

THE EFFICACY OF NEEM, *AZADIRACHTA INDICA* SEED OIL EXTRACT IN THE CONTROL OF *AULACOPHORA* SPECIES (COLEOPTERA: CHRYSOMELIDAE) ON *CORCHORUS OLITORIUS* L.

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ABSTRACT

Cochorusolitorius is one of Africa's most common leafy vegetables with high nutritional and socio-economic value. Despite being frequently used, its varietal variety is unknown. Insect pests, notably *Aulacophora* species, continue to pose a threat to its domestication at various phases of development. As a result, a field trial was conducted to assess the control potential of Neem seed-oil extracts (NOE) for the management of cucurbit leaf beetles (CLB) on *cochorus*. Lambda-cyhalothrin at 2.5 ml/25m² and water as controls were used to bioassay three concentrations of neem-seed-oil extracts (NOE) against *Aulacophora* species: 0.25ppm, 0.5ppm, and 0.75ppm. The experiment was set up in a randomized complete block design, with five treatments replicated three times. The reduction in the adult insect population demonstrated the efficacy of the NOE treatments. The results showed that aqueous NOE and synthetic insecticide reduced the number of CLB significantly ($p < 0.05$) when compared to the control. The synthetic-insecticide-treated plots had the best CLB control, but it was not significantly different ($p < 0.05$) from the yield recorded in the plots sprayed with 0.75ppm aqueous NOE. Field trials revealed that both NOE and λ -cyhalothrin treatments significantly suppressed CLB infestations throughout the study period. However, the most effective NOE formulation was aqueous NOE at 0.75ppm, which caused a significant reduction in leaf damage and field infestation when compared to the untreated control, but was not significantly different from the effectiveness of λ -cyhalothrin. This implied that NOE at 0.75ppm was suitable as an environmentally friendly control measure.

Keywords: Treatments, efficacy, infestation, reduction, leaf, pests, insecticide, insect, beetles

INTRODUCTION

Cochorusolitorius is one of Africa's most common leafy vegetables with high nutritional and socio-economic value. Despite being frequently used, its varietal variety is unknown. Insect pests, notably *Aulacophora* species, continue to pose a threat to its domestication at various phases of development. This crop was used to meet people's food, nutritional, and health demands. It provides an additional source of income and work (FAO, 2012; Joosten et al., 2015; Feyemet al., 2016). Some of these vegetables are available in the form of traditional leafy vegetables (LFTs). They had a significant impact on consumer food, nutrition, and

health. In addition to proteins and energy, they provide minerals, vitamins, and certain hormonal precursors to the body (Antia et al., 2006; Adjatinet al., 2013; Onuminya et al., 2018). *Cochorusolitorius* is a traditional leafy vegetable that helps feed the world's population by meeting their nutritional and medicinal needs (Dansiet al., 2011; Odhavet al., 2007; Uusiku et al., 2010; Dada et al., 2021). Its mineral content is especially important for health benefits in countries where there is a high prevalence of anaemia caused by iron deficiency, as well as to counteract the effects of malaria and immune deficiency (Ndlovu and Afolayan, 2008; Ojiewo et al., 2013; Agoyi,

