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Analysis of the insecticidal effect of botanical extracts on mealybugs (*Phenacoccus solenopsis*)

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Abstract

The Cotton mealybug, also known as *Phenacoccus solenopsis*, poses a significant threat to agricultural productivity, particularly in cotton cultivation, where infestations cause substantial yield losses. This study examined the effectiveness of botanical extracts from neem (*Azadirachta indica*), clove (*Syzygium aromaticum*), black pepper (*Piper nigrum*), and bay leaf (*Laurus nobilis*) in controlling *P. solenopsis*. Neem extract showed the highest mortality effect at 86.6% followed by clove (68.3%) and black pepper (58.3%). Bay leaf extract had the lowest mortality effect at 36.6%. These findings highlight the potential of neem, clove, and black pepper extracts as effective options for managing *P. solenopsis* infestations in agriculture.

Keywords: Cotton mealybug; Infestation; Biological control; Agricultural productivity; Natural pesticides

1. Introduction

Indian textile mills rely heavily on cotton (*Gossypium hirsutum*), constituting 60% of raw material use. This sector contributes 5% to GDP, 14% to industrial production, and 11% to exports. It's the second-largest employer, with 51 million direct and 68 million indirect jobs. However, challenges such as *P. solenopsis* resistance due to insecticide misuse persist. (Shwetha et al, 2022). *P. solenopsis* threat prompts widespread, imprudent insecticide use in Asian cotton production. Consequently, populations evolve resistance to both conventional and innovative insecticides (Nagrare et al., 2020).

Phenacoccus solenopsis Tinsley, classified within the family Pseudococcidae, exhibits pronounced polyphagy. Studies have documented its substantial pest status, with documented infestations exceeding 154 plant species spanning over 52 plant families. *P. solenopsis*, known for its copious honeydew excretion, fosters sooty mold development, disrupting affected plants' photosynthetic apparatus. Its prevalence poses a significant obstacle to global cotton cultivation, threatening crop integrity and productivity (Mamoon et al.,2012). It acts as vectors for various plant pathogens such as viruses, bacteria, and fungi. This amplifies their impact on crop health and yield severity (Subramanian et al.,2021). *Phenacoccus* species are highly efficient vectors for plant viruses, particularly *Ampelovirus*, facilitating the spread of a wide range of viral pathogens (Ahmed et al.,2023).

Mealybug infestations have significant economic consequences due to their phloem-feeding behaviour, which compromises the vigour and productivity of host plants (Subramanian et al.,2021). Farmers are presently employing hazardous chemical insecticides to combat cotton mealybugs in both extensive and small-scale cultivation. Overreliance on synthetic chemicals for pest control harms beneficial organisms like parasitoids and predators, disrupting the food chain and reducing biological diversity. Indiscriminate pesticide use can also trigger secondary pest outbreaks by disrupting natural pest control mechanisms (Prishanthini & Vinobaba, 2014). Estimates suggest that approximately 0.1% of pesticides effectively reach the intended target organism, while the majority contaminates the surrounding

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environment (Carriger et al.,2006). Continued use of persistent, non-biodegradable pesticides contaminates the ecosystem, entering the food chain and accumulating in higher trophic levels via bioaccumulation. (Gill & Garg, 2014). Numerous acute and chronic human ailments have been linked to exposure to pesticides (Mustafalou & Abdollahi, 2017). Botanical pesticides are inherently degradable (Devlin and Zettel, 1999). Botanical extracts, deemed safer for their biodegradability, still require careful management to minimize impacts on non-target species and ensure effective pest control.

Neem (*Azadirachta indica*) is a potent biocontrol agent with low toxicity and high efficacy against plant pests and diseases. Its active ingredient, azadirachtin, disrupts insect metabolic processes, including protein synthesis, biological fitness, sexual communication, and chitin synthesis (Adusei et al., 2022). Clove (*Syzygium aromaticum L.*) stands out among the plant species with insecticidal potential for insect management (Afonso et al., 2012) (El Gohary et al.,2021), characterized by gas chromatography revealed eugenol as the major component (Elzayat et al., 2018). Column chromatography of the polar fraction of bay leaf (*Laurus nobilis*) essential oil isolated two compounds: eugenol and 7,7-dimethyl-3-methylenebicyclo heptan-4-ol. Their structural elucidation was achieved through spectral analysis, and subsequent evaluation for insecticidal activity was conducted. Black pepper (*Piper nigrum*), commonly employed as a culinary spice, along with its primary alkaloid piperine, has previously demonstrated larvicidal properties (Samuel et al., 2016). Ethyl alcohol pesticides penetrate the waxy layer, leading to mealybug mortality (Mani & Shivaraju, 2016).

