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Synergistic Modulation of Neuronal Responses in the Cockroach Periplaneta americana by Fenvalerate and Azadirachtin: An Electrophysiological Study

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Abstract

Pesticides play a crucial role in modern agriculture by protecting crops from pests and increasing yields. Synthetic pyrethroids such as fenvalerate are widely used as pesticides due to their high efficiency, rapid action, and relatively low mammalian toxicity. However, their persistent usage has raised concerns regarding ecological imbalance, resistance development, and off-target effects. To address these concerns, neem-derived biopesticide azadirachtin, have emerged as viable alternatives due to their biodegradable nature and selective toxicity towards pests.

This study investigates the neurotoxic effects of fenvalerate, azadirachtin, and their synergistic interaction using electrophysiological recordings in the cockroach Periplaneta americana. Electrophysiological responses were measured in Giant fibers (ventral nerve cord VNC) exposed to fenvalerate, azadirachtin, and their synergist. Results revealed that Fenvalerate shown inhibitory effects on the giant fiber responses, reducing neuronal excitability and causing conduction block, which involves prolonging sodium channel activation, leading to repetitive firing and eventual nerve impairment. In contrast, azadirachtin shown excitatory effects, increasing the frequency of action potentials to alter neurotransmitter levels there by affecting neuromuscular coordination. Interestingly, the synergistic combination of fenvalerate and azadirachtin produced an amplified effects with greater inhibition of sensory-mediated giant fiber responses.

INTRODUCTION : Pesticides are chemicals designed to eliminate pests detrimental to human interests, particularly in agriculture. They are classified as poisons and defined as substances used to prevent, destroy, or repel pests during food production, processing, transportation, and distribution (Gupta et al., 2020). While pesticides are crucial for crop protection and yield improvement, their indiscriminate use has led to critical concerns, including resistance development, environmental contamination (Chen et al., 2018), and toxicity to non-target organisms, including humans (Rathod et al., 2019). Synthetic pesticides have gained popularity due to their effectiveness, ease of application, and economic benefits. However, their overuse has raised issues of ecological imbalance, contamination of the food chain, and negative impacts on beneficial organisms (Sharma et al., 2021,). Residues of pesticides have now been documented globally, including remote regions like the Arctic (Li et al., 2019; Wang et al., 2022). In response to these challenges, there is a growing need for safer,

environmentally sustainable alternatives, driving increased research into natural products like neem-derived biopesticides.

Neem (Azadirachta indica) has emerged as a promising alternative to synthetic insecticides. Its active component, azadirachtin, exhibits antifeedant, growth regulatory, and neurotoxic properties (Singh et al., 2017). Neem-based products are not only biodegradable but also show low toxicity toward beneficial organisms, making them ideal for Integrated Pest Management (IPM) programs (Kumar et al., 2020). These properties make neem derivatives particularly suitable for addressing pesticide resistance while maintaining environmental safety.

Pyrethroids act primarily on the insect nervous system, targeting sodium channels and leading to prolonged depolarization, hyperexcitation, paralysis, and mortality (Song et al., 2021). Despite these advantages, pyrethroids can have off-target effects on non-target species and have been associated with increasing cases of resistance (Zhang et al., 2020). The nervous system of insects, consisting of sensory structures, ganglia, and nerve cords, is highly sensitive to insecticides. It provides an excellent model for studying neurotoxic effects and electrophysiological changes induced by pesticides (Huang et al., 2019). Electrophysiology has emerged as a critical tool to investigate alterations in nerve excitability, synaptic transmission, and giant fiber conduction following exposure to insecticides. In models such as Periplaneta americana, electrophysiological studies have revealed changes in sensory neuron firing patterns, synaptic dysfunction, and impaired neuromuscular responses upon exposure to synthetic and botanical insecticides (Wang et al., 2022).

The electrophysiological effects of pyrethroids and azadirachtin have gained attention in recent years, particularly for their impact on sodium channel kinetics, neurotransmitter pools, and neuronal excitability (Reddy et al., 2020; Reddy et al., 2021). For instance, pyrethroids have been shown to prolong sodium channel activation, leading to repetitive neuronal firing and eventual nerve block (Song et al., 2021). In contrast, azadirachtin disrupts neuromodulation by altering the levels of neurotransmitters like acetylcholine, serotonin, and dopamine, thereby impairing neuronal and muscular function (Khan et al., 2020).

Challenges of pesticide resistance, environmental toxicity, and food safety, the present study focuses on understanding the neurotoxic effects of the pyrethroid fenvalerate, neem-derived azadirachtin, and their synergistic interaction by using the well-characterized nervous system of Periplaneta americana, electrophysiological changes were examined in Giant fibres (VNC). These studies aim to elucidate the neurophysiological mechanisms underlying pesticide toxicity and their implications for safer and more sustainable pest management strategies.