Olajubu, and Osuntokun, 2019). This vegetable's high carotenoids content would confer antioxidant properties beneficial to human health (Obohet al., 2009; Poljsaket al., 2021). *Corchorus olitorius* is commonly known as "Jew's mallow", "Tossa jute", "bush okra", *Corchorus* "ewedu", "West African sorrel" and "jute mallow" to mention a few. It is one of the most widely consumed traditional vegetables in Africa (Ngomuo et al., 2017a & b) and consumed in Asia (Kar et al., 2009; Mukul and Akter, 2021). There are cultivated and wild sources of *Corchorus olitorius*; it is rich in vitamins, mineral salts, and folic acid, constituting an important nutritional benefit (Zeghichi et al., 2003a & b; Guzzetti et al., 2021). The leaves were a component of 87 vegetable-based dishes that accompany starchy dishes. It may be prepared alone or in mixtures with legume leaves (East Africa) or other wild vegetables or in a mixture enriched with fish sauce. Thus, it could play an important role in food security and the fight against poverty in Africa (Attere, 1999; Arlette et al., 2017). Despite this socio-economic and nutritional importance, several constraints are linked to its development in the wild and cultivated. Previous studies have focused on the agro-morphological and genetic evaluation of accessions towards boosting a breeding program (Kiebre, 2016) on the agro-morphological evaluation of *C. olitorius* accession in Burkina Faso and Ivory Coast. Other studies on *C. olitorius* were on morphotype diversity (Akoroda, 1985; Ngomuo et al., 2017), seed production in Nigeria (Akoroda and Akintobi, 1983; Salacket al., 2015) and, domestication, ethnobotany, and production constraints in Ghana (Nyadanu et al., 2017). As a plant in the breeding process, there is little to learn from genetic variation, which means that existing studies may not be applicable throughout the natural range of this remedy. Similarly, most of these studies do not address the diversity and the potential impacts of insect pests that feed off this plant in the wild and cultivated environments. Since the plant is collected from the wild and consumed, economic thresholds are tough to determine. *C. olitorius* plants were susceptible to a wide range of insect pests. Insect and mite pests attack these plants at seedling, growth and fibre development stages (Selvaraj et al., 2016). Among these, *Aulacophora indica* and *Aulacophora Africana* (Coleoptera: Chrysomelidae) are the most

damaging insect pest after inspection on a neighbouring farm on the same agro-ecological zone. The adult beetles feed on the leaves and make numerous holes (Shot holes) that lead to yellowing, drying, and falling of the leaves. This caused a reduction in the photosynthetic activity of leaves as well as plant growth and grain yield. The use of chemical pesticides in most pest control programs is inevitable as they boost agricultural production, but their constant use causes harmful effects on human health (Hassan et al., 2007; Soomro et al., 2008; Nicolopoulou-Stamatiet al., 2016). Researchers worldwide have been exploring alternatives to chemical pesticides because of the many risks associated with their usage. Such risks include; environmental persistence and health hazards related to their toxicity (Scott et al. 2004, 2005; Lushchaket al. 2018), toxicity to non-target organisms, toxicity to humans and animals, and high cost of procurement (Adesina and Idoko, 2013). The development of resistance of leaf beetles to synthetic insecticides in crucifers has been identified (Fan and Huang, 1991; Kortbeek, van der Gragt and Bleeker, 2019). In response to the high costs of pesticides and their adverse effects, there is a need for a paradigm shift to the development of non-chemical technologies which may eliminate the use of insecticides and could have economic and health benefits to the applicators, consumers, and the environment (Murdock et al., 1997; Pretty and Bharucha, 2015). *Azadirachta indica* is a native plant in Nigeria and other parts of the world. It is called neem or dongoyaro, and it has traditionally been used as a pesticide and medicine and has been proven safe for humans (Mondal and Chakraborty, 2016). The neem tree (*Azadirachta indica* A. Juss) was known as a wonder tree for a very long time in the Indian subcontinent. It is now known worldwide mainly for its relevance in food production and medicinal properties (Mugnai, 2009; Iduet al., 2017). The Neem plant is the most diverse and versatile tree with maximum useful non-wood products (leaves, barks, flowers, fruits, seeds, gum, oil and neem cake) (Francine, Jeannette, and Pierre, 2015). Neem plant is popularly known as village plant medicine because it contains limonoids in its seeds, bark and leaves. The plant was confirmed to exhibit; antiseptic, antiviral, antipyretic, anti-inflammatory, antiulcer, antifungal (Nix, 2007;

Alzohairy, 2016), insecticidal and nematicidal properties. Most of these bio-pesticidal activities of neem oil were illustrated by Chaudhary et al., 2017 (Fig. 1) with modification.