2. Materials and methods

Insect Culture: Large-scale culture of mealybugs. Fresh, uncontaminated twigs with female mealybug infestation were procured from their natural habitat. The twigs were placed onto water-saturated oasis substrates within a glass enclosure to maintain optimal moisture levels conducive for mealybug viability. Following this, the mealybug colony was inoculated onto *Hibiscus calyphyllus* plant at 25.4°C±4.6°C and 53.5% ±14.8% humidity. The adult female mealybugs were observed to die after laying eggs. The 2nd and 3rd instar nymphs were identified and carefully separated from the adult females. These nymphs were then utilized in subsequent experimental procedures.

Collection of Botanicals: The botanical specimens employed in this experimental investigation included *Syzygium aromaticum* (Myrtaceae), *Piper nigrum* (Piperaceae), and leaves of *Laurus nobilis* (Lauraceae), which were procured from the local market. Additionally, leaves of *Azadirachta indica* (Meliaceae) were gathered from the garden in the department of Zoology, Isabella Thoburn College. The botanicals were subjected to a thorough cleansing regimen utilizing sterile distilled water to eliminate any extraneous contaminants. Following this, they were allowed to desiccate under shaded conditions for a period of one week to ensure complete removal of moisture. Subsequently, the desiccated materials were finely pulverized employing a domestic mixer grinder, until attaining a powdered consistency. The resulting powder stored in light-opaque containers in anticipation of subsequent extraction processes.

Preparation of Extracts: 10 grams of powered botanicals were measured using a calibrated precision balance and is subjected to thermal extraction by boiling with approximate volume of 100 ml of water over controlled flame for 5-10 minutes. The resulting aqueous solution was allowed cool at room temperature and strained through a fine mesh sieve into a glass jar. A precisely measured volume of 150 ml of high proof ethanol is added to the glass jar containing the aqueous infusion. The jar is tightly sealed and stored in cool, dark place for 2 weeks to undergo fermentation with occasional shaking to mix the content. The botanical extract, formulated as described, was transferred into a sprayer bottle for use as a pesticide. This methodical process adheres to scientific principles, enabling precise application of the extract's pesticidal properties onto targeted areas (Madanat, et al., 2016) (Erdogan, et al., 2020) (Khalid, et al., 2020).

2.1. Bioassay 1: Investigating the toxicological impact of individual botanical extracts on *Phenacoccus solenopsis*

Small plastic containers, with dimensions of 72 mm in diameter and 60 mm in height, were thoroughly washed and dried. Then oasis material was trimmed using a bottle cap slightly smaller in dimensions compared to the test containers, ensuring precise adaptation for a snug fit. To ensure proper identification of different botanical extracts' each container was labelled with name of the extract used. Following this procedure, a 40 mm twig, adorned with leaves infested by *P. solenopsis*, was delicately inserted into the hydrated oasis material. Third instar female mealybug were selected and 20 mealybugs were introduced in each container with a camel brush and the mouth of the container was covered with a muslin cloth secured by rubber bands. To ensure the precision of the results, each botanical extract was replicated in five distinct sets. To establish a reference point for comparison, untreated control groups comprising 20 mealybugs in each container were also set up. The natural climatic conditions were maintained by positioning the test containers on a table adjacent to a window, ensuring ample sunlight exposure and optimal humidity levels. The administration of the extracts was performed via direct spraying onto the infested leaves (0.5 ml approx.) at 12-hour

intervals, and the mortality rate was assessed through systematic enumeration of mealybug after 12, 24, 36, 48, 60, and 72 hours. Subsequently the control was sprayed with tap water.

$$Mortality\ \% = \frac{Number\ of\ dead\ insects}{Total\ number\ of\ insect} X\ 100$$

