International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 03-02-2024, Accepted: 27-02-2024, Published: 20-03-2024
Volume 9, Issue 3, 2024, Page No. 79-86

Ovicidal and toxicological potential of neem and *Harad* Extracts against *Megacopta cribraria* (F.) (Hemiptera: Heteroptera: Plataspidae)

Anjana Singha Naorem[1*], Rubina Azmeera Begum[1], Santana Saikia[2], Anandita Buragohain[3]
[1] Department of Zoology, Cotton University, Guwahati, Assam, India
[2] Department of Zoology, Handique Girls' College, Guwahati, Assam, India
[3] Department of Zoology, Pandu College, Guwahati, Assam, India

Abstract
Megacopta cribraria is a pest of major concern in soybean-producing nations and to curb its population build-up, use of cultural practices, resistant varieties, biological control agents and synthetic insecticides are generally practiced. The present study was undertaken to assess the ovicidal and toxicological potential of NeemOz Gold formulations and crude *harad* extract against *M. cribraria*. Treatment on the 1-day old egg batches with Neem formulation showed no hatching in all the test concentrations whereas *harad* extract also inhibited egg hatching but only at the highest two concentrations of 7.5% and 10%. Treatment on the 3-day old egg batches showed dose-dependent inhibition of egg hatching in both treatments. Neem formulation was much more effective in its ovicidal potency as compared to *harad* extract and the potency of the extract was also dependent on the age of the treated egg. The toxicity assay with neem at the sub-lethal doses resulted in the development of several body deformities along with the arrestment of moulting in some nymphs. *Harad* extract did not result in any body deformities but was significantly toxic at higher concentrations. A positive dose-dependent mortality was observed in both treatments. LC_{50} of neem and *harad* was 0.088% and 5.45% respectively, indicating higher toxicity of neem as compared to *harad.* The present findings shows the potential of these extracts as a valuable environment friendly tool for managing *M. cribraria* infestations in the crop fields.

Keywords: Neem, *harad*, ovicidal, toxicity, mortality, body deformities, intermoult, longevity, integrated pest management

Introduction

Megacopta cribraria (F.) commonly called as kudzu bug or lablab bug, is primarily a pest of legume crops like soybean, kudzu, pigeon pea, lima bean, pinto bean, white clover, red clover, alfalfa, perennial peanut but its association with other non-leguminous crops are also reported (Srinivasperumal *et al*. 1992 [37], Wang *et al*. 1996, Thippeswamy and Rajagopal 1998 [44], Sujithra *et al*. 2008 [41], Thejaswi *et al*. 2008 [43], Xing *et al* 2008 [49], Zhang *et al* 2008 [50], Lahiri and Reisig 2016) [20, 21]. The native distribution of the lablab bug includes Australia, China, India, Indonesia, Japan, Korea, Malaysia, Myanmar, New Caledonia, Pakistan, Sri Lanka, Taiwan, Thailand and Vietnam (Eger *et al*. 2010) [8]. In Asia, *M. cribraria* tends to be an occasional pest of legumes but can be serious at times depending on the infestation density of the bug as well as the stage at which the infestation occurs (Thippeswamy and Rajagopal 1998) [44]. The soybean yield losses of up to 50% with 80 to 100 adults per plant has been reported in the Asian region whereas in the southeastern United States yield losses has been estimated to be 59.6% (Seiter *et al*. 2013) [33]. Synthetic insecticides like pyrethroids, organophosphates, neonicotinoids are reported to be used to control this bug (Wang *et al*. 2004 [48], Miao *et al*. 2016) [21]. Though in the United States management is being carried out with synthetic insecticides, by manipulating cultivation practices, working on resistant cultivars, assessing the spatial and temporal distribution of this pest, encouraging the use of biological control (Lahiri and Reisig 2016) [20, 21] but not much has been done on its management aspect in its native region, particularly due to its minor or occasional pest status (Seiter *et al*. 2013) [33]. This bug is not just an agricultural crop pest but has been reported as a nuisance in urban landscape (Suiter *et al*. 2023) [40] releasing a foul odor when disturbed or crushed like other stink bugs and can also stain skin (Ruberson *et al*. 2013) [31]. Therefore, there is a need to find out eco-friendly ways of its population control so that it can be easily used in agricultural farms as well as in human dwellings without much impact on non-target organisms and human health.

In the present study, two plants, *Terminalia chebula* Retz. (commonly known as *harad* in India) and *Azaditachta indica* A. Juss (commonly known as neem) have been chosen to assess their efficacy for the control of *M. cribraria.* These plants have been used since time immemorial for several therapeutic, pharmaceutical and insecticidal properties (Isman and Ketkar 1990 [18], Schmutterer 1990 [32], Mordue (Luntz) and Blackwell 1993 [23], Kumar 2006 [19], Singha *et al*. 2007 [36], Raju *et al*. 2009 [28], Gupta 2012 [11], Senthilnathan *et al*. 2017, Veni *et al*. 2017 [47], Das *et al*. 2020 [6], Kim *et al*. 2022) [16]. But none of these plant extracts or their formulations have been tested against *M. cribraria* so far. So, this study aimed at assessing the toxic effect (both lethal and sub-lethal effect) of crude extracts of *harad* and commercially available neem formulations on *M. cribraria* and also to check their ovicidal potential.

Materials and methods

Insect Collection and Rearing

M. cribraria adults were collected from the different host plants *Cajanus cajan, Phaseolus vulgaris* and *Lablab purpureus* from Horticulture Research Station, Guwahati (Assam). Collected adult bugs were brought to the laboratory and the culture was maintained in a plexiglass cage and the bugs were fed the French bean, *P. vulgaris* and the culture condition was maintained at 27 ± 2ºC, 60 ± 5% R.H. and a photoperiod of 14:10 (L:D) h.

