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Research Article

One pot chemical co-precipitation preparation of magnetic graphene oxide-deltamethrin nanoformulations for management of *Aedes aegypti*

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Abstract

Aedes-borne diseases are of worldwide concern due to the lack of effective medicine and vaccination. Frequent use of chemical intervention has developed insecticide resistance in mosquitoes and posed health risks to humans and the environment, necessitating an effective and safer intervention. Graphene Oxide (GO) is an efficient material that can absorb pesticide particles and release pesticide macromolecules in a controlled manner. With the proposition that magnetic graphene oxide (MGO)-based nanoformulations can be an eco-safe and effective material for pesticide conjugation, the present study synthesized these nanoformulations conjugated with a pyrethroid, deltamethrin (DL) through chemical co-precipitation method. The formulations were validated using biophysical techniques and investigated for their efficacy against *Aedes aegypti*. The X-ray diffraction (XRD) pattern of nanocomposites showed six intense diffraction peaks of Fe_3O_4 particles, X-ray photoelectron spectroscopy (XPS) displayed the C1s, O1s, and Fe2p photoelectron lines in the MGO nanocomposite's spectra, while Field Emission Scanning Electron Microscopy (FESEM) revealed the small size and uniformity of Fe_3O_4 nanoparticles on the GO surface. The individual MGO and DL, as well as MGO-DL binary combinations (1:1, 1:2, and 1:3) imparted significant larval toxicity, demonstrating 30%, 50%, and 85% CTC (Co-toxicity coefficient), respectively. High corresponding Synergistic factor (SF) values indicated significant synergism increasing with the rise in deltamethrin proportion. The MGO-DL combinations also increased irritancy and flight response in adults, the notable synergistic effects imparted by the 1:3 combination. The effective actions of MGO-DL nanoformulations against mosquitoes suggest their possible use for mosquito management as a safer and more operative intervention.

Keywords: *Aedes aegypti,* Contact irritancy, Deltamethrin, Larvicidal, Magnetic Graphene oxide, Nanoformulation

INTRODUCTION

Insect pests and vector control have become a worldwide issue in recent years. Chemicals are used more frequently than other control measures due to their quick action and effective results. The increasing frequency of disease outbreaks, such as Chikungunya, yellow fever, dengue fever, and Zika, spread globally by *Aedes aegypti* mosquitoes, has increased human mortality and health issues. Furthermore, different chemical insecticide formulations being used more frequently than other control interventions have exacerbated these problems. Nonetheless, indiscriminate pesticide application not only resulted in a variety of environmental, social, and health consequences but has also intensified insecticide resistance in the target organisms. Besides, the loss of a great proportion of chemicals due to degradation, photolysis, and volatilization has reduced mosquito lethality (Monteiro *et al*., 2021). Hence, more frequent application with increased dosages of insecticides has aggravated the associated issues. All these complications have diverted the interest of researchers towards nanopesticides as a new technique for insect vector and pest control, offering a viable answer to the present global pesticide-related issues.

Nanopesticides, formulated with much lower pesticide concentrations, are assumed to be more advantageous than conventional insecticides in terms of safety, durability, better solubility, and stronger activity against the target (Lu *et al*., 2021). Additionally, in comparison to their bulk equivalents, their modest surface-to-volume ratio attributes those improved chemical and physical properties (Samadi *et al*., 2017). It is believed that nanotechnology's promising application in mosquito management has the potential to reduce the detrimental effects of currently employed pesticides. Keeping this in view, it is proposed that the conjugated formulation of nanomaterials and insecticides, utilizing a lower amount of toxicant, could be a suitable and synergistic delivery method for mosquito management effectively with minimized negative effects of insecticides.

