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Efficacy of aqueous Aegle marmelos leaf extract for the control of Megacopta cribraria

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Abstract

Megacopta cribraria, a new pest of concern worldwide, has been studied extensively to understand its binomics and control. Research on efficacy of botanical pesticides for its control, however is lacking. This study focuses on the efficacy of a plant-based extract derived from *Aegle marmelos* leaves. The ovicidal potential of crude aqueous *A. marmelos*leaf extract was tested at five concentrations (1.25%, 2.5%, 5%, 7.5%, and 10%), resulting in a maximum of 30% ovicidal action at the 10% concentration which is significantly higher than the control as in the natural condition 100% egg hatching is observed. The extract also exhibited deterrence activity against adult bugs. At all concentrations, significant deterrence was observed in terms of time spent on the treated surface and the frequency of visitation. At the highest concentration, only 2.5% of the total 10 min observation time was spent on the treated surface as compared to the control. The frequency of visitation to the treated surface (14% visit) also declined significantly at 10% concentration clearly exhibiting the deterrent effect of the extract to the bugs. This deterrence activity was further evident by frequent grooming behavior exhibited by the bugs, trying to cleanse away the extract stuck to their body receptors. The study shows that *A. marmelos* leaves extract has the potential as a biopesticide against *M. cribraria* and can be researched further in different aspects of its insecticidal potential and can be considered by agriculturists for the pest control program.

Introduction

Megacopta cribraria (F.), is a pest of leguminous crops but has been found associated with several non-leguminous plants as well (Ishihara, 1950; Schaefer et al., 2000; Thippeswamy and Rajagopal, 2005; Hosokawa et al., 2007; Eger et al., 2010; Lahiri and Reisig, 2016). It is known by different names in different places- kudzu bug, lablab bug, bean plataspid, and globular stink bug owing to its association with the kind of host plant or the globular structure of the bug. This pest is native to Asia but has invaded several places in the US and has become a serious threat to soybean production (Zhixing et al., 1996; Ruberson et al., 2013; Blount et al., 2015). The yield reduction of the crop plants by them is dependent on the growing season of the plant and accordingly, the severity of the damage also varies. Feeding by this pest on the plants results in the crinkling of the damaged soybean leaves, improper development of pods, or development of undersized seeds in field beans, development of brownish patches at feeding locations that primarily appear whitish, and in case of severe damage, withering and drying of shoots take place (Thippeswamy and Rajagopal, 2005). Apart from the damage caused to plants, they cause annoyance to people in urban areas by entering houses and generating rashes on the skin upon exposure, as well as staining surfaces yellow when it is crushed (Ruberson et al., 2013).

As with majority of the insect pests, synthetic insecticides, particularly pyrethroids are used for controlling the *M. cribraria* population (Lahiri and Reisig, 2016) but the drawbacks associated with the long-term use of any synthetic insecticides are much more than the benefit accrued from it (Aktar et al.,

2007). In recent times, there has been growing interest in utilizing botanical pesticides for pest control owing to their environmental safety, minimal residue effect, fewer risks to non-target organisms, etc. (Damalas and Koutrabas, 2020; Souto et al., 2021; Guinti et al., 2022), making botanical pesticides one of the best sustainable candidate in IPM approaches.

Aegle marmelos (L.), commonly known as *bael* in India is a slow-growing, deciduous, tree belonging to the family Rutaceae (Maity et al., 2009) and possess a variety of phytochemicals such as alkaloids, flavonoids, carotenoids, coumarins, saponins, terpenoids, and tannins (Badam et al., 2002; Mujeeb et al., 2014; Ganapathy and Karpagam, 2016). The *bael* tree holds great religious significance among Hindu people and its importance in ancient Indian medicine is well scripted in *Vedas, Puranas, Charaka Samhita,* etc (Mandal et al., 2020) due to the presence of a plethora of phytochemicals in it and hence it has been researched to a great extent for its nutritional and pharmacological properties (Sarkar et al., 2020; Venthodika et al., 2021). However, there are very few reports on the insecticidal activity of this plant extracts on insect pests or vectors. The essential oils obtained from the leaves of this tree, such as β- terpinyl acetate, hepten-2-ol, etc., have been demonstrated to exhibit insecticidal effects against mosquitos (Sarma et al., 2017). The leaf extracts have also shown oviposition deterrence, ovicidal and repellent activity against *Anopheles subpictus* (Elango et al., 2009). This plant extract has also exhibited insecticidal and repellent effects against a few agricultural and stored grain insect pests (Hiremath et al., 1997: Chaubey, 2017: Murasing et al., 2017) but no such activity has been reported against *M. cribraria* so far.