Test extracts preparation

NeemOz Gold formulation: Utkarsh Neemoz-Gold formulation, is a neem oil, containing 1% (10000 ppm) azadirachtin and it was obtained from Amazon e-commerce platform. Test concentrations of 0.75%, 0.5%, 0.25%, 0.125%, 0.06% and 0.03% azadirachtin were made by serial dilution of 1% NeemOz Gold (NOG) with the control solution. Control solution was made with distilled water containing 0.5% Triton X-100.

Harad hexane extract (HHE) preparation: Harad powder was also obtained from Amazon e-commerce platform. 20g of *harad* powder was mixed with 200ml hexane in a conical flask and the mixture was stirred thoroughly in a magnetic stirrer for an hour and then kept covered with aluminium foil and left it overnight. The next day, mixture was stirred again and then filtered through Whatman filter paper no. 1. The filtrate so obtained was concentrated in the rotary evaporator at 40ºC at reduced pressure until a viscous fluid was obtained (Singha *et al*., 2007) [36]. This extract was kept for 30 minutes in room temperature to allow any remaining hexane to evaporate. Control solution was made with distilled water containing 0.5% Triton X-100. A stock solution of 20% (vol: vol) was prepared from the extract obtained by mixing with the control solution. From the stock solution, the test concentrations of 12.5%, 10%, 7.5%, 5% and 2.5% were made by serial dilution with the control.

Ovicidal assay

1day old and 3day old egg batches were taken for the ovicidal test and each egg batch was randomly assigned to the treatment. For each of the toxicity test, 8 egg batches were treated by dipping the egg batches in the test extracts for 1sec and then air dried them by keeping them on a filter paper for 10 min. Post-treatment, egg masses were transferred individually to small plastic containers (9.5 cm diameter X 4 cm height) lined with tissue paper and a moist cotton swab was provided in a small plastic lid inside the container for humidity. Percent egg hatching was noted.

Set up for toxicity assay

2day old fourth instar nymphs were topically applied with 1µl of the test solution or control on their dorsum with the help of Hamilton's syringe (10 µl). After the application of the test extracts, the nymphs were allowed to air dry and then transferred to their container (9.5 cm diameter X 4 cm height) lined with tissue paper and the food was provided in the container ensuring that bugs didn't die of hunger. For every concentration of the two test extracts –NOG and HHE, and for the control 3 replicates were made containing 10 nymphs in each. Daily observations on body deformities, nymphal duration and mortality were noted.

Statistical Analysis

Data were subjected to One Way- Analysis of Variance (ANOVA) to check for differences among the various treatments using SPSS 27.0 and means were separated using Tukey's post-hoc test ($P < 0.05$). The mortality data was corrected using Abbott's formula (Abbott 1925) [1] and data on nymphal mortality and for the ovicidal action were subjected to Probit analysis (Finney 1971) [9] to calculate the median lethal concentrations (LC_{50}) and median effective concentration (EC_{50}) respectively. 95% upper confidence limit (UCL) and lower confidence limit (LCL) and Chi square values were calculated using the same package.

Results

Ovicidal Assay

The treatment of the 1-day-old egg batches with NOG test extracts resulted in 100% mortality of eggs in all the concentrations except at 0.03% and 0.06% concentration where hatching did initiate but could not be completed and the nymphs were seen arrested while hatching in some of the egg batches. A total of only 12 such eggs were seen arrested (7 eggs in 0.03% treatment and 5 eggs in 0.06% concentration) out of the total 509 eggs that were treated. So, no statistical analysis was done on this data.

The treatment of 3-day-old egg batches with NOG extracts, resulted in a significant ($P<0.01$) reduction in egg hatching with the increasing concentration and no hatching of eggs was observed at the highest test concentration of 0.5% concentration (5000 ppm) (Table 1). Decline in egg hatchability was gradual from 0.03% to 0.125% concentration but a sharp decline in egg hatchability was observed at 0.25% concentration. EC_{50} of the ovicidal activity of NOG was 0.34% (Table 2). Since the significance level was greater than 0.05 ($P=0.340$), no heterogeneity factor was used in the calculation of confidence intervals.

In the case of HHE treatment, on 1-d old egg batches, almost no hatching was observed in the higher two concentrations of 7.5% and 10% except that few eggs hatched from some of the egg batches but they died during hatching. At 5% concentration also, out of 8 egg batches, hatching was observed in 4 egg batches with an average egg hatchability of ~29% only, so no statistics were performed on this set of data. *Harad* treatment was not very effective in ovicidal property for the treatment of 3-day old eggs as compared to NOG treatment so only three test concentrations were chosen keeping 10% concentration as the highest concentration. A significant difference in the egg hatchability was seen amongst the different treatments showing 100% mortality at 10% concentration (Table 1). Only three concentrations were used for the ovicidal tests so no EC_{50} was calculated for HHE.

Table 1: Ovicidal effect of the extracts on 3d old egg batch

Extract	Concentration (%)	Percent hatchability Mean ± SE
NOG	Control	$100+0^{a}$
	0.03	87 ± 2.14^{b}
	0.06	73.64 ± 0.51^{c}
	0.125	64.11 ± 2.44^{d}
	0.25	31.51 ± 0.47^{e}
	0.5	0
HHE	Control	100 ± 0^{a}
	5	91.28 ± 0.71^{b}
	7.5	49.36 ± 1.16^{c}
	10	0

Note: Means followed by different superscripts in a column are significantly ($P<0.01$) different from each other (Tukey HSD) for a particular extract.