Graphene Oxide (GO) is considered an efficient nanomaterial that responds well to environmental stimuli, can absorb pesticide particles, and release pesticide macromolecules in a controlled manner (Lu *et al*., 2021). Magnetic Graphene Oxide (MGO) synthesized from graphene oxide is made up of a monolayer of sp^2 linked carbon atoms, which can display higher efficiency because of its huge surface area and excellent thermal, mechanical, and electrical properties (Wang *et al*., 2019a). As a result, the present study hypothesized that MGO could be used to create highly effective nanopesticides for managing insect vectors. Use of synergistic mixes of graphene oxide and pesticides as an approach for mosquito management has not been attempted yet, though their efficacy has been investigated against a few other organisms. The contact toxicity of GO-synergized chlorpyrifos, pyridaben, and β-cyfluthrin rose against *Tetranychus urticae* and *T. truncates* by 1.78, 1.75, and 1.50-fold, and 1.55, 1.56, and 1.77-fold more, respectively (Wang *et al*., 2019a). Similar synergistic action of graphene oxide with β-cyfluthrin, monosultap, and imidacloprid has been reported against Asian corn borer resulting in 2.10, 1.51, and 1.83-fold higher contact toxicities than the individual insecticides (Wang *et al*., 2019b). Novel results have been obtained with an individual amalgam of graphene oxide with malathion and endosulphan, resulting in 80.43% and 6.43-fold increased efficacy of both insecticides against *Ae. aegypti*, respectively (Gupta *et al*., 2021b). The novelty of this technique has not, however, been properly investigated particularly against mosquito populations and as per our knowledge, no such studies have been performed with MGO.

Thus, nanoformulations prepared with synergistic combinations of MGO and deltamethrin were investigated against an Indian strain of *Ae. aegypti*. Deltamethrin, a pyrethroid, in its purest form, is an odorless, colorless/white to light beige crystalline pyrethroid, used widely outdoors. It is primarily a contact nerve poison which rapidly paralyses the insects with an efficient knockdown effect; though it can act as stomach poison too. It is considered a safe insecticide because of its low toxicity to mammals (Ray, 2003; Dai *et al*., 2010). This study aimed to compare the individual effectiveness of graphene oxide-based nanoparticles and insecticide (deltamethrin) and their combinations against the Indian strain of dengue vector *Ae. aegypti*, to develop a strategy to combat dengue hazard and insecticide resistance in mosquitoes. The present study deals with the magnetization of graphene oxide, followed by characterization of the material using X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS) and Field Emission Scanning Electron (FESEM); and *in-vitro* studies of binary mixtures (MGO and deltamethrin) against *Aedes aegypti.* The larvicidal potential and contact irritancy of synergistic deltamethrin-MGO combinations against *Ae. aegypti* were investigated with the hypothesis that magnetic graphene oxide-pesticide nanoformulations can result in more effective insecticidal action than the lone insecticide.

MATERIALS AND METHODS

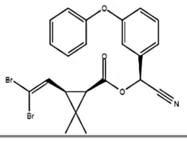
Materials

All the reagents used were of analytical grade and were used without further purification. Iron (III) chloride ($FeCl_3$, 96.0%), Iron (II) chloride ($FeCl_2$, 96.0%), Hydrochloric acid (HCl, 35%), Sodium Hydroxide (NaOH, 97%), concentrated sulphuric acid (Conc. H_2SO_4, 98%), Hydrogen Peroxide (H_2O_2, 30%) and Ammonia solution (NH_3, 25 wt%), were purchased from Merck. Sodium nitrate ($NaNO_3$, 99%) and potassium permanganate ($KMnO_4$, 99%) were purchased from Hi-media. Graphene oxide was synthesized from graphite (99%) purchased from Sigma Aldrich, India. Distilled water was used throughout the experiments.

Synthesis of Graphene oxide nanomaterial

The graphene oxide was prepared from graphite powder following the Hummers' method (Chen *et al*., 2013). A mixture of 0.5 g of $NaNO_3$ and 1.0 g of graphite powder was added to the concentrated H_2SO_4 (23 mL) and

Table 1. Structural and functional details of Deltamethrin [Source: Insecticide Resistance Action Committee (IRAC)]

IUPAC name	Chemical Structure	Mode of action	% purity	Target site	Route of
[(S)- Cyano-(3-phenoxyphenyl)-methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethyl-cyclopropane-1-carboxylate		Modulates sodium channels	98.0	Nerve axon	Contact

kept in an ice bath at 5 °C (Gautam *et al.*, 2020). After 5 mins of continuous stirring, $KMnO_4$ (3g) was added to the mixture gradually with close monitoring the rate of addition to prevent the temperature rise higher than 15 °C. After 2 h, the mixture was removed from the ice bath and regularly stirred for the next 30 min till the temperature reached 35 °C. The temperature was further raised to 98 °C by the subsequent addition of deionized water which was sustained by continuously swirling the mixture against the grain for 30 min. The reaction was stopped by adding H_2O_2 (10% v/v, 10 mL) and deionized water, yielding a yellow colour product that was vacuum filtered to create a brownish-yellow precipitate. The unreacted Mn^{2+} and sulphuric acid were removed by the separation of the precipitate, followed by washing 5 times with HCl (5%, 200 mL) and warm deionized water. The graphene oxide nanopowder was made by drying the precipitate for 12 h at 60 °C.