So, the present study was conducted to assess the ovicidal efficacy and deterrence effect of leaf extracts of *A. marmelos* on the adult bugs of *M cribraria.*

Materials And Methods

Insect rearing

M. cribraria eggs and adults were collected from the pigeon pea (*Cajanus cajan*) field of Assam Agricultural University, Azara, Assam, India. The eggs and adult bugs collected from the field were reared in the laboratory conditions of temperature 27±2°C, relative humidity (R.H.) of 60-70%, and a photoperiod of 14:10h (Light: Dark). Adults were reared following the method of Singha et al. (2007) with slight modifications. Adults were maintained in a cubical plexiglass cage (20cm × 19.5cm), with two meshed windows (10cm×10cm) on opposite walls of the cage. The floor of the cage was lined with tissue paper cut into equal halves according to the size of the cage to ensure the absorption of the liquid excreta of the insect. The tissue was changed periodically to avoid fungal growth. The cage was provided with three small cups (2cm dia X 3cm ht) with moist water-soaked cotton as a source of water. The cage was provisioned with small plant stem cuttings in small bottles to serve as a site for oviposition for the bugs. Adults were fed on two diets, pigeon pea *(C. cajan*) and French beans (*Phaseolus vulgaris*), which were replaced by fresh food every third day. The pigeon peas were collected from Assam Agricultural University's fields, and the French beans were collected from a local market. Eggs laid on tissue paper,

leaves, stems, and cage walls were transferred to a plastic container (7cm dia X 11.6cm ht) having moist cotton in a cup (2cm dia X 3cm ht) for humidity and was covered from the top**.** Nymphs on hatching from the egg were transferred using a camel hair brush (no. 00) to another container of the same dimension as the egg container. Nymphal rearing containers were lined with filter paper and bean was provided as food and the containers were covered with a muslin cloth. Food and filter paper were changed every third day and were reared until they became adults. The adults were transferred into the cage.

Plant material collection and extract preparation

The leaves of *A. marmelos* were collected from the tree in the Department of Zoology, Cotton University. Collected leaves were thoroughly washed under running tap water and allowed to shade dry at room temperature (~27°C) for one week**.** Subsequently, the dried leaves were mechanically crushed into a powder. To prepare the crude aqueous *Aegle* leaf extract (AALE), a fresh stock solution of 20% (wt/vol) was made by mixing 10gm of powdered *A. marmelos* leaves with 50ml of distilled water. The mixture was stirred using a magnetic stirrer for 1hr and then kept undisturbed for 10h before filtering with Whatman No.1 filter paper (Singh and Singh, 2000). Distilled water served as the control. Test concentrations of 1.25%, 2.5%, 5%, 7.5%, and 10% were prepared by serial dilution of the 20% AALE stock solution with the control solution.

Ovicidal assay

For the ovicidal assay, freshly laid eggs (0-24hr) were collected from the rearing cage and the eggs were counted in each egg batch. AALE was applied by dipping the eggs in the test concentrations for 10s and then air-drying them for 10-15 minutes before transferring them to experimental containers (7cm X 11.6cm) with moist cotton placed in a small cup (2cm diameter X 3cm height) for humidity. For each test concentration, five replicates were made, and each replicate contained one egg batch. The eggs in each egg batch were counted and noted. Egg batches were observed daily for any hatching and the percent hatchability of the eggs was calculated using the following formula:

$$\text{Percentage Hatchability} = \frac{\text{Number of hatched eggs}}{\text{Total no of eggs in an egg batch}} \times 100$$

Deterrence Experiment

To test the deterrence effect of the AALE, a clean petri dish measuring 7.8cm in diameter was divided equally into a treatment zone (TZ) (2.9cm), a control zone (CZ) (2.9cm) and a central neutral zone (NZ)

(2cm) (Fig. 1). The test concentrations were evenly applied to the TZ, while the CZ was uniformly coated with distilled water using a camel hairbrush No. 2. After coating, the surfaces of the petridish was allowed to dry completely. NZ was not coated with anything. For the experiment, one adult was introduced into the NZ, and before the release of the insect, they were immobilized by keeping them in the deep freezer for ~ 3min. After the release of the bugs in the NZ, the petridish was covered with a slightly bigger petridish to prevent bugs from escaping and their movement was noted for 10min. In 10min duration, the number of times, the bug visited each zone along with the duration of the time spent in that zone was noted. The movement pattern and behavior of the insects were also recorded for all the five concentrations during the 10m observation time. For each concentration, five replicates were made, and each replicate consisted of 1 insect.