Table 2: Ovicidal effect of the NOG extract on the eggs of *M. cribraria*

Extract	Slope ± SE	EC_{50} (95%CI)	χ^2 (*P*)
NOG	1.313 ± 0.178	0.340 (0250-0.551)	2.160 (0.340)

Toxicity assay

Topical application on the 4th instar nymphs of *M. cribraria* with the test concentrations of NOG and HHE resulted in mortality at both the fourth instar and the fifth instar and in the intermoult stages. Mortality was seen 3-day post treatment. Dose-dependent mortality was observed in the treatments. A significant (P<0.05) increase in mortality was observed with the increasing concentrations in both the NOG and HHE treatments (Table 3). However, HHE treatment resulted in the maximum mortality of 90% at the highest test concentration of 12.5% and no bodily deformities were observed in this treatment.

In the case of the lowest test concentration of 0.03% of NOG treatment only ~7% mortality was observed in the fourth instar and the rest of the nymphs survived to adulthood but two adults were deformed with poorly developed hind wings which could not be folded properly to rest under the forewings. The forewings were more transparent in texture and coloration as compared to the wings of the normal bugs (Fig. 1). Rest of the adults died within 11 days of moulting. However, from 0.06% concentration onwards, the mortality was observed in both the fourth and the fifth instars and some died during the 4-5th intermoult stage and a very few at 5th-adult intermoult stage, though the highest mortality was observed in fourth instars. At the highest two concentrations of 0.5% and 0.75%, none of the nymphs could reach upto the adult stage resulting in 100% mortality till 5th instar.

The sub-lethal concentration of the NOG extracts resulted in body deformities of various degrees in the surviving bugs but these deformities were not manifested immediately in the 4th nymphal instar (the stage of treatment) but post-moulting the deformities were evident in 5th instar and in the adults. The nymphs that died during the fourth nymphal instar developed dark colouration and body became black and delicate to touch (Fig. 2). The surviving nymphs/adults showed deformities in all the body parts and the deformities were of varying degrees in different bugs (Fig. 3) which ultimately resulted in the early death of the insect. Some of the major deformities observed were- drooping and dragging antennal segments, separated stylets of the mouthparts to varying degree, thoracic region either incompletely formed or lifted up from margins, wing pads was ill-formed in the nymphs, wings deformed in the case of adults, legs weak, abdomen shrunken, poorly developed genitalia, etc.

Many of the fifth instar nymph at 0.25% and 0.5% showed deformed and separated stylets and only 2 such cases were noted in 1.25% concentration. NOG treatment resulted in reduced longevity of the surviving adults with a maximum survival period of 14 days.

Fig 1: Deformed adult (hindwings not folded and forewings translucent and not pigmented)

Fig 2: Dark coloured 4th instar nymph

Table 3: Mortality at different stages post NeemOz Gold (NOG) and *Harad* hexane extract (HHE)Treatment on *M. cribraria*

Concentration (%)		Percent mortality (Mean)*		
		N4	N5	Total Mortality (± SE)
NOG	Control	0	0	0
	0.03	6.67	0	6.67 ± 3.33^{a}
	0.06	20	20	40 ± 5.77^{b}
	0.125	43.33	23.33	66.67 ± 3.33^{c}
	0.25	70	13.33	83.33 ± 6.67^{cd}
	0.5	76.67	23.33	100 ± 0^{d}
	0.75	96.67	3.33	100 ± 0^{d}
HHE	Control	0	0	0
	2.5	13.33	0	13.33 ± 3.33^{a}
	3.5	13.33	3.33	16.67 ± 3.33^{a}
	5	33.33	13.33	46.67 ± 3.33^{b}
	7.5	36.67	36.67	73.33 ± 3.33^{c}
	10	43.33	43.33	86.67 ± 3.33^{cd}
	12.5	83.33	6.67	90 ± 0^{d}

Note

- *Mean of three replicates and each replicate consisted of 10 insects
- Percentage mortality is calculated based on the initial number of insects taken
- For a particular extract, mean values followed by different superscripts are significantly different ($P<0.05$) down the column (Tukey HSD)

The LC_{50} value of the total nymphal mortality with NOG and HHE extract was 0.088% and 5.455%, respectively (Table 5). Since the significance level of NOG extract treatment ($P=0.034$) was lesser than the critical value $P=0.05$, a heterogeneity factor was used in the calculation of confidence intervals but no such heterogeneity factor was used in the calculation of confidence intervals for HHE extract treatment as the significance level was greater than 0.05 ($P=0.387$).

Table 4: LC_{50} and other associated parameters of the NOG and HHE extract

Extract	Slope ± SE	LC_{50} (95%CI)	χ^2 (*P*)
NOG	2.773 ± 0.196	0.088 (0.064-0.114)	10.405 (0.034)
HHE	3.893 ± 0.273	5.455 (5.079-5.846)	8.119 (0.087)