MATERIAL AND METHODS

Selection and maintenance of the test animals : A colony of cockroaches (Periplaneta americana) was maintained in the animal house for a steady supply of animals for experimentation. Adult cockroaches were selected from the colony and acclimatized to

laboratory conditions (30 ± 2°C) for one week prior to use. They were fed ad libitum on bread and rice according to a fixed feeding schedule. Feeding was stopped one day before the experiment to avoid any metabolic variations. Male cockroaches were used as females may exhibit erratic rhythmicity influenced by their reproductive cycle (Hoffmann et al., 2017; Yin et al., 2019).

Selection of test chemicals: The synthetic pyrethroid **fenvalerate (20EC)** (Northern Minerals Ltd., India) and neem derivative **azadirachtin (0.15EC)** (Godrej, India) used in this study were of commercial grade and obtained from the local market.

Preparation and application of different concentrations: A stock solution of 20 ppm azadirachtin and 10 ppm fenvalerate were prepared in acetone. From these, appropriate dilutions were prepared in distilled water for different test concentrations. A synergist was created by mixing equal volumes of fenvalerate and azadirachtin.

The toxicity of an insecticide to an organism is usually expressed in terms of 24hr LD_{50} . The 24hr LD_{50} (dose required to kill 50% of the animals with in 24 hrs after exposure) was determined using "Probit method" (Finney. 1964). and 1/3 of 24hr LD_{50} was selected as the sublethal dose for fenvalerate, azadirachtin and synergist.

Determination of electrophysiological activity: Healthy and active adult cockroaches were selected for electrophysiological recordings. Animals were fixed to wax-filled dissection boards with the dorsal side up. The dorsal abdominal body wall was removed to expose the **ventral nerve cord (VNC)**. All exposed nerves and ganglia were immersed in cockroach Ringer solution throughout the procedure (Wang et al., 2016; Zhang et al., 2021).

Experimental Procedure: : Preparation and Recording Setup: The giant interneuron responses in the **ventral nerve cord** were recorded following mechanical stimulation of the **cercal sensilla** using controlled air puffs. The nerve cord was continuously bathed in cockroach Ringer solution to maintain physiological function (Khan et al., 2015; Kumar et al., 2015).

1.Application of Test Chemicals: Each test solution i.e **fenvalerate**, **azadirachtin**, and their **synergist**, was applied separately to the ventral nerve cord using perfusion. The chemicals were allowed to act for 5 minutes. For topical exposure studies, sublethal doses of the chemicals were directly applied to the cockroach cuticle, and recordings were conducted after the designated exposure times (Chen et al., 2018;).

2.Recording and Measurement: Electrophysiological responses were amplified using a **Grass PS AC preamplifier** and visualized on a **Systronics 5100 dual-beam oscilloscope**. Recordings were simultaneously fed into a **tape recorder** for analysis. A **Grass C4 kymograph camera** captured the oscilloscope traces onto photographic film. To quantify activity, spike counts (number of action potentials) were obtained manually and presented as **spikes/second** (Huang et al., 2019; Reddy et al., 2021, Zhou et al., 2022).

ISSN PRINT 2319 1775 Online 2320 7876
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3.Statistical Analysis: The data is analyzed using statistical tools such as **mean**, **standard deviation**, and **Student's t-test** for significance, following the methods of Snedecor and Cochran (1967). Results were considered significant at a p-value <0.05.

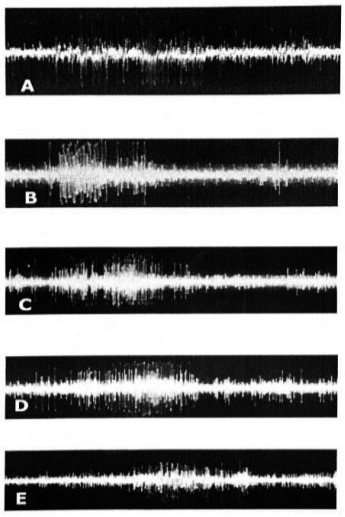
TABLES AND GRAPHS: Changes in the giant fibre responses in the VNC of the cockroach Periplaneta americana exposed (topical and bath applications) to fenvalerate, azadirachtin and synergist.

S.N	Insecticide	Changes in the giant fibre responses in the VNC of cockroach Periplaneta americana				
		Control	Topical	Control	Bath application	
					Sublethal	lethal
1.	Fenvalerate SD PDC	72 ± 5.2	43 ± 4.6 -40.28	79 ± 7.2	67* ± 6.4 -15.20	48 ± 4.6 -39.24
2.	Azadirachtin SD PDC	52 ± 6.4	64* ± 7.8 +23.07	48 ± 5.6	63* ± 8.2 +31.25	74 ± 5.2 +54.17
3.	Synergist SD PDC	67 ± 7.4	50 ± 4.8 -25.37	62 ± 7.2	47 ± 6.4 -24.19	26 ± 5.4 -58.06

The values are mean SD of 4 separate recordings.
The values marked with * are not significant

Fig. 1: Effect of lethal and sublethal doses (topical and bath application) of fenvalerate on the giant fibre response sin the cockroach Periplaneta americana

Extracellular recordings were made from ventral nerve cord (VNC) in control animals and animals exposed to lethal and sub lethal doses of fenvalerate**.**



A: Airpuff evoked giant interneuron responses (burst of high amplitude spikes) in control animal.