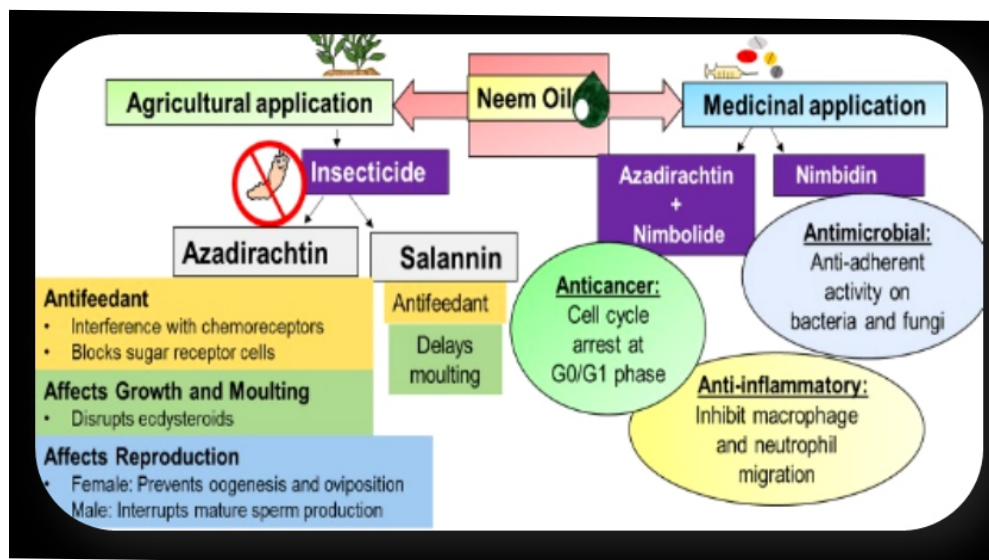


Figure 1: Neem oil's bio-pesticidal activity (Chaudhary et al., 2017)

Given the importance of cochorus, the economic damage caused by *Aulacophora* species, and the scarcity of research on its control, particularly in Nigeria, this study sought to investigate the efficacy of neem, *Azadirachta indica* seed oil extract for the management of *Aulacophora* spp. on *Cochorus solitorius* L.

MATERIALS AND PROCEDURES

Field experiments were conducted at the Federal University of Agriculture Abeokuta (FUNAAB) School of Agriculture Teaching and Research Farm in Abeokuta, Ogun State, Southwest Nigeria, which is located between Lat. 7°20'N and Long. 5°30'E and has a bi-modal precipitation model with a long rainy season, generally between March and July, and a short rainy season from September to the beginning of November, following a short drought in August

Site Preparation: Four weeks before planting, the land was cleared by mechanical tillage and harrowing, and the stones and stumps were removed. The prepared field was set up in experimental units with measuring tape, stakes, and twine.

The prepared land was laid out into experimental plots and blocks with measuring tape, stakes, and string. The experimental design was Randomized Complete Block Design (RCBD) with five treatments and three replications. A total land area of 351m² measuring 27m x 13m was used in the study. The land was partitioned into four blocks, and each block was subdivided into fifteen plots measuring 3 m x 3 m each.

Planting Operation: The planting operation was completed by June 2021, and the seeds were obtained from a good seed and agrochemical store in Abeokuta. The seed dormancy was broken by tying the seeds in a piece of cotton fabric and immersing them in nearly boiling water for five seconds before sowing. The seeds were then combined with sand in a ratio of 1:10 to ensure even distribution throughout sowing.

Lambda-cyhalothrin (-cyano-3-phenoxy -benzyl-3-(2-chloro-3, 3, 3-trifluoro-1-propenyl)-2, 2-dimethylcyclopropanecarboxylate) is a synthetic pyrethroid that is 99.8 per cent active and was obtained from a reputable agrochemical store in Abeokuta, Ogun State, Nigeria.