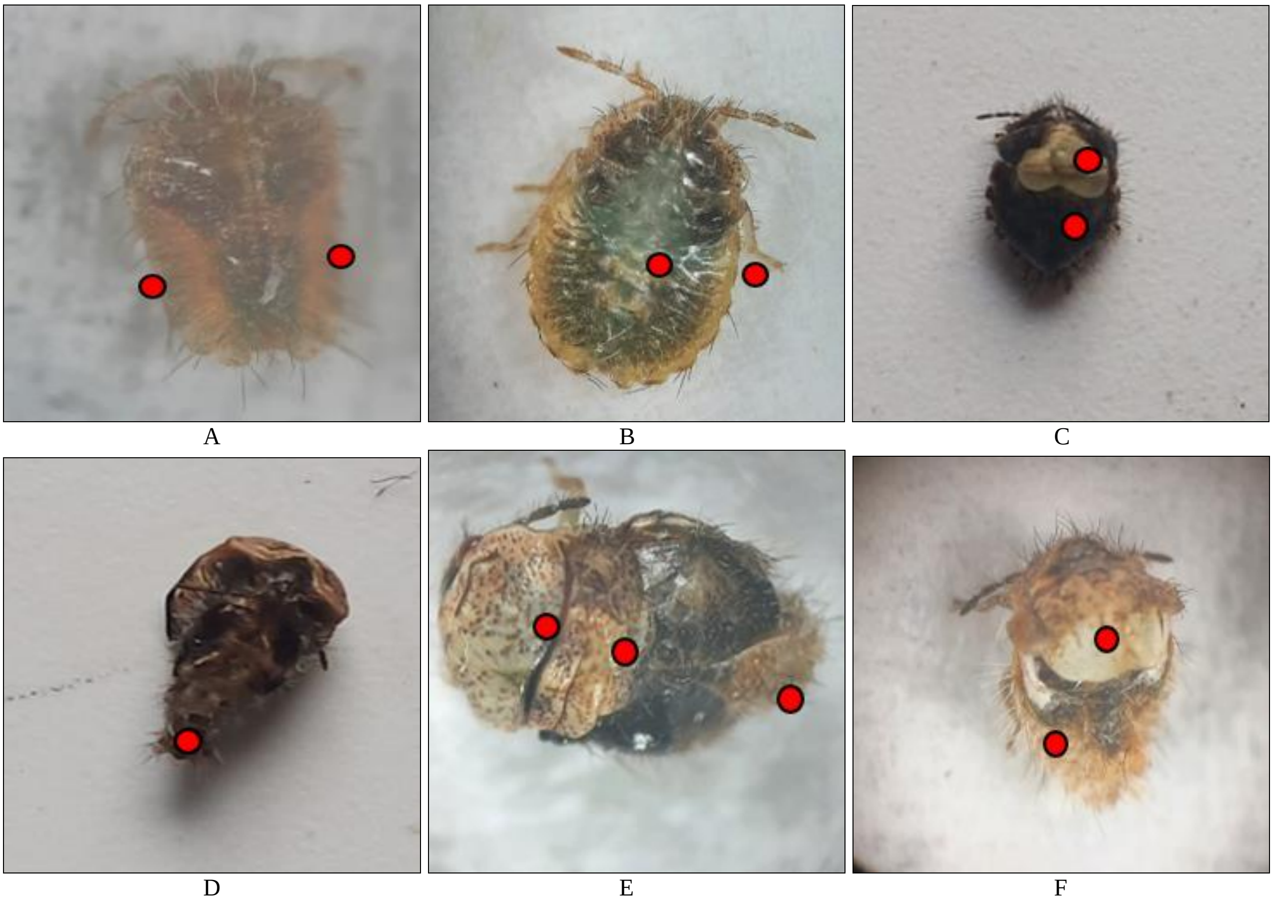

Fig 3: (A-F) Deformed stages of the insect post NOG application

A: Laterally compressed fifth instar nymph (N5); B: Incomplete wing pad and thoracic region deformed (N5); C: Thoracic region ruptured, bloated up and body pigmented; D: N5-adult intermoult; E: Body malformed, thoracic region incompletely developed, abdominal region bloated (adult); F: Malformed adult, forewings lifted up and hindwings ill-formed. All the regions in the insect body are marked in red circle.

Discussion

With the increasing societal concerns of the impact of pesticides on human health and environment (United Nations Environment Programme, 2022) there is a growing interest in searching and adopting alternative pest management strategies that can align well with the sustainable agricultural practices (Guleria and Tiku, 2009 [10], Ngegba *et al*., 2022) [26]. Plant derived extracts and botanical pesticides serve as one of the better alternative to synthetic insecticides owing to their multi-prong benefits to the farmers without compromising the health of the ecosystem (Mitchell *et al*., 2004 [22]; Kilani-Morakchi *et al*., 2021) [15]. Using these extracts for targeting insect eggs as a part of pest control program offers several advantages. Firstly, eggs are immotile and are often more vulnerable to pesticide spray than the other stages of the insect as they will not show any escape behaviour making them easier to target without the need for frequent reapplications. Secondly, targeting the egg stage can ultimately have a negative cascading impact on pest population buildup and hence reduction in the pest population. So the eggs were the prime target in the present study to see the efficacy of the selected plant extract/formulation on them. It was observed that the ovicidal potency of both the extracts, NOG and HHE, varied with the age of the eggs in the present study showing that younger eggs were more vulnerable to the toxic effect of the extract than the older eggs (Campbell *et al*., 2016) [5]. This could be due to hardening of the eggshell with the passage of time thereby resulting in lesser penetration of the chemicals and in the late stage egg the embryo may produce enzymes that break down insecticides. (Su and Mulla, 1998 [39], Jacobs *et al*., 2013) [13]. Studies on *Dysdercusa koenigii* using four different plant extracts *viz.* thionemone (from neem), heartwood extract (from neem), *Swietenia* macrophylla King seed extract and *Calophyllum inophyllum* seed extract showed the ovicidal action on the freshly laid 1 day old eggs than the advanced stage of the egg (5d old) where egg hatching was not affected (Agrawal, 1993) [3]. In the present study also, almost no egg hatching was observed post-neem application on 1d-old eggs in all the concentrations except at the lowest two concentrations where, hatching did initiate but could not be completed due to the toxic effect of the neem. This is in contrast to some of other findings where the azadirachtin from neem did not have any effect on the egg hatching in *D. koenigii* and *Oncopeltus fasciatus* (Koul, 1984 [17], Dorn, 1986) [7] and this could be due to the difference in the type of the solvent chosen for the treatment as well as different architectural texture and pattern of eggs of these insects which probably made these eggs immune or resistant to the phytochemical. However, neem and its extracts have been reported to be effective for their ovicidal action on the eggs of many other insects (Su and Mulla [39], 1998; Hassan, 1999 [12]; Kavitha *et al*., 2008) [14]. In any case, the efficacy of neem formulation on *M. cribraria* eggs in laboratory condition was established for the first time in the present study. The structure and architecture of insect egg chorion varies according to the insects which will result in a variable level of permeability of the pesticides affecting the egg hatchability and embryo development (Campbell *et al*., 2016) [5]. Therefore, despite neem's established efficacy in inducing ovicidal effects in numerous insect species, it becomes imperative to assess and validate its potency against *M. cribraria* as well and hence the relevance of the present study.