ISSN PRINT 2319 1775 Online 2320 7876
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B: Decrease in giant interneuron responses in animals topically exposed to sublethal doses of synergist.

C: Airpuff evoked giant interneuron responses (burst of high amplitude spikes) in control animal which was further used for bath application effect of fenvalerate.

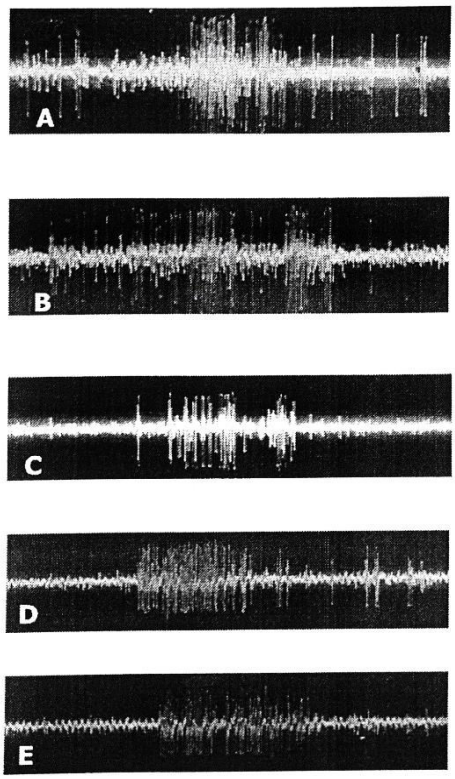
D: Decrease in giant interneuron responses in animal under bath application of sublethal doses of synergist.

E: Decrease in giant interneuron responses in animals under bath application of lethal doses of synergist.

Three separate animals were used for each set of recordings. Above is a representative picture from recordings of 4 separate experiments.

Fig. 2: Effect of lethal and sublethal doses (topical and bath application) of azadirachtin on the giant fibre responses in the cockroach Periplaneta americana

Extracellular recordings were made from ventral nerve cord (VNC) in control animals and animals exposed to lethal and sub lethal doses of azadirachtin.



A: Airpuff evoked giant interneuron responses (burst of high amplitude spikes) in control animal.

B: Increase in giant interneuron responses in animals topically exposed to sublethal doses of synergist.

C: Airpuff evoked giant interneuron responses (burst of high amplitude spikes) in control animal which was further used for bath application effect of azadirachtin.

D: Increase in giant interneuron responses in animal under bath application of sublethal doses of synergist.

ISSN PRINT 2319 1775 Online 2320 7876
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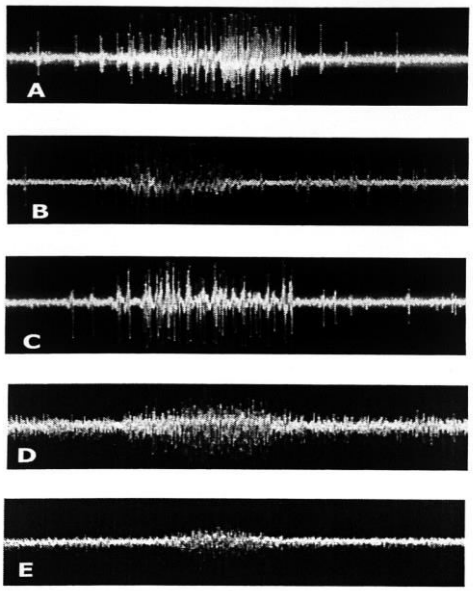
E: Increase in giant interneuron responses in animals under bath application of lethal doses of synergist.
Three separate animals were used for each set of recordings. Above is a representative picture from recordings of 4 separate experiments.

Fig. 3: Effect of lethal and sublethal doses (topical and bath application) of synergist on the giant fibre responses in the cockroach Periplaneta americana

Extracellular recordings were made from ventral nerve cord (VNC) in control animals and animals exposed to lethal and sub lethal doses of synergist.



A: Airpuff evoked giant interneuron responses (burst of high amplitude spikes) in control animal.
B: Decrease in giant interneuron responses in animals topically exposed to sublethal doses of synergist.
C: Airpuff evoked giant interneuron responses (burst of high amplitude spikes) in control animal which was further used for bath application effect of synergist.
D: Decrease in giant interneuron responses in animal under bath application of sublethal doses of synergist.
E: Decrease in giant interneuron responses in animals under bath application of lethal doses of synergist.
Three separate animals were used for each set of recordings. Above is a representative picture from recordings of 4 separate experiments.