Preparation of MGO Nanocomposite

MGO Nanocomposite was made using the co-precipitation method (Gautam *et al.*, 2018). The mixture of 1 M $FeCl_3$, (2 mL) and 0.5 M $FeCl_2$ (2 mL) was agitated for 20 min at room temperature to dissolve the iron salts. The mixture was then re-agitated for another 20 min after adding GO (10 ml, 0.01 mg/mL) followed by adding 20 mL ammonia solution, creating a black MGO nanocomposite almost instantly. The MGO powder was rinsed many times with deionized water, separated using a permanent external magnet, and dried in an air oven for 12 h at 60°C.

Characterization

A Field Emission Scanning Electron Microscopy (FESEM) picture of MGO was obtained at a voltage of 20 kV using a JEOL JSM 6610. The powder X-ray diffraction (XRD) pattern was obtained on a Bruker D8 Discover, X-ray source Cu, 3KW. Further, X-ray photoelectron spectroscopy (XPS) (Model PHI-5700, Physical Electronics Inc. USA) with Al K-excitation radiation was used to determine the chemical composition (1486.6 eV) of MGO.

Insecticide used in the study

The investigation employed deltamethrin (pyrethroid) obtained from M/s Sigma-Aldrich, India. The structural formula, chemical name and mode of action of deltamethrin are presented in Table 1.

Preparation of nanomaterials-insecticide conjugated formulation

The nanomaterial (MGO) and deltamethrin were mixed in the ratios of 1:1, 1:2, and 1:3 and dispersed in 2 mL acetone. The volume of 0.1 mL of each solution was ultrasonicated in 100 mL of water and mixed in the dark for 24 h. Subsequently, the mixtures were dissolved in 100 mL of autoclaved water to obtain the conjugated formulation.

Culture establishment of *Aedes aegypti*

The culture of *Ae. aegypti* was maintained in the insect rearing laboratory of Acharya Narendra Dev College, University of Delhi, India, under controlled conditions of 28 °C, 85% RH, and 14 L/10 D photo regime based on the described protocol (Samal and Kumar, 2021). Adult mosquitoes were fed on the sugary juice of water-soaked raisins, while females were fed intermittently on blood. The eggs laid and hatched in dechlorinated water were raised to larvae in water-filled trays containing yeast and powdered dog biscuits (3:1) for nutrition. The pupae were housed in clothed cages until they emerged as adults (Samal and Kumar, 2021; Gupta *et al.*, 2021a)

Effect of MGO and MGO-deltamethrin formulations on *Aedes aegypti*

The effects of the binary combinations of MGO nanocomposites and deltamethrin (MGO-DL - 1:1, 1:2, and 1:3) were assessed on the survival, morphology and irritancy of *Ae. aegypti*.

Larval toxicity bioassay

The method was followed while performing larvicidal assays on *Ae. aegypti* (WHO, 2016). MGO-DL formulations were prepared with acetone as a solvent. Batches of 20 early fourth instar larvae were added to a mixture of 199 mL distilled water and either 1 mL of deltamethrin alone, MGO alone or MGO-DL combinations at a particular concentration. Each dilution was investigated four times in a row. For controls, 1 mL of acetone was substituted for the required formulation. Dead and moribund larvae were counted as percent larval mortality after 24 h. The control larval mortality was corrected as per Abbott's formula represented in Eq.1 (Abbott, 1925). Lethal concentrations (LC) causing 50% and 90% larval mortality (LC_{50} and LC_{90}, respectively) along with the 95% confidence intervals (CI) were computed from a log dosage–probit mortality regression line using computer software programs SPSS 22.0. In the case of assays with binary combinations of MGO-DL, the co-toxicity coefficient, and synergistic factors were calculated as per the formulae given below in Eq. 2 and Eq. 3 (Kalyanasundaram and Das, 1985; Trisyono and Whalon, 1999).