The Neem Plant: The neem plant,



Figure 2: A Neem Tree

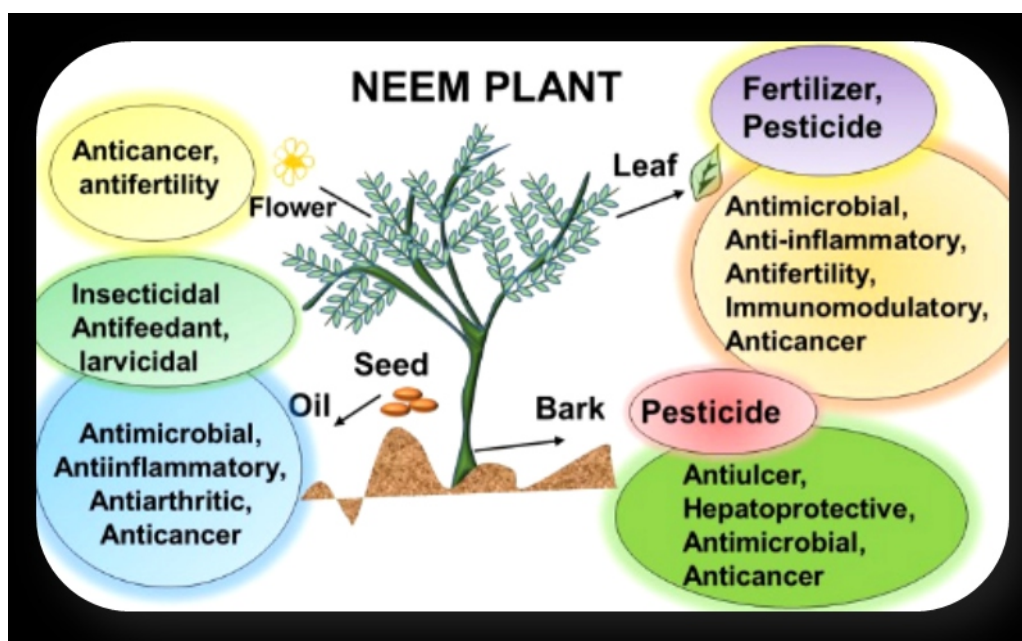


Figure 3: The neem tree's potential as a bio-pesticidal and medicinal agent
(Chaudhary *et al.*, 2017)

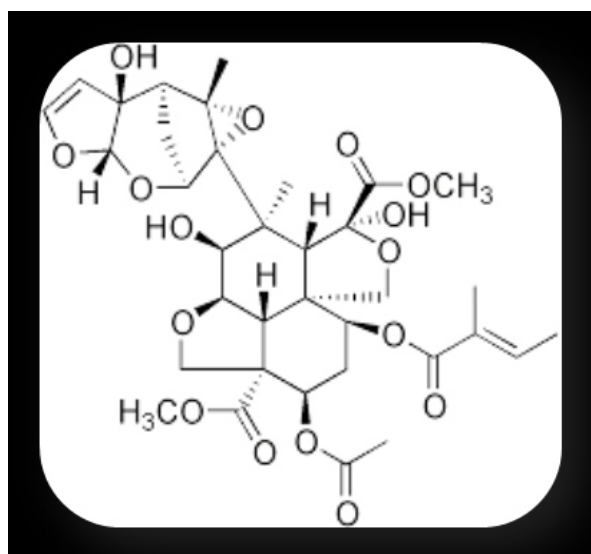


Figure 4 depicts the chemical structure of azadirachtin-A tetranortriterpinoid (Atawodi and Atawodi, 2009)

Lambdacyhalothrin (-cyhalothrin): The active ingredients in cyhalothrin are gamma-cyhalothrin and lambda-cyhalothrin. Lambda-cyhalothrin (-cyano-3-phenoxy -benzyl-3-(2-chloro-3, 3, 3-trifluoro-1-propenyl) -2, 2-dimethylcyclopropanecarboxylate) is a manufactured pyrethroid insecticide. Pyrethroids initially stimulate nerve cells to produce repetitive discharges and eventually cause paralysis in the

insect. Such effects are caused by their action on the sodium channel, a tiny hole through which sodium ions are permitted to enter the axon to cause excitation. These effects are produced in the insect nerve cord, which contains ganglia and synapses, as well as in giant nerve fibre axons. The stimulating effect of pyrethroids is much more pronounced than that of DDT.

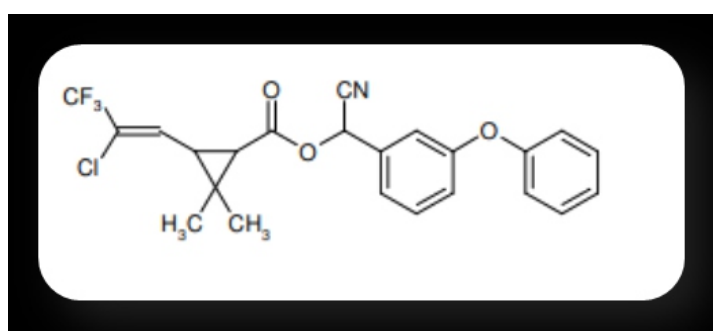


Figure 5 depicts the chemical structure of lambda-cyhalothrin (Zhenzhong et al. 2015)

Data collection: Data on the population of *Aulacophora* spp. and the damage caused to *Corchorus* leaves were determined by observing visual symptoms from 21DAS, and then weekly

until maturity. The severity of pest damage by *Aulacophora* spp. was assessed using a modified Peterson's scale for damage assessment, as used by other workers kirsch (1986) Table 1.