RESULTS AND DISCUSSION

The LD for 24 hr value was found to be 2.16 µgig body weight for fenvalerate, 12.1 µg/g body weight for azadirachtin and 5.68 µg/g body weight for synergist. The intermediate LD_{50} value of the synergist (5.65 µg/g body weight) compared to fenvalerate (2.18 µg/g) and azadirachtin (13.12 µg/g) suggests an enhanced efficacy when these compounds are combined. This synergistic action may result from their combined modulation of sodium and potassium channels, disrupting neuronal function more effectively than individual treatments. The enhanced efficacy observed in this study supports the potential for combining synthetic and natural insecticides to improve pest control strategies while reducing environmental impact.

Electrophysiological Effects of Fenvalerate, Azadirachtin, and Synergist

The results of the present study revealed that topical exposure of fenvalerate, azadirachtin, and their synergist to the cockroach Periplaneta americana caused significant electrophysiological changes. Fenvalerate and the synergist demonstrated inhibitory effects on giant fiber responses of the ventral nerve cord (VNC), while azadirachtin exhibited excitatory effects. These findings align with previous research indicating that pyrethroids act on voltage-gated sodium channels, leading to the inhibition of spontaneous and sensory-evoked neuronal responses (Zhang et al., 2021; Kumar et al., 2021).

The synergist prepared from fenvalerate and azadirachtin showed an intermediate LD50 value of 5.65 µg/g body weight, compared to fenvalerate (2.18 µg/g) and azadirachtin (13.12 µg/g). This result confirms that combining the two compounds enhances efficacy, as seen in the amplified electrophysiological effects (Huang et al., 2019; Wang et al., 2023).

Mechanisms Underlying Neurophysiological Effects

Fenvalerate, a synthetic pyrethroid, is known to target sodium channels, causing prolonged activation that leads to repetitive neuronal firing and eventual conduction block (Song et al., 2021). In this study, fenvalerate's inhibitory action on the giant fibers supports its mechanism as a neurotoxic agent disrupting central nervous system coordination. Similarly, azadirachtin altered electrophysiological activity by modulating voltage-gated potassium currents and interfering with calcium ion regulation, consistent with earlier studies (Reddy et al., 2021).

The synergist amplified these effects, causing greater inhibition of sensory-mediated giant fiber responses. This synergistic action may be attributed to combined effects on sodium and potassium channel modulation (Li et al., 2019; Zhou et al., 2022). The use of synergists, as demonstrated in this study, provides a promising strategy for improving the efficacy of biopesticides. The observed electrophysiological effects underscore the potential of combining pyrethroids with neem-based compounds like azadirachtin to enhance pest control efficacy while potentially reducing overall chemical usage (Reddy et al., 2021).

Recovery from Sublethal Exposure

Cockroaches exposed to sublethal doses exhibited milder behavioral changes and showed recovery over time. This recovery suggests the presence of detoxification mechanisms that mitigate the toxic effects of insecticides. The ability of the animals to restore near-normal neuronal activity highlights their physiological resilience and supports the idea of employing synergists at lower doses to minimize environmental and non-target organism impacts (Wang et al., 2016).

CONCLUSION

The present investigation highlights the neurotoxic effects of fenvalerate, azadirachtin, and their synergist on the cockroach Periplaneta americana. Topical exposure to these insecticides resulted in significant electrophysiological changes, with fenvalerate and the synergist causing inhibitory effects on giant fiber responses in the ventral nerve cord, while azadirachtin produced excitatory effects. These observations suggest differential impacts of these compounds on sensory-mediated neuronal activity (Huang et al., 2019; Reddy et al., 2021).

The synergist demonstrated enhanced efficacy, combining the strong inhibitory effects of fenvalerate with the unique excitatory properties of azadirachtin. This combination not only improved pest control but also offered a potential strategy to minimize the dosage of individual components, thereby reducing environmental impact and resistance development (Li et al., 2019; ; Song et al., 2021; Zhou et al., 2022). Electrophysiological recovery observed in cockroaches exposed to sublethal doses indicates the presence of detoxification mechanisms that mitigate the neurotoxic effects of these insecticides. This finding underscores the resilience of insect nervous systems and supports the development of safer, synergistic formulations for integrated pest management (Kumar et al., 2020). The findings of this study highlight the utility of synergists in enhancing insecticidal efficiency while mitigating issues related to resistance and environmental safety. Further research on such synergistic formulations could pave the way for more effective and sustainable pest control strategies (Hoffmann et al., 2017; Zhang et al., 2021).

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