% Corrected mortality = (% Test mortality - % Control mortality) - (100 - % Control mortality) …...Eq. 1

CTC (Co-toxicity coefficient) = Observed % mortality - Expected % mortality / Expected % mortality x 100 …...Eq. 2

CTC value ≥ 20 indicate synergism; CTC value ≤ (-) 20 indicate antagonism; CTC intermediate value of ≤ (-) 20 to ≥ 20 indicate an additive effect

Synergistic factor (SF) = Toxicity of deltamethrin alone/ Toxicity of deltamthrin with MGO nano formulation …...Eq. 3

Values of SR > 1 indicate synergism

Value of SR < 1 indicate antagonism

Contact irritancy against adult females

After preliminary testing, 0.01% dosage of MGO-deltamethrin formulation was selected for irritancy assays. The 0.01% concentration of each formulation was diluted with acetone and independently saturated on Whatman filter paper circles. A carrier (Silicon oil) was used to prepare a thin, homogeneous layer of the nanocomposite and insecticide to spread on the filter paper and prevents crystallization of the insecticide, which is usually solid at room temperature. The formulation-impregnated circles were dried in the air. Each circle was placed on separate glass plates and covered with an inverted destemmed conical glass funnel with a hole on the top. A 3-day-old unfed female mosquito was introduced into the funnel using an aspirator and the hole was closed with a cotton plug to prevent the escape of the mosquito. After a 3-min settling period, the first flight time and the total number of flights were recorded for the next 15 minutes. The contact irritancy of each formulation was tested a total of ten times (Warikoo *et al*., 2012). The acetone-impregnated Whatman filter sheets, with silicon oil as carrier, were used in the control experiments. The assays were repeated with blood-fed mosquitoes. Similar investigations were performed with individual MGO and deltamethrin-impregnated papers on both unfed and blood-fed mosquitoes. Relative irritability was calculated to assess the comparative efficacy of all the binary mixtures and individual compounds.

RESULTS

Characterization of MGO Nanocomposites

The XRD pattern of MGO nanocomposite (Fig. 1) shows six intense diffraction peaks at 2θ values of 30.62, 35.74, 42.95, 54.09, 57.26, and 62.41, which correspond to the (220), (311), (400), (422), (511), and (440) crystal planes of Fe_3O_4 particles, respectively. The GO diffraction peak at around 2θ = 10.5 which implied that the Fe_3O_4 nanoparticles damaged the GO substrate's natural lattice structure. The X-ray photoelectron spectroscopy (XPS) used to examine the chemical structure of the MGO nanocomposite displayed the C1s, O1s, and Fe2p photoelectron lines in the MGO nanocomposite's spectra (Fig. 2). The $Fe2p_{3/2}$ and $Fe2p_{1/2}$ peaks were found at 718.21 and 730.29 eV, respectively, indicating the presence of the Fe_3O_4 phase in the MGO nanocomposite, as shown in Fig. 2. Furthermore, the Fe_3O_4 nanoparticles were uniformly dispersed across the smooth surface of the GO as revealed from a FESEM image of MGO nanocomposite (Fig. 3).

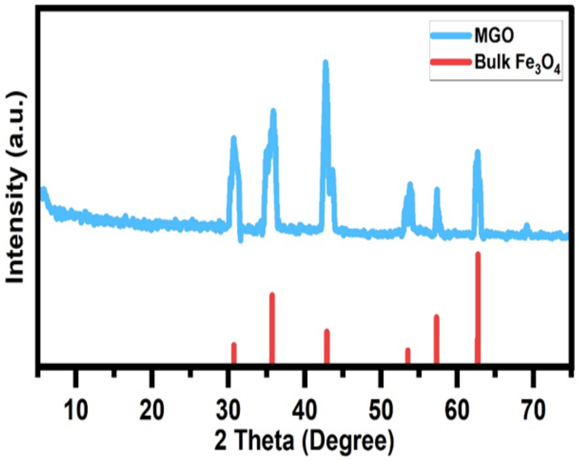


Fig. 1. *Comparative Powder XRD pattern of Bulk Ferric Oxide and Magnetic Graphene Oxide nanocomposite*

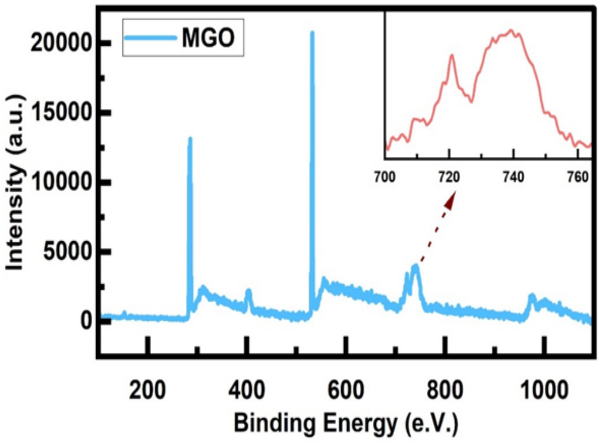


Fig. 2. *XPS spectrum and high-solution spectrum of Fe2p peak of Magnetic Graphene Oxide nanocomposite*