Statistical analysis

The statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS, v.28). The data on percentage egg hatchability and the time spent on the TZ were subjected to One-Way Analysis of Variance (ANOVA), and the means were separated using the Tukey HSD method ($P < 0.05$). To compare the time spent on untreated versus treated surfaces and the frequency of insect visits to either surface, paired Student t-test was applied. Graphs were generated using Microsoft Excel (MS-Excel).

Results

After treating the eggs and releasing the adults in the experimental arena, the ovicidal effect and deterrent effect of AALE were observed.

Ovicidal effect

A negative dose-dependent effect of AALE on the egg hatchability of *M. cribraria* was observed (Table 1). Among the concentrations tested, the highest concentration of 10% resulted in only 70.14% hatchability of eggs whereas the control showed 100% egg hatchability. Egg hatchability in control was significantly greater ($P<0.05$) than all the tested concentrations except at 1.25%. The lowest concentration did not seem to have any significant effect on egg hatchability whereas from 2.5% concentration onwards a significant ($P<0.05$) decrease in egg hatching was observed.

Deterrence activity of *A. marmelos* leaf extracts against *M. cribraria* adults

The deterrence effect of AALE on the adult bugs was observed in all the tested concentrations. The deterrence activity was evident from the longer duration spent by the bugs on the untreated surface

(CZ+NZ) as compared to the treated surface at all the concentrations from 1.25% to 10% (Fig. 2). In the highest test concentration of 10%, the bugs spent only 2.5% of their time on the treated surface than on the untreated surface which showed a highly significant difference in the time spent on the two surfaces (t=80.840, P<0.001). Even at the lowest concentration of 1.25%, bugs spent 59% of the total time on the untreated surface and 41% time on the treated surface which was statistically significant (t=4.305, P=0.013).

Table 1 Percentage egg hatchability of *M. cribraria* eggs post AALE treatment

Concentration	*Percentage hatchability (Mean±SD**)
Control	100%±0.00^{a}
1.25%	97.92%±3.60^{a}
2.5%	84.97%±0.63^{b}
5%	81.56%±1.35^{bc}
7.5%	77.18%±5.04^{cd}
10%	70.14%±7.33^{d}

Note- *Each replicate had one egg batch and five such replicates were made.

**Means followed by different superscripts in the column are significantly (P<0.05) different (Tukey HSD)

With the increasing concentrations, time spent on the TZ decreased significantly (F= 173.565, P<0.001) (Fig. 2). There was no significant difference between 1.25% and 2.5% concentration in the amount of time spent on TZ, but the time spent on these two concentrations were statistically more than at the other higher concentrations.

The deterrent effect was observed not only in the amount of time spent on a specific surface but also in the frequency of visits to the treated surface. During the 10min observation, the frequency of visits by the bugs on different zones was also noted. The frequency of visits on the treated surface was much lesser than on the untreated surface in all the concentrations (Table 2). The ratio of untreated to treated surface visits was always high for all the concentrations. At the lowest concentration of 1.25%, bugs transitioned between zones 13 times, with 9 of those instances (approximately 69% of the frequency) involving movement to the untreated surface, and the remaining 4 times (around 31% of the frequency) to the treated surface (t= 5.270, P=0.006). With increasing concentrations, the bugs' frequency of visits to the treated surface decreased significantly. At the 10% concentration, they visited the treated surface only around 14% of the time. Across all test concentrations, the bugs showed much lower visitation rates on the treated surface compared to the untreated surface (P≤0.006).

