, NSKE = Neem seed kernel extract.

Table 2: Oviposition Deterrence Effect of Neem Formulations Against Cypermethrin-Resistant *Helicoverpa armigera* Moths

Treatment	**Concentration (L ha-1)**	**Eggs Laid Per Female**	**Deterrence (%)**
Control	-	487±23.4[a]	-
Azadirachtin 0.15% EC	1.0	198±18.7[b]	59.3
Azadirachtin 0.15% EC	2.0	124±12.5[c]	74.6
Neem oil 0.03% EC	2.5	178±16.3[b]	63.4
NSKE 5%	5.0	200±19.1[b]	58.9

Values are mean ± SE of five replications. Means followed by same letter within column are not significantly different at $p<0.05$ (Tukey's HSD test).

Table 3: Field Efficacy of Neem-Based Insecticides Against Resistant *Helicoverpa armigera* in Cotton (Pooled Data of 2022-2023)

Treatment	Dose (L ha-1)	Larval population per 5 plants	Fruiting body damage (%)	Seed cotton yield (kg ha-1)
		7 DAS	15 DAS	7 DAS
Untreated control	-	10.8±0.84[a]	11.4±0.92[a]	32.4±2.1[a]
Cypermethrin 10% EC	0.5	5.2±0.43[b]	6.8±0.56[b]	18.7±1.4[b]
Azadirachtin 0.15% EC	1.0	4.8±0.38[b]	5.4±0.48b[c]	16.3±1.2[b]
Azadirachtin 0.15% EC	2.0	3.6±0.31[c]	3.2±0.29[d]	12.8±0.9[c]
Neem oil 0.03% EC	2.5	6.4±0.51[b]	6.1±0.53[c]	20.5±1.6[b]
NSKE 5%	5.0	6.8±0.54[b]	6.9±0.58[b]	22.1±1.7[b]

DAS = Days after second spray. Values are mean ± SE of five replications. Means followed by same letter within column are not significantly different at $p<0.05$ (Tukey's HSD test).

Authors' Contribution

Conceptualization of research: Frederick Sidney Correa, Dr. Subhalaxmi Roy.

Designing of the experiments: Frederick Sidney Correa, Alok Kumar Yadav, Mahaveer Prasad Ola.

Contribution of experimental materials: Mahaveer Prasad Ola, Dr. BALASANKAR KARAVADI.

Execution of field/lab experiments and data collection: Supriya Awasthi, Dr. D. Neelamegam, Alok Kumar Yadav.

Analysis of data and interpretation: Dr. BALASANKAR KARAVADI, Frederick Sidney Correa.

Preparation of the manuscript: Frederick Sidney Correa, Dr. Subhalaxmi Roy, Supriya Awasthi.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

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