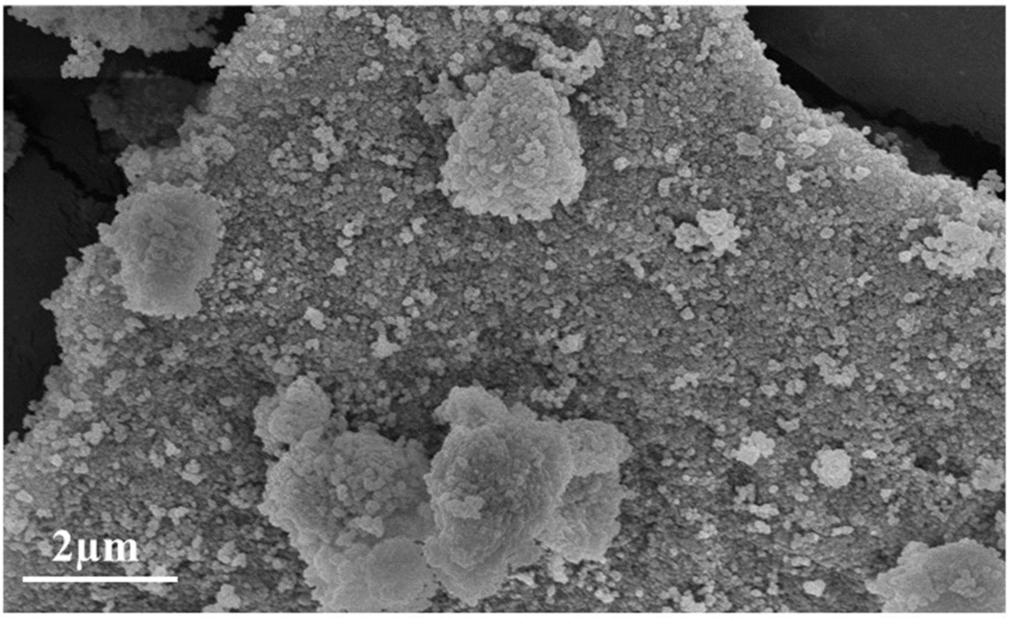


Fig. 3. *FESEM image of magnetic graphene oxide nanocomposite*

Table 2. Relative larvicidal efficacy of MGO and deltamethrin alone and their binary mixtures against early fourth instars of *Aedes aegypti*

Compound/ Binary Mixture	LC_{50} (ppm)	Relative Larvicidal Efficacy	CTC	Type of action (Based on CTC)	SF	Type of action (Based on SF)	LC_{90} (ppm)	Relative Larvicidal Efficacy	CTC	Type of action (Based on CTC)	SF	Type of action (Based on SF)
Nanocomposite and Insecticide												
MGO	11.24925 (9.01223-14.04257)	-	-	-	-	-	61.20025 (49.02911-76.39326)	-	-	-	-	-
Deltamethrin	0.00321 (0.00109-0.01368)	-	-	-	-	-	0.01152 (0.00511-0.85327)	-	-	-	-	-
MGO + Deltamethrin												
1:1	0.00011 (0.00007-0.00015)	0.036	30	Synergism	29.18	Synergism	0.00405 (0.00277-0.00591)	0.368	0	Additive	2.84	Synergism
1:2	0.00042 (0.00028-0.00061)	0.14	50	Synergism	7.64	Synergism	0.00382 (0.00261-0.00557)	0.347	3.33	Additive	3.02	Synergism
1:3	0.00021 (0.00015-0.00031)	0.07	80	Synergism	15.29	Synergism	0.00362 (0.00247-0.00528)	0.329	10	Additive	3.18	Synergism

N=600;No mortality was observed in the control, LC_{50} - Lethal Concentration that kills 50% of the exposed larvae, LC_{90} - Lethal Concentration that kills 90% of the exposed larvae, CTC= Co-toxicity coefficient SF = Synergistic Factor

Larvicidal bioassay with deltamethrin and magnetic graphene oxide

The larvicidal potentialities of DL and MGO were evaluated against the early fourth instar of *Ae. aegypti* and presented in Table 2. The larval subjection to deltamethrin resulted in LC_{50} value of 0.00321 mg/L and LC_{90} value of 0.01152 mg/L, which was 3500-fold more effective than MGO at 50% toxicity level ($p < 0.05$).