Harad extract has not been experimented much for its insecticidal and ovicidal effect. A single report of *harad* leaf extract’s ovicidal effect exist on mosquitoes- *A. stephensi*, *A. aegypti,* and *C. quinquefasciatus* (Veni *et al*., 2017) [47] where the egg hatchability was dependent on the type of extract and the type of insects. The methanol solvent was found to be most effective in reducing egg hatching as compared to benzene, hexane, ethyl acetate, and chloroform, resulting in 100% egg mortality of *A. stephensi, A. aegypti and C. quinquefasciatus* at 200 ppm, 250 ppm and 300 ppm of the extract, respectively. In the present study, *harad* hexane extract though exhibited egg mortality but the effect was moderate and only at a very high concentration of 10%, all the eggs died when the treatment was done at 3-day old stage. When eggs are exposed to higher concentration, more of the extract will enter the eggshell affecting the embryogenesis (Broadbent and Pree, 1984) [4] so at the highest concentration only 100% egg hatching inhibition was observed in the present study. However, in the freshly, laid eggs, 100% egg mortality was achieved at 7.5% concentration and even at the lowest concentration of 5% only 29% egg hatching took place, indicating that freshly laid eggs could be targeted to achieve a better ovicidal action with *harad* extract. This clearly shows that the effectiveness of a plant extract varies depending on the kind of the solvent and insect chosen as *harad* extract showed a good potential in ovicidal action against the three mosquito species but in the present study, the potency was poor in aged eggs and a moderate effect was seen when the treatment was done on the freshly laid eggs.

There are several reports on the toxicity studies of neem on large number of insect pests (Mordue and Nisbet, 2000 [25]; Singha *et al*., 2007) [36]. However, studies pertaining to phytochemical effect on heteropteran pests are severely sparse when compared to many other insect groups, and *M. cribraria* is one such insect where neither the direct effect of neem nor *harad* extract has been studied so far. Therefore, studies relating to the efficiency of such important insecticidal plants, on its potential impact on this bug needs to be studied. However, a field application of three biopesticides- Azera, Neem, and Pyganic to see their efficacy in the reduction of *M. cribraria* pest density and egg hatching along with its effect on the egg parasitoid has been reported (Olson, *et al*., 2021) [27]. Both the neem and Azera were found to be significantly effective in reducing the egg hatching percentage as well as reducing the density of second instar nymph but Neem had a considerable negative impact on egg parasitism than Azera so neem could be used as a better alternative to a biopesticide for *M. cribraria* control.

In the present study neem formulation proved to be effective against this bug resulting in significant mortality in concentrations as low as 0.06% and 100% mortality in concentrations above 0.5%. Higher mortality observed in the fourth instar as compared to the subsequent stages was not immediate but observed after 3-days post treatment and it could be due to toxicity of the neem extract that penetrated through the insect cuticle and led to the death of the insect. It is also well established that azadirachtin, the active component in neem, is responsible to affect the sensory mechanism of the insects post topical applications that might affect its food searching ability and hence the feeding (Schmutterer, 1990) [32] and eventually may result in mortality in insects due to starvation. Moreover, neem is not known for its immediate knockdown effect but it takes more than two days to do so. Such observations have been reported earlier in *Nezara viridula*, *Oebalus poecilus* and *Clavigralla scutellaris* (Sutherland *et al*. 2002 [42], Abudulai *et al*. 2003 [2], Mitchell *et al*. 2004 [22], Singha *et al*. 2007) [36]. Due to the toxicity at the highest two concentrations, none of the nymphs could reach to the adult stage and died in nymphal stage only. Similar failure in reaching to adulthood post neem treatment was reported in *N. viridula* (Riba *et al*., 2003 [30], Singha *et al*., 2007) [36]. Death in the fifth nymphal instar could be associated due to the mouthpart deformities observed and hence ability to feed was hampered that could have led to the death due to starvation (Singha *et al*., 2007) [36]. Change in the body colouration to darker colour or black is a cuticular melanization process which is one of the common manifestations of neem on insects. This change is related to the absence of juvenile hormone and the low level of ecdysteroids (Hori *et al*., 1984; Koul *et al*., 1990 [18], Mordue (Luntz) and Blackwell, 1993) [23], and has been observed in several other insects (Stark and Rangus, 1994; Singha *et al*., 2007) [36]. Neem pesticides are better known for its growth regulatory properties (Mordue (Luntz) and Blackwell, 1993) [23] and development to intermoult stage is one of the key changes observed across several insects, post-neem application indicative of the hampering of the endocrine regulation of metamorphosis in insects. Treated nymphs during moulting are unable to swallow enough air to create the correct hydrostatic pressure for ecdysis resulting in intermoult (Mordue (Luntz) *et al*., 1986) [24].