Table 2 Frequency of visit by the adult bugs of *M. cribraria* on the three surfaces in deterrence test

Concentration (%)	Frequency of Visit*			Ratio of Visit Untreated**/ Treated	% Visit by the insect#	
	Control	Neutral	Treated		Untreated	Treated
1.25	2	7	4	9:4	69.23[a]	30.77[b]
2.5	2	4.2	2	6.2:2	75.61[a]	24.39[b]
5	1.8	4.6	2	6.4:2	76.19[a]	23.81[b]
7.5	4.2	7.8	2.4	12:2.4	83.33[a]	16.67[b]
10	4.4	8	2	12.4:2	86.11[a]	13.89[b]

Note: * value in each concentration represents the mean of frequency of visits by 5 insects

**Control and Neutral are together considered as Untreated category

#Values followed by different superscripts in a row are significantly different at P<0.05 with a paired Student *t*- test

The deterrence effect of the extract was also well exhibited in the avoidance behavior and movement of the bugs after contact with the treated surface. After entering the TZ, the bugs halted briefly, changed direction, and repeated this process until they reached either the NZ or CZ, where they began their grooming behavior. The frequency at which the antennae were cleansed with the help of forelegs was more at 7.5% and 10% than at other lower concentrations (5%, 2.5%, and 1.25%).

Discussion

Exploring the potential of the plethora of phytochemicals for the control of insect pests opens vistas for research as well as finding eco-friendly solutions to many of the problems faced by mankind. In the present study, *A. marmelos* leaves were found to be ovicidal as well as deterrent to the adult bugs of *M. cribraria*. The efficacy of aqueous *bael* leaf extract in controlling the egg hatchability of *M. cribraria* has not been reported so far but the organic extracts of the leaves of this plant have been tested on other insects. Ethyl acetate and methanol extract of *A. marmelos* leaf extract showed 100% egg-hatching inhibition of the mosquito, *Anopheles subpictus* at 1000 ppm (Elango et al., 2009). 30% egg hatching inhibition at the highest test concentration of 10% AALE in the present study as against 100% inhibition in the studies by Elango et al., (2009), could be due to the use of organic solvents by them. Organic solvents easily penetrate through the waxy layers of insect egg coats (Campbell et al., 2016). Insect eggs

are designed in such a manner to limit water loss (Smith and Salkeld, 1966), and the eggs are designed in a well-waterproof manner- with a very thin layer of wax or lipid covering the inner surface of the chorion and another layer of waterproofing occurs by wax impregnation in the embryonic membrane during early embryonic development in the case of Hemiptera (Beament, 1949). So even at the highest concentration of 10%, there was only 30% inhibition of egg hatching as it could be related to less permeability of the egg chorions to the water which was used as a solvent for the plant extract in the present study. Despite the low percentage of inhibition on egg hatching, it is remarkable to observe a significant reduction compared to the control. Generally, 100% egg hatchability is observed in natural conditions, indicating that any change in hatching percentage is but the outcome of the treatment. Ovicides in the case of hemipteran insects can enter through only micropyle though the penetration level differs with respect to the kind of solvent used- lipophilic being faster in penetrating than the aqueous medium (Beament, 1948). The decreasing egg hatchability with increasing concentrations observed in the present study may be due to the phytochemicals in the *A. marmelos* leaf extract that may have penetrated the eggshell through micropyles and at the higher concentrations the toxic compound of the extract would have hampered the embryogenesis process affecting its hatchability. Dose-dependent effect of aqueous extracts of *A. marmelos* was seen in the stored grain pest *Callosobruchus chinesis* where egg hatchability inhibition of nearly 70% was observed at 5% concentration (Murasing et al., 2017). The ovicidal action of other plants on *M. cribraria* eggs is also reported using other botanical pesticides. Azera, Neem, and Pyganic biopesticides exhibited a variable effect on the egg hatchability of this bug. In comparison to control, Neem, and Pyganic biopesticides, treatment with Azera reported the lowest percentage of egg hatchability (Olson et al., 2021).

Aqueous extract of leaves of *A. marmelos* showed deterrence activity against adult *M. cribraria* at all the tested concentrations. For all the tested concentrations, the time spent on the treated surface decreased progressively, with the least time (2.5%) spent at 10% and a maximum time (41%) spent at 1.25%. The deterrence activity could be due to the higher concentration of the test extracts on the treated surface, which compelled the insect to stay away from the treated zone showing its repellent activity. The essential oil of leaves of *A. marmelos* and *Allium sativum* exhibited repellence to *Sitophilus zeamais* (Chaubey, 2017). They reported 100% repellence at 0.8% and 1.6% which is a much lower concentration than that used in the present study. The difference could be due to the kind of extract used in the two studies but in either case, the deterrence of the leaf extract of *A. marmelos* is well established. The study by Elango et al. (2009) on the repellent activity of the acetone, ethyl acetate, and methanol extracts of *A. marmelos,* reported 100% protection from *Anopheles subpictus* for 120 and 150min at 500ppm of the methanolic extract of *A. marmelos*.