Table 1: Pest damage to celosia caused by *Aulacophora* spp.

Score	% Damage	Description
0	0	No visible damage
1	25	Almost 1/4 of the total leaf area was damaged
2	50	Almost 1/2 of the total leaf area was damaged
3	75	Almost 3/4 of the total leaf area was damaged
4	95	A small number of leaves green, stem green
5	100	Entire leaves and stem were eaten

Data Analysis: Data was collected and transformed using the square root transformation method to ensure homogeneity of variance and normal distribution of the data before being subjected to analysis of variance (ANOVA) using Gen Stat Release version 12.1.

RESULTS

The effect of neem-seed oil at various concentrations on several leaves of *Corchorus*

olitorius

The effect of neem-seed oil on *Corchorus olitorius* shows that the least number of leaves on the field at 4WAP was recorded on the plot with no treatment (control: 6.917), while the plot treated with neem-oil at 0.75 ppm had the highest (7.833) number of leaves (Table 3). The least number of leaves on the field at 5WAP was recorded on the (control) plot, while *Corchorus olitorius* treated with

Table 2 shows the effect of neem -seed oil application on the number of leaves of *Corchorus olitorius*.

Treatments	4 WAP	5 WAP	6 WAP
2.5 ppm	7.417 ^b	9.920 ^{bc}	12.670 ^b
5.0 ppm	7.417 ^b	11.080 ^{ab}	13.080 ^a
7.5 ppm	7.833 ^a	12.420 ^a	14.330 ^a
2.4 ppm (L: positive control)	7.667 ^{ab}	12.170 ^a	14.250 ^a
Control	6.917 ^c	9.250 ^c	11.170 ^b

a–b: Means in the same column with different superscripts are significantly different ($p > 0.05$) using the least significant difference (LSD) as mean separation. L = λ -cyhalothrin; Control = Water. The effect of neem-seed oil application on *Corchorus olitorius* plant height: The value of plant height

does not differ significantly ($p > 0.05$) among the concentration of the treatment from 4WAP to 5WAP, but it differed significantly at 6WAP. The effect of neem-seed oil on *Corchorus olitorius* indicates that the least value of plant height in the field at 4WAP was recorded on the plot with no

Table 3 shows the effect of neem-seed oil application on the plant height of *Corchorus olitorius*.

Treatments	4WAP	5WAP	6WAP (cm)
2.5 ppm	9.657 ^a	11.740 ^a	13.260 ^{ab}
5.0 ppm	10.147 ^a	11.330 ^a	13.490 ^{ab}
7.5 ppm	10.153 ^a	12.000 ^a	14.280 ^a
2.4 ppm (L: +ve control)	10.430 ^a	12.110 ^a	14.040 ^{ab}
Control	9.527 ^a	11.620 ^a	12.860 ^b

a–b: Means in the same column with different superscripts are significantly different ($p>0.05$) using the least significant difference (LSD) as mean separation. L = -cyhalothrin; Control = Water

Effect of neem-seed oil application on *Corchorus olitorius* leaves damaged by

***Aulacophora* spp.**

At 6WAP, the plot treated with neem-seed oil at 7.5 ppm had the least amount of leaves damaged (12.860 cm), while the plot with no treatment (control) had the most amount of leaves damaged (14.280 cm).

Table 4: Effect of neem-seed oil application on *Corchorus olitorius* leaves damaged by *Aulacophora* spp.