Synergistic larvicidal studies with MGO Deltamethrin nanomaterial (1:1, 1:2 and 1:3)

The results of larvicidal bioassays using binary mixtures of MGO with deltamethrin against *Ae. aegypti* larvae are presented in Table 2. The results showed that conjugation of deltamethrin with MGO increased its efficacy by 7.64 to 29.18-fold. After 24 h, the MGO-DL nanomaterial (1:1) lowered the LC_{50} value displayed by

Table 3. Response of 3-day old adult females of *Aedes aegypti* (blood-fed and unfed) to papers impregnated with deltamethrin and MGO nanocomposite (0.01%) in the contact irritancy assays

Female *Aedes aegypti*	Mean Time lapse before first take-off (in seconds) ± SEM	Mean number of take-offs by females (in 15 min) ± SEM	Relative irritability
Deltamethrin			
Control	1.33 ± 0.33 a	6.33 ± 0.88 a	1
unfed	2.33 ± 0.33 b	25 ± 2.08 b	3.95
Blood-fed	5.66 ± 1.20 c	41.67 ± 2.60 c	6.58
MGO Nanocomposite			
Control	1.00 ± 0.59 a	4.33 ± 0.33 d	1
unfed	3.67 ± 0.33 b	13.33 ± 0.88 e	3.08
Blood-fed	5.00 ± 0.59 c	16.00 ± 0.58 f	3.69

Values followed by different letters within the same column are significantly different, whereas the same letter depicts no significant difference (p< 0.05), one-way ANOVA followed by Tukey's all pair-wise multiple comparison test.

Table 4. Response of 3-day-old adult females of *Aedes aegypti* (blood-fed and unfed) to papers impregnated with binary mixtures of MGO-DL during contact irritancy assays

Parameters	MGO + Deltamethrin								
	1:1			1:2			1:3		
	C	UF	BF	C	UF	BF	C	UF	BF
Mean Time lapse before first take-off (in sec) ± SEM	1.67 ± 0.33 a	2.67 ± 0.88 a	5.67 ± 1.20 b	1.00 ± 0.58 a	1.33 ± 0.33 a	4.67 ± 0.33 b	1.67 ± 0.33 a	2.67 ± 0.33 a	4.00 ± 0.58 b
Mean number of take-offs by females (in 15 min) ± SEM	4.67 ± 0.81 a	24 ± 1.15 b	37.33 ± 1.45 c	6.00 ± 1.00 a	22.67 ± 0.67 b	44.67 ± 1.33 d	4.67 ± 0.33 a	26.67 ± 1.45 b	45.67 ± 1.45 d
Relative irritability wrt control	1	5.14	8.01	1	3.78	7.44	1	5.71	9.78
SF	-	1.04	1.12	-	1.10	0.93	-	0.94	0.91
Type of action	-	A	A	-	A	S	-	S	S

SF-Synergistic Factor; A- Antagonism; S-Synergism; C-Control; UF-Unfed; BF- Blood-fed. Values followed by different letters within the same row are significantly different, whereas the same letter depicts no significant difference (p < 0.05), one-way ANOVA followed by Tukey's all pair-wise multiple comparison test

deltamethrin and boosted the larvicidal effects against *Ae. aegypti* by 29.18 times. Besides, 24 h larval exposure to 1:2 and 1:3 combinations of MGO and deltamethrin boosted larvicidal efficiency by 7.64 and 15.29 times, respectively. These results indicate a correlation between larvicidal potency and the proportion of deltamethrin in the mixture. MGO-DL combinations in 1:1, 1:2, and 1:3 ratios demonstrated 30%, 50%, and 85% respective CTC, with high corresponding SF values indicating a high level of synergism. At the LC_{90} level, all the three combinations of MGO and deltamethrin could impart additive effects on *Ae. aegypti* larval survival, with CTC % ranging from 0 to 10 and SF ranging from 2.84 to 3.18 (Table 2).

Contact irritancy bioassay with MGO and deltamethrin

The female adults of *Ae. aegypti* subjected to contact irritancy assays showed a significant behavioral response to deltamethrin as well as to the MGO-DL (p < 0.05); a delayed but higher irritant response was obtained in blood-fed mosquitoes in comparison to the unfed ones (Table 3). The deltamethrin exposure resulted in the first flight only after 2.33 and 5.66 sec in unfed and blood-fed mosquitoes, respectively, stimulating an average total of 25 and 41.67 take-offs against the control (Table 3). Significant response was also observed with MGO nanocomposite in eliciting first flight only after 3.67 and 5.00 sec in respective unfed and blood-fed mosquito (p < 0.05) and an average total of 13.33 and 16 take-offs, displaying 3-4-fold higher irritability as compared to the respective control.