Other body deformities like partially formed thoracic segments, ill-formed wing pad, distorted abdomen, etc are also observed in other insects post neem treatment (Koul, 1984 [17]; Mordue (Luntz) and Blackwell, 1993 [23]; Abudulai *et al*., 2003 [2], Riba *et al*., 2003 [30], Singha *et al*., 2007) [36] as azadirachtin is known to modify the ecdysteroid titer (reduction or delay in release) in the hemolymph which is manifested in the form of developmental aberrations in the young stages (Redfern *et al*., 1982 [29], Sieber and Rembold, 1983 [34]; Mordue (Luntz) *et al*., 1986) [24]. Limping legs observed in the present study could be due to muscle paralysis, or necrosis of cells, as the azadirachtin uptake by cells and tissues of the insects results in such effect and it also affects the enzyme productions, protein synthesis, and many such molecular and physiological changes (Mordue (Luntz) and Nisbet, 2000) [25], which may ultimately affect the overall health of the test insects. Such body deformities post neem treatments are reported by several other authors (Singha *et al*., 2003 [35], Riba *et al*., 2003 [30], Singha *et al*., 2007) [36].

LC_{50} of 0.088% (880 ppm) observed in the present study is comparable to the LC_{50} (810 pm for mortality on 5d) of Neemix® treatment on 4th instar nymph of *Nezara viridula* (Abudulai *et al*., 2003) [2]. LC_{50} of the neem kernel crude extract in hexane and water on *N. viridula* was 1.32% and 0.32% respectively for the adult bugs when the treatment was done at the fourth nymphal instar (Singha *et al*., 2007) [36]. This indicates that the type of extract or the solvent choice also results in variable responses in the same insects. *M. cribraria* is known to survive for 24-56 days (Eger *et al*, 2010) [8] but the reduced longevity of the bugs (maximum of 14 days) in the lowest test concentration of NOG is indicative of the manifestation of the toxic effect of neem.

This is the first report where the toxicity of *harad* extract on *M. cribraria* has been assessed and has been found toxic though at higher doses and interestingly intermoults were also observed but as such no marked morphological deformities were found indicating that this plant can be further explored and different phytochemicals present in *Terminalia* can be tested for its potential as a biopesticide. Probably, a single active ingredient of this plant could be more potent than its crude extract to bring about the toxic or sub-lethal effect on the pests thereby reducing its LC_{50}. This study has left a more scope for research in this direction using *Terminalia* as a potent plant for IPM besides the already established neem tree.

Conclusion

Conventional pesticides being associated with several risks to the environment, plants, a repertoire of secondary metabolites, serve as one of the potent candidates in insect pest management with negligible impact on the ecosystem. Neem is one such plant and *Terminalia* being pharmacologically active, its use as a botanical pesticide was assessed against *M. cribraria* which at present is a seasonal pest in India but has attained a serious pest status in some countries. In the present study, both the neem formulations and *harad* hexane extracts were found to be considerably toxic to the bug as well as in suppressing the hatchability of the egg. Targeting insect eggs in pest control programs offers a proactive and ecologically sound approach that can lead to long-term reduction of pest populations while minimizing environmental impacts and the development of resistance. If insect pests are targeted in this stage, then the future build-up of the pest population can be mitigated to a larger extent. Hemipteran bugs kudzu bug are often neglected for their importance as a pest due to their unnoticeable damage to the plant and probably, due to the same factor the volume of research pertaining to their control using any form of insecticides is comparatively sparse when taken other insect orders like Lepidoptera into consideration. This insect group has invited less attention despite many of them being important crop pests, so assessing and analyzing different ways of their management is imperative to safeguard our crops from invisible damage as well as saving the environment with sustainable and eco-friendly ways of pest management.

Acknowledgements

We are thankful to Cotton University for the financial support granted for this in-house research project (CU/DEAN/R&D/2019/05/8141-45) and to Dr. Akalesh K. Verma of Cotton University for letting the team use his laboratory facilities.

Declaration of Competing Interest

Authors declare that they have no conflicting interest of any kind.