Treatments	6WAP (%)
2.5 ppm	17.12 ^{ab}
5.0 ppm	16.42 ^{bc}
7.5 ppm	15.18 ^d
2.4 ppm (L: +ve control)	15.79 ^{cd}
Control	17.47 ^a

a–b: Means in the same column with different superscripts are significantly different ($p>0.05$) using the least significant difference (LSD) as mean separation. L = λ -cyhalothrin; Control = Water

The effect of the neem-seed-oil application on the abundance of *Aulacophora* spp. and other *Corchorus olitorius* pests

At 6WAP, the plot treated with neem-seed oil at 0.75 ppm had the lowest value (12.830 per cent, 0.667 per cent, and 0.000 per cent) for the abundance of *Aulacophora* spp. and other pests of *Corchorus olitorius*, while the plot with no treatment (control) had the highest value (14.280 per cent, 4.667 per cent, and 2.000 per cent).

Table 5: The effect of neem-seed oil application on the abundance of *Aulacophora* spp. and other *Corchorus olitorius* pests.

Treatments	6 WAP % of <i>Aulacophora</i> spp.	6 WAP % of <i>Lixusspp.</i>	6 WAP % of <i>Podagricaspp.</i>
2.5 ppm	14.040 ^{ab}	3.000 ^{ab}	2.000 ^a
5.0 ppm	13.490 ^{ab}	2.000 ^b	1.667 ^a
7.5 ppm	12.830 ^b	0.667 ^b	0.000 ^b
2.4 ppm (-ve ctrl)	13.260 ^{ab}	1.000 ^b	1.333 ^a
Control	14.280 ^a	4.667 ^a	2.000 ^a

a–b: Means in the same column with different superscripts are substantially different ($p>0.05$) using the least significant difference (LSD) as mean separation. L = λ -cyhalothrin; Control = Water

The effect of neem-seed oil application on the

yield of *Corchorus olitorius*

At 6WAP, the plot treated with neem-seed oil at 7.5 ppm had the highest yield (17.830 g/plot), while the plot with no treatment (control) had the lowest yield (13.330 g/plot).

Table 6 shows the effect of neem-seed oil application on the yield of *Corchorus olitorius*.

Level of conc.	6 WAP (g/plot)
2.5 ppm	14.920 ^c
5.0 ppm	16.250 ^b
7.5 ppm	17.830 ^a
2.4 ppm (L: positive control)	17.670 ^a
Control	13.330 ^d

a–b: Means in the same column with different superscripts are substantially different ($p>0.05$) using the least significant difference (LSD) as mean separation. L = λ -cyhalothrin; Control = Water

DISCUSSION

ambda-cyhalothrin at 2.5 ml/25m² is commonly used to control insect pests of *Corchorus*

olitorius; however, indiscriminate use may result in direct health risk to the applicator; depletion of soil vitamins; accumulation of residues in flora; unfit water for consumption; acute and persistent toxicity; eradication of natural enemies, environmental degradation, and; bioaccumulation and biomagnification of the poisonous substance within the food-chain (Ecobichon, 2001 and Carmoet *al.*, 2010).

Conversely, neem-seed oil is used as an alternative pest control strategy to this synthetic insecticide (Riffat *et al.*, 2012 and Usharani *et al.*, 2019). The principal energetic aspect in *A. Indica* confirmed to have insecticide properties had been diagnosed as azadirachtin-A, a tetranortritarpinoid of neem seed-kernel. A number of plants are known to have biocidal activity against insect pests but are friendly to natural enemies and the environment. These pesticide plant extracts increase yield and suppress insect pest infestation on *Corchorus olitorius* without harming natural enemies.

However, there was a significant difference ($P > 0.05$) between the other neem seed oil rates. Neem seed oil at 0.25ppm and 0.5ppm were significantly different ($P > 0.05$), indicating that NOE at 0.25ppm had a relatively lower effect on the population of *Aulacophora* species in this study. However, treatment with neem seed oil at 0.25ppm for suppressing the insect population might induce resistance and subsequent pest resurgence.

Moreover, the lowest population of *Aulacophora* species and other insects in the *Corchorus olitorius* treated with λ -cyhalothrin when compared to the control (water) but was not significantly different ($P > 0.05$) from neem seed oil at 0.75ppm. This suggests that neem oil is more active at a higher dose (0.75ppm) and compared favourably with the standard dosage of lambda-cyhalothrin. This implied the suitability of neem seed oil at 7.5ppm as an eco-friendly control measure (Welch and Harwood, 2014; Roubos *et al.*, 2014).