Synergistic irritancy studies with MGO-DL (1:1, 1:2, 1:3) against *Aedes aegypti* adult females

The MGO-deltamethrin binary mixtures also induced a similar irritant response in female adults of *Ae. aegypti*. The MGO-DL (1:3) induced first flight only after 2.67 sec in unfed females with an average total of 26.67 take-offs (Table 4). In comparison, the mixture induced 19 additional take-offs leading to 45.67 take-offs in their blood-fed counterparts with the first flight taken after 4 sec. In comparison to lone DL, the MGO-DL (1:3) combinations increased corresponding irritability by 1.1-fold in blood-fed adult female *Ae. aegypti*. On the other hand, the rest of the binary mixtures showed antagonism.

DISCUSSION

Researchers are continuously exploring environmentally-sustainable solutions for *Aedes* management due to the cosmopolitan breakout of *Aedes*-borne diseases with continuously increased incidences of dengue, Chikungunya, yellow fever and Zika. While many chemical insecticides have been employed to eliminate mosquito life stages and limit reproduction to keep a check on their threshold level, their widespread usage has severely harmed our environment, non-target organisms and even humans. Consequently, they are focusing on developing and testing nanomaterials as transporters of insecticides to formulate an effective and environmentally friendly mosquito management option (Samal *et al.,* 2022). The findings of the present study shows that combining graphene-based nanoparticles with insecticides can result in highly effective insecticidal action, and they could have major implications for the development of graphene-based insecticides and other insecticide applications.

Magnetic graphene oxide (MGO) has been reported as a suitable material and an effective sorbent of toxicants, dyes, insecticides, etc. The compound has been demonstrated to have the capacity to adsorb various insecticides, such as neonicotinoids, pyrethroids and emamectin benzoate, etc. present in the water and food samples (Lingamdinne *et al*., 2019; Ghiasi *et al*., 2020; Sha *et al*., 2022). Considering the advantages and possibility of MGO being used as an efficient toxicant delivery vehicle, attempts were made to prepare MGO nanoformulations by conjugating it with a widely used pyrethroid, deltamethrin different proportions. These formulations were evaluated for their contact irritant effects and larvicidal potential against *Ae. aegypti*.

The uniform distribution of Fe_3O_4 particles over MGO nanocomposite was completely explained by XRD, XPS and SEM techniques. The presence of Fe_3O_4 nanoparticles over GO surfaces completely damaged the GO substrate's natural lattice structure. The present investigation showed that the conjugation of deltamethrin with graphene-based nanomaterials increased its efficacy considerably. All three combinations (1:1, 1:2, and 1:3) imparted notably augmented larvicidal effects and irritancy against *Ae. aegypti* adult females, the maximum effects were obtained with 1:3 combination. It indicates that the efficacy of binary mixtures directly corresponded to the amount of deltamethrin in the combination. These results are in accordance with the effects of GO-insecticide binary mixtures (1:2) against *Ae. aegypti* larvae, using malathion (ML) and endosulphan (EN) as the insecticide component (Gupta *et al*., 2021a). The investigations showed that as compared to ML and EN alone, GO-ML and GO-EN (1:2) imparted higher larval toxicity by 80.43% and 6.43-fold, respectively, while conjugates in 1:1 ratios displayed 2.71-fold and 1.38-fold increase. In addition, studies reported cuticular deposition of black soot in the larvae exposed to the GO-ML mixture while disintegrated gut viscera in the larvae exposed to GO-EN.