In the present study, the frequency of visits to the treated surface decreased with increasing concentrations. High concentrations led to a higher presence of the active component in the extract, resulting in more residue on the petri dish. This increase in residue could have made the bugs find the surface deterrent, leading them to avoid it and visit less frequently. Pesticides can adhere to the insect's body surface, including its antennae, legs, and wings and the chemoreceptors present on legs, antennae, and mouthparts can easily sense the suitability of their environment (Sokolinskaya et al., 2020)

In our present study, we observed avoidance and grooming behavior in the adult bug in the form of frequent cleaning of antennae and rubbing of tarsi when in contact with the treated surface during the 10-minute observation period. Insects often like to self-groom to remove any foreign material or unwanted material sticking to their body parts, especially from their sensory appendages to enable them to better sense their surroundings (Rebora et al., 2018). By actively cleaning themselves, insects can remove pesticide residues and reduce their exposure to these harmful substances. This grooming behavior aids in minimizing the toxic effects of pesticides and preserving their physiological health. The cleaning of antennae and rubbing of tarsi by insects after contact with pesticides serve as a critical adaptive response. Insect antennae and tarsi apart from other body parts contain specialized chemoreceptors that help insects detect chemical cues, pheromones, and environmental signals (Chapman, 2013). Upon contact with the pesticides by the antennae, pesticides can disrupt the sensory receptors and signals thereof, impairing the insect's ability to navigate, locate food sources, or detect predators. Cleaning their antennae helps insects remove pesticide residues and restore the functionality of their sensory organs (Böröczky et al., 2013; Yanagawa et al., 2014)

Conclusion

The ovicidal effects and deterrence studies are crucial to evaluating botanical insecticides' efficacy against insect pests. The ovicidal effect pertains to the ability of an insecticide to kill insect eggs which is the starting point of insect population growth. Disrupting egg development using botanical pesticides can significantly impact pest population build-up without much impact on the environment. This effect is particularly important in integrated pest management strategies, as it helps lower the number of insects in future generations and reduces the need for further interventions. Deterrence studies help determine the ability of these insecticides to repel pests, preventing them from reaching and damaging the target crops or areas. By deterring pests, botanical insecticides can reduce the risk of infestations and subsequent crop losses. In the present study, establishing the role of leaf extract of *A. marmelos* as a potent agent for ovicidal effect and against the establishment of *M. cribraria* on the host plants owing to its deterrent effect opens the scope for further research using this plant. Overall, for effective pest control, pest establishment if targeted, can be one of the best approaches in bringing about a significant reduction in pest population on agricultural crops. Therefore, studies pertaining to the deterrent effect and understanding the ovicidal effect contribute to the successful implementation of botanical insecticides as an effective tool at the initial stage of pest establishment, providing sustainable and environmentally friendly solutions for pest control in agriculture and other sectors.

Declarations

Author’s Contributions

ASN conceived and designed the research. MM conducted experiments. RAB and SS helped in taking observations along with MM. ASN analyzed the data. MM, RAB, and SS prepared the manuscript which was proofread and finalised by ASN. All authors read and approved the final manuscript.

Declarations

Competing interests The authors do not have any competing interests to report.