However, with visual observations during the preliminary survey, the NOE formulations had far less influence on natural enemies. Although, the population of natural enemies were reduced by the NOE when compared to untreated controls, however, the reductions were not as dramatic as those seen with lambda-cyhalothrin. These deductions were arrived at based on mummified larvae with postmortem indications of parasitoid infestation.

At this stage, the pest population was below the economic threshold level, so NOE control in conjunction with natural pest regulation was justified (Arditi and Ginzburg, 1989; Ruschet *et al.*, 2010). NOE treatments provide continuous knock-down, allowing beneficial species to contribute to pest management on a large scale (Crowder *et al.*, 2010).

The effect of neem-seed oil extract (NSE) on *Corchorus olitorius* revealed a significant difference ($P > 0.05$) in the number of leaves and plant height at 4WAP, with the plot treated with NSE at 0.75 ppm having the highest number of leaves and plant height, which followed a similar trend until harvesting.

There were significant differences ($P > 0.05$) in the severity of leaf damage caused by *Aulacophora* species in this study. Leaf damage assessment of *Corchorus olitorius* in each treatment agreed with the result of the modified Peterson's scale for damage assessment used in this study. The highest severity of leaf damage was observed in the leaves of *Corchorus olitorius* treated with negative control (water), followed by a *Corchorus olitorius* treated with 0.25ppm neem.

Furthermore, *Corchorus olitorius* treated with λ -cyhalothrin and 0.75ppm neem seed oil had the lowest leaf damage, which was not significantly different ($P > 0.05$) from λ -cyhalothrin. This suggests that the 0.75ppm neem seed oil treatment is more effective than the lower doses in protecting the *Corchorus olitorius* against damage by *Aulacophora* species.

Furthermore, broader leaves and healthier plant growth were observed on all *Corchorus olitorius* treated with neem seed oil extracts, implying that the neem seed oil provides additional benefits as it decays quickly and adds nutrients to the soil. It could also be deduced that neem seed oil served as nematicides, reducing the nematode population in the soil to enhance the healthy growth of *Corchorus olitorius*.

This study reveals that neem seed oil is biocidal (0.25ppm, 0.5ppm, and 0.75ppm) against insect pests, but its misuse may become toxic to natural enemies, the environment, and humans at a comparatively higher dose. The neem seed oil has been reported to show acute toxicity in rats and rabbits with LD50 of 14 mg/kg and 24 mg/kg, respectively, possibly targeting organs for toxic effects, especially the central nervous system and the lungs

The most effective NOE formulation was aqueous NOE at 0.75ppm, which caused a significant reduction in leaf damage and field infestation compared to the untreated control, but was not significantly different from λ -cyhalothrin effectiveness, implying the suitability of NOE at 0.75ppm as an eco-friendly control measure.

The use of neem oil is an alternative strategy to chemical insecticides in terms of environmental hazards, cost rates, and natural enemies (Riffat *et al.*, 2012). To date, many plant extracts have been utilized for pest control. Among these, neem oil has attracted special attention to entomologists worldwide because neem is assorted with some biologically active ingredients, of which azadirachtin is one of the best known (Riffat *et al.*, 2012).

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CONCLUSION

The results of this study show that using neem oil extracts to control *Aulacophora* spp. can be as effective as λ -cyhalothrin in terms of improved *Corchorus olitorius* yields as well as a reduction in tritrophic effects, thereby conserving non-target arthropods that provide important ecosystem services such as pollination and natural enemies acting as pest regulators.

RECOMMENDATIONS

More research is needed to determine the toxicity profile (bio-safety) of neem seed oil extracts as a biocide against *Aulacophora* spp. on *Corchorus olitorius*, as well as the application of the neem extracts for soil amendment and nematicides. In addition, the effect of neem seed oil on natural enemies should be investigated further as there is no empirical evidence to justify this assertion in the present study.

Declaration

The authors declared that there is no conflict of interest

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of vegetable Gbolo [*Crassocephalum rubens* (Juss. ex Jacq.), *S. moore* and *C. crepidioides* (Benth.)

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