Studies on mosquitoes are limited to date, according to our present knowledge and available literature. However, the bioassay with GO combined with another pyrethroid, β-cyfluthrin, against spider mites, *T. urticae* and *T. truncatus* induced 1.50 and 1.77-fold higher toxicity, respectively in comparison to the pesticide alone (Wang *et al*., 2019) which, though considerable, is much lower than observed in the present study. A noticeable decrease in the lethal toxicity of monosultap (LC_{50} = 1.96 μg/mL) and imidacloprid (LC_{50} = 4.23 μg/mL) was recorded against *Ostrinia fumacalis,* corn borer, when conjugated with GO, the respective LC_{50} values decreasing to 1.23 μg/mL and 2.31 μg/mL (Wang *et al*., 2019b). The use of carbendazim–graphene oxide (CBZ–GO) and carbendazim–ultrasonic graphene oxide (CBZ–UGO) against *Magnaporthe oryzae* mycleliae demonstrated 2.29-fold and 1.56-fold higher activity than carbendaziium, respectively. They proposed that nanocomposites could potentially destroy the glutathione on the cell membrane which reduced the host cell activity (Hu *et al*., 2021). It is proposed that the increased efficiency of the synergistic mixtures may be due to the adsorption of GO on the mosquito cuticle leading to enhanced uptake and utilization of deltamethrin, a contact pyrethroid.

The present investigation also revealed the enhanced irritancy response of mosquitoes with MGO synergized mosquitoes than deltamethrin alone. In addition, a higher flight response was elicited in blood-fed mosquitoes than in their unfed counterparts, which increased with the proportion of deltamethrin in the combination. These results corroborate the outcomes obtained with similar studies carried out with GO-malathion and GO-endosulphan conjugates against *Ae. aegypti* adults eliciting respective increase in take-offs by 28-32% and 24-33% (Gupta *et al*., 2021a). They also concluded that the addition of GO synergized the deltamethrin activity and increased the irritancy effects against blood-fed adult females in comparison to unfed females. Similar results, however, have been reported on exposure of *Ae. aegypti* with *Citrus sinensis* extract, inducing a higher behavioral response in blood-fed mosquitoes as against non-blood-fed ones (Warikoo *et al*., 2012). Nevertheless, such studies are not available in the literature with MGO nanomaterials which denotes the significance of current investigations as bed nets impregnated with MGO-deltamethrin formulation can reduce human-vector contact more efficiently, alleviating disease transmission.

It has been proposed that the avoidance response in mosquitoes to different toxicants is influenced by the insecticide susceptibility status and physiological, especially nutritional, condition of the mosquitoes (Gupta *et al*., 2021b). In addition, the response may vary based on the duration between feeding, the contact with the insecticide, and even the amount of blood-fed. The higher susceptibility of *Ae aegypti* to the MGO-deltamethrin than the deltamethrin alone might be one of the reasons for enhanced flight response. However, the effect of blood feeding on the flight response is still not apparent as contrary to our results, and the reports have also revealed higher irritability in unfed mosquitoes than blood-fed mosquitoes on insecticide contact (Warikoo *et al*., 2012). It is proposed that combining MGO nanocomposite with toxic deltamethrin, imparting enhanced larvicidal effects and irritant response in *Ae. aegypti*, could be a long-term and eco-safe mosquito control intervention.

The present study is of high significance as this approach not only minimizes the amount of insecticide to be used but also makes the conjugate mixture more operative, cost-effective and comparatively less harmful to the environment. The multifunctional mechanism that allows MGO-based nanomaterial to serve as a synergist for the existing insecticides demonstrates the excellent application potential in vector management. It is proposed that combining nanomaterials with insecticide leads to increased larvicidal and irritant effectiveness of the insecticides against *Ae. aegypti* may provide a sustainable and eco-friendly solution for mosquito management. This study allows us to further investigate the uses of nanotechnology in controlling dengue vector as the promising usage of nanomaterials will help to mitigate the harmful effects of insecticides.

Conclusion

The present study used a one-pot co-precipitation method to prepare a promising magnetic graphene oxide-deltamethrin nanomaterial with a proposition that it will help to battle the dengue hazard and counter insecticide resistance in mosquitoes. The investigation revealed the use of MGO-DL in various proportions (1:1, 1:2, and 1:3) against *Ae. aegypti* resulted in more efficient larvicidal action and irritating reaction in adults, with the 1:3 combination having the greatest effects indicating that the amount of deltamethrin in the binary mixtures was directly proportional to their efficacy. The present study is significant in the field of mosquito management as the proposed formulation utilizes a lower amount of pesticide, increases the toxic effects, augments the stability and is safer for the environment. This study allows to investigate further the promising use of nanotechnology in dengue vector management to mitigate the harmful effects of insecticides. It is suggested that MGO nanocomposite can serve as an effective conjugate for the existing insecticides demonstrating the excellent application potential in vector management. Finally, it is proposed that field trials with these formulations would aid in the standardization of dosage and administration mode in *Ae. aegypti*.

Conflict of interest

The authors declare that they have no conflict of interest.

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