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Figures

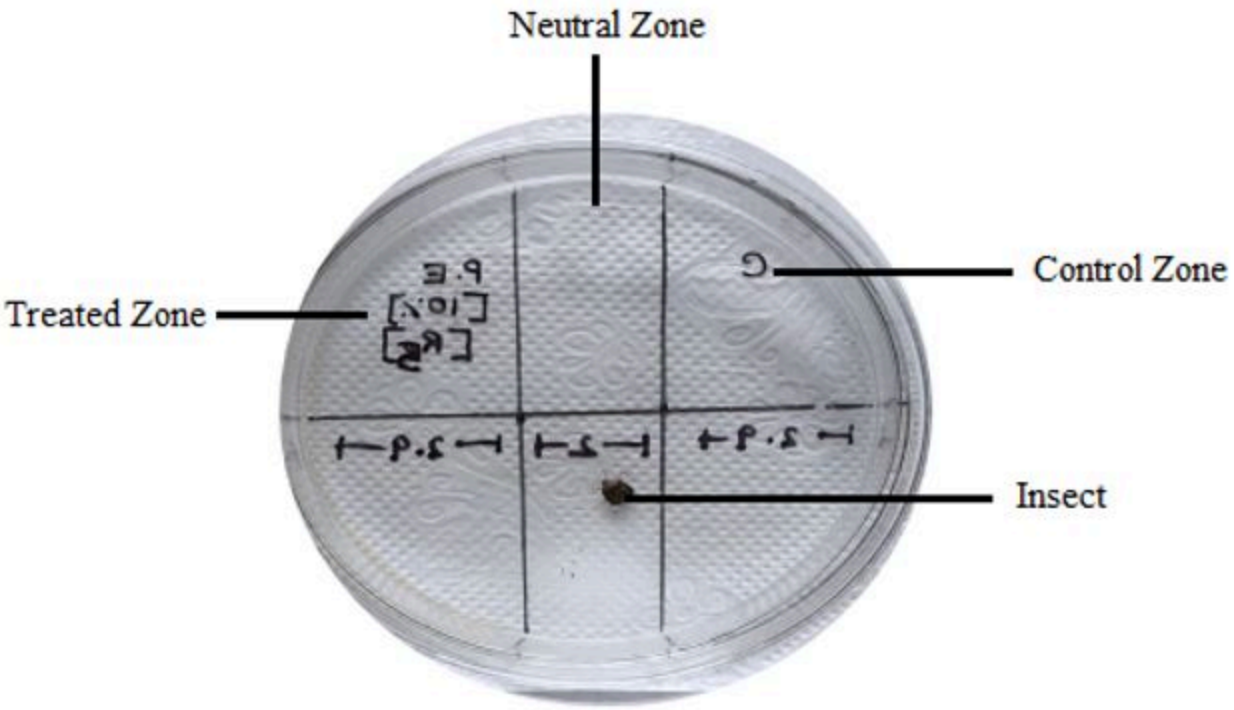


Figure 1

Deterrence experiment setup

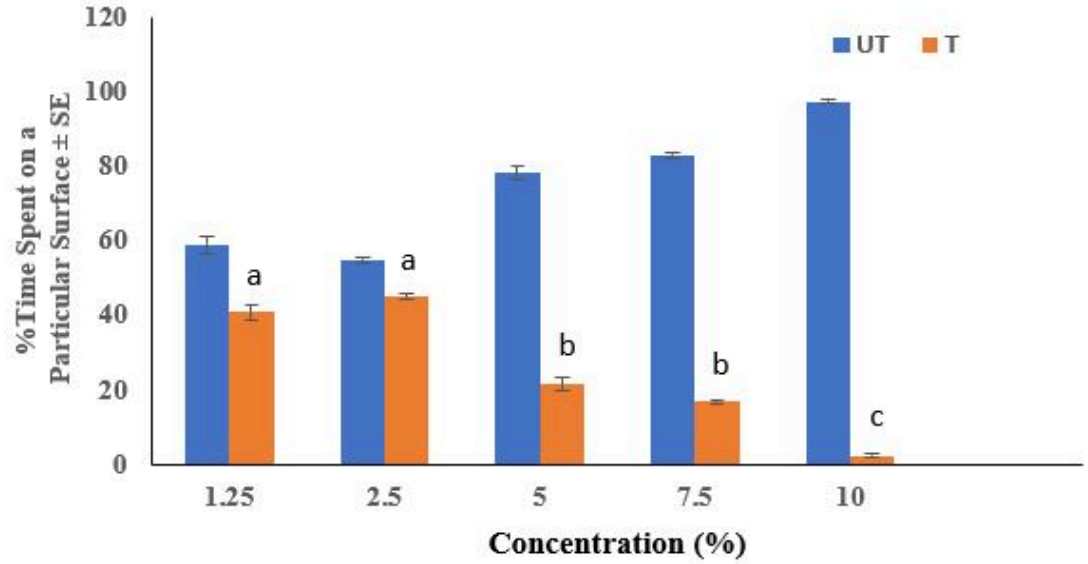


Figure 2

Percentage deterrence of *A. marmelos* leaf extract at different concentrations against *M. cribraria* adults

Note:

1. Each bar represents the average time spent by 5 insects on a particular surface per concentration.

2. Control and Neutral are together considered as Untreated category**.**

3. All pairs of a particular concentration showed significant differences at $P < 0.05$ based on a paired t-test.

4. Treated bars with different superscripts indicate significant differences from each other at $P < 0.05$ (One Way ANOVA followed by Tukey HSD)