References

1. Abbott WS. A method for computing the effectiveness of an insecticide. J Econ Entomol,1925:18:265–267.
2. Abudulai M, Shepard BM, Mitchell PL. Antifeedant and toxic effects of a neem (Azadirachta indica A. Juss)– based formulation Neemix® against Nezara viridula (L.) (Hemiptera: Pentatomidae). J Entomol Sci,2003:38:398-408.
3. Agrawal IL. Ovicidal activity of four plant extracts on Dysdercus koenigii Fabr. (Heteroptera: Pyrrhocoridae). Indian J Entomol,1993:55(4):440-443.
4. Broadbent AB, Pree DJ. Effect of diflubenzuron and BAY SIT8514 on the Oriental Fruit Moth (Lepidoptera: Olethreutidae) and Oblique banded Leafroller (Lepidoptera: Tortricidae). J Econ Entomol,1984:77(1):194–197.
5. Campbell BE, Pereira RM, Koehler PG. Complications with controlling insect eggs. In: Trdan S, editor. Insecticide Resistance. Intech: 2016. p. 83-96. http://dx.doi.org/10.5772/61848.
6. Das G, Kim D-Y, Fan C, Gutiérrez-Grijalva EP, Heredia JB, Nissapatorn V, Mitsuwan W, Pereira ML, Nawaz M, Siyadatpanah A, Norouzi R, Sawicka B, Shin H-S, Patra JK. Plants of the Genus Terminalia: An Insight on Its Biological Potentials, Pre-Clinical and Clinical Studies. Front Pharmacol,2020:11:561248. doi: 10.3389/fphar.2020.561248.
7. Dorn A. Effects of azadirachtin on reproduction and egg development of the heteropteran Oncopeltus fasciatus Dallas. J Appl Entomol,1986:102:313-319. https://doi.org/10.1111/j.1439-0418.1986.tb00928.x.
8. Eger JE, Ames LM, Suiter DR, Jenkins TM, Rider DA, Halbert SE. Occurrence of the Old-World bug Megacopta cribraria (Fabricius) (Heteroptera: Plataspidae) in Georgia: A serious home invader and potential legume pest. Insecta Mundi,2010:121:1–11.

9. Finney DJ. Probit analysis. London: Cambridge University Press: 1971. pp. 68–78.
10. Guleria S, Tiku AK. Botanicals in Pest Management: Current Status and Future Perspectives. In: Peshin R, Dhawan AK, editors. Integrated Pest Management: Innovation-Development Process. Springer, Dordrecht: 2009. pp: 317-329. DOI 10.1007/978-1-4020-8992-3_12.
11. Gupta PC. Biological and pharmacological properties of Terminalia chebula Retz. (Haritaki)- An overview. Int J Pharm Pharm Sci,2012:4(3):62-68.
12. Hassan E. The Insecticidal Effects of Neem Seed Kernel Extract on Eggs and Larvae of Helicoverpa armigera (Hübner). J Plant Dis Prot,1999:106(5):523–529. http://www.jstor.org/stable/43215326.
13. Jacobs CGC, Rezende GL, Lamers GEM, van der Zee M. The extraembryonic serosa protects the insect egg against desiccation. Proc R Soc Lond B,2013:280(1764):1-8. doi:10.1098/rspb.2013.1082.
14. Kavitha VS, Revathi N, Kingsley S. Egg susceptibility of brinjal pest Leucinodes orbonalis Genue to neem extracts. Asian J Bio Sci,2008:3(2):308-310.
15. Kilani-Morakchi S, Morakchi-Goudjil H, Sifi K. Azadirachtin-Based Insecticide: Overview, Risk Assessments, and Future Directions. Front Agron,2021:3:676208. doi: 10.3389/fagro.2021.676208.
16. Kim HL, Choi BK, Yang SH. Terminalia chebula Medicinal Uses: A Review of *in vitro* and *in vivo* Studies. Biotechnol Bioproc E,2022:27:729-739. https://doi.org/10.1007/s12257-022-0090-0.
17. Koul O. Azadirachtin: I-interaction with the development of red cotton bugs. Entomol Exp Appl,1984:36(1):85-88.
18. Koul O, Isman MB, Ketkar CM. Properties and uses of neem, Azadirachta indica. Can J Bot,1990:68(1):1-11.
19. Kumar KJ. Effect of geographical variation on contents of tannic acid, gallic acid, chebulinic acid and ethyl gallate in Terminalia chebula. Nat Prod Indian J,2006:2(3-4):170–175.
20. Lahiri S, Reisig DD. Ecology and Management of Kudzu Bug (Hemiptera: Plataspidae) in Southeastern Soybeans. J Integr Pest Manag,2016:7(1):1–6. doi:10.1093/jipm/pmw013.
21. Miao J, Reisig DD, Li G, Wu Y. Sublethal Effects of Insecticide Exposure on Megacopta cribraria (Fabricius) Nymphs: Key Biological Traits and Acetylcholinesterase Activity. J Insect Sci,2016:16(1):99. doi: 10.1093/jisesa/iew083.
22. Mitchell PL, Gupta R, Singh AK, Kumar P. Behavioral and Developmental Effects of Neem Extracts on Clavigralla scutellaris (Hemiptera: Heteroptera: Coreidae) and Its Egg Parasitoid, Gryon fulviventre (Hymenoptera: Scelionidae). J Econ Entomol,2004:97(3):916–923. https://doi.org/10.1093/jee/97.3.916.
23. Mordue (Luntz) AJ, Blackwell A. Azadirachtin: an update. J Insect Physiol,1993:39(11):903-924.
24. Mordue (Luntz) AJ, Evans KA, Charlet M. Azadirachtin, ecdysteroids and ecdysis in Locusta migratoria. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol,1986:85(2):297-301.
25. Mordue (Luntz) AJ, Nisbet AJ. Azadirachtin from the neem tree Azadirachta indica: its action against insects. An Soc Entomol Bras,2000:29(4):615-632. https://doi.org/10.1590/S0301-80592000000400001.
26. Ngegba PM, Cui G, Khalid MZ, Zhong G. Use of Botanical Pesticides in Agriculture as an Alternative to Synthetic Pesticides. Agriculture,2022:12(5):600. https://doi.org/10.3390/agriculture12050600.
27. Olson DM, Tillman G, Toews M. Biopesticide and Parasitoid Effects on Megacopta cribraria (Hemiptera: Plataspidae) Life Stage Density and Egg Parasitism in Soybean. J Entomol Sci,2021:56(2):272-277. https://doi.org/10.18474/0749-8004-56.2.272.
28. Raju D, Ashish K, Ilango K, Chitra V. Evaluation of antiulcer activity of methanolic extract of Terminalia chebula fruits in experimental rats. J Pharm Sci Res,2009:1:101-107.
29. Redfern RE, Kelky TJ, Borkovec AB, Hayes DK. Ecdysteroid titres and moulting aberrations in last stage Oncopeltus nymphs treated with insect growth regulators. Pestic Biochem Physiol,1982:18:351-356.
30. Riba M, Marti J, Sans A. Influence of azadirachtin on development and reproduction of Nezara viridula L. (Het., Pentatomidae). J Appl Entomol,2003:127:37–41.
31. Ruberson JR, Takasu K, Buntin GD, Eger JE, Gardner WA, Greene JK, Jenkins TM, Jones WA, Olson DM, Roberts PM, *et al*. From Asian curiosity to eruptive American pest: Megacopta cribraria (Hemiptera: Plataspidae) and prospects for its biological control. Appl Entomol Zool,2013:48(1):3-13.
32. Schmutterer H. Properties and potential of natural pesticides from the neem tree, Azadirachta indica. Annu Rev Entomol,1990:35:271-297.
33. Seiter NJ, Greene JK, Reay-Jones FPF. Reduction of soybean yield components by Megacopta cribraria (Hemiptera: Plataspidae). J Econ Entomol,2013:106:1676-1683.
34. Sieber KP, Rembold H. The effects of azadirachtin on the endocrine control of moulting in Locusta migratoria. J Insect Physiol,1983:29:523-527.
35. Singha A, Thareja V, Singh AK. Effect of neem seed extract on development of Nezara viridula. In: Proceedings of the National Symposium on Frontier Areas of Entomological Research. IARI, New Delhi,2003. p. 589-590.
36. Singha A, Thareja V, Singh AK. Application of neem seed kernel extracts result in mouthpart deformities and subsequent mortality in Nezara viridula (L.) (Hem: Pentatomidae). J Appl Entomol,2007:131:197-201. https://doi.org/10.1111/j.1439-0418.2006.01134.x.
37. Srinivasperumal S, Samuthiravelu P, Muthukrishnan J. Host plant preference and life table of Megacopta cribraria (Fab.) (Hemiptera: Plataspidae). Proc Indian Natl Acad Sci B,1992:58:333-340.
38. Stark JD, Rangus TM. Lethal and sub-lethal effects of the neem insecticide formulations, 'Margosan- O', on the pea aphid. Pestic Sci,1994:41:155-160.
39. Su T, Mulla MS. Ovicidal activity of neem products (azadirachtin) against Culex tarsalis and Culex quinquefasciatus (Diptera: Culicidae). J Am Mosq Control Assoc,1998:14:204-209.
40. Suiter DR, Gardner WA, Ames LM, Eger JE, Kudzu Bugs as a Nuisance Pest. https://www.pctonline.com/article/pct0811-kudzu-bugs-nuisance-pest-difficult-control/, 2023 (accessed on 18 July 2023).

41. Sujithra M, Srinivasan S, Hariprasad KV. Outbreak of lablab bug, Coptosoma cribraria Fab. on field bean, Lablab purpureus var. lignosus Medikus. Insect Environ,2008:14:77-78.
42. Sutherland JP, baharally V, Permaul D. Use of the botanical insecticide, neem to control the small rice stinkbug Oebalus poecilus (Dallas, 1851) (Hemiptera: Pentatomidae) in Guyana. Entomotropica,2002:17(1):97-101.
43. Thejaswi L, Naik MI, Manjunatha M. Studies on population dynamics of pest complex of field bean (Lablab purpureus L.) and natural enemies of pod borers. Karnataka J Agric Sci,2008:21:399-402.
44. Thippeswamy C, Rajagopal BK. Assessment of losses caused by the lablab bug, Coptosoma cribraria (Fabricius) to the field bean, Lablab purpureus var. lignosus Medikus. Karnataka J Agric Sci,1998:11:941-946.
45. United Nations Environment Programme, World Health Organization, Food and Agriculture Organization of the United Nations. Chapter 5/12: The Environmental, Human Health and Economic Impacts of Pesticides,2022. Available from: https://wedocs.unep.org/20.500.11822/40354.
46. Vel T, Prabhakaran V-S, Sengottayan S-N, Sam E-E, Athirstam P, M Chellappandian, Rani S, Escaline L, Kalaivani K. Impact of Terminalia chebula Retz. against Aedes aegypti L. and non-target aquatic predatory insects. Ecotoxicol Environ Saf,2017:137:210-217. https://doi.org/10.1016/j.ecoenv.2016.11.004.
47. Veni T, Pushpanathan T, Mohanraj J. Larvicidal and ovicidal activity of Terminalia chebula Retz. (Family: Combretaceae) medicinal plant extracts against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. J Parasit Dis,2017:41:693-702.
48. Wang HS, Zhang CS, Yu DP. Preliminary studies on occurrence and control technology of Megacopta cribraria (Fabricius). China Plant Prot,2004:24:45-46.
49. Xing GN, Zhou B, Zhao TJ, Yu DY, Xing HH, Chen SY, Gai JY. Mapping QTLs of resistance to Megacopta cribraria (Fabricius) in soybean. Acta Agron Sin,2008:34:361-368.
50. Zhang YZ, Hanula JL, Sun JH. Survey for potential insect biological control agents of Ligustrum sinense (Scrophulariales: Oleaceae) in China. Florida Entomol,2008:91:372-382.