2.2. Bioassay 2: Investigating the toxicological impact of botanical residues on: Phenacoccus solenopsis

To assess the residual impact of chosen botanical extracts on cotton mealybugs, fresh and juvenile leaves of *Hibiscus* were procured from a domestic garden and subjected to thorough washing with tap water followed by complete air-drying. Subsequently, these leaves were immersed in botanical extracts for a duration of 25-30 minutes and allowed to air-dry fully. Control leaves underwent a parallel process but were submerged solely in tap water. The treated leaves were then situated in individual glass petri dishes, each receiving an inoculum of precisely 20 mealybugs at the 2nd instar and 3rd nymphal stage with the help of a soft camel brush and the petri dish was covered with muslin cloth. Mortality rates were recorded at 12,24,36,48,60 and 72 hours post-treatment. To forestall desiccation, the treated leaves were substituted after 12 hours. There were 5 different replication setups to ensure the precision of the results. Untread control groups were also established to provide a reference point.

2.3. Bioassay 3: Investigating the contact toxicological impact of different (Mixed) botanical extracts: *Phenacoccus solenopsis*

To examine the contact effect of different botanical extract on 1st instar cotton mealybugs, petri dishes were uniformly sprayed using a sprayer until runoff, followed by a 30-minute air drying period. Subsequently, 1st instar cotton mealybug nymphs were placed in the petri dish using a fine brush onto a single treated leaf and the petri dish was covered with muslin cloth. To forestall desiccation, the treated leaves were substituted after 12 hours. After 72 hours, the cotton mealybug nymphs were extracted, and the mortality count was conducted, with the results expressed as the percentage of dead nymphs for different botanical extract. There were five distinct replication configurations established to ensure result accuracy. Additionally, untreated control groups were implemented to serve as a baseline reference (Mamoon-ur-Rashid et al., 2012).

Statistical Analysis: The recorded data was analyzed using a completely randomized design, aiming to detect significant differences between clove, neem, bay leaf and black pepper treatment. The data was then subjected to Analysis of Variance (ANOVA), the test significant value is $P < 0 \cdot 05$, the calculation was performed by using IMB SPSS statistical ver. 27 software and graphs are plotted using the GraphPad prism 9 software for Microsoft.

Figure 1 Rearing of female mealybugs

Figure 2 Experimental set up for mortality effect of prepared extract

Figure 3 Experimental set up for mortality effect of prepared extract

Figure 4 Prepared extracts

Figure 5 Experimental set up for Residual effect

Figure 6 Experimental set up for Contact effect

3. Result

The research aimed to assess the efficacy of four distinct extracts as eco-friendly biopesticides against *P. solenopsis*. The above experiment focused on investigating the mortality rates involving individual effects, residual effects, and contact effects of selected botanical extract. The analysis revealed significant mortality rates across all treatment groups, with *A. indica* (neem) exhibiting most notable insecticidal effect, followed by *S. aromaticum* (clove). *P. nigrum* (black pepper) was proved effective against *P. solenopsis* but exhibited lower efficacy compared to clove. Conversely, *L. nobilis* was identified as the least efficient among the tested extracts (Neem>Clove>Black pepper>Bay leaf). The effects of the extracts, highlighting the highest recorded mortality rates at 72 hours post-treatment. Notably, neem extract treatments demonstrated the most substantial impact, resulting in a mortality rate of 86.6%, followed by clove with 68.3%. Black pepper treatment followed closely with mortality rate of 58.3%. Bay leaf treatment yield the lowest mortality rate by 36.6% (Table 1 and Figure 7). The residual effect of the extracts, with neem and clove extract exhibiting highest toxicological effect securing 53.3% and 41.6% mortality respectively. Black pepper and bay leaf exhibiting least toxicological effect securing 28.3% and 11.6% mortality respectively (Table 2 and Figure 8). The contact effect of extracts, neem extracts displayed highest mortality of 71.6% followed by clove with 56.6% mortality. Black pepper and bay leaf treatments showed 43.3% and 23.3% mortality respectively (Table 3 and figure 9).

Table 1 Mean mortality effect of different extracts on *Phenacoccus solenopsis*

S.N	Extract	No. of *Phenacoccus solenopsis* (N)	Mortality ±S.E					
			12HR	24HR	36HR	48HR	60HR	72HR
1	Control	20	0.33±0.33	1.00±0.33	1.33±0.33	1.66±0.33	2.00±0.33	2.33±0.33
2	*A. indica*	20	1.33±0.33	3.66±0.33	7.33±0.33	11.66±0.33	14.00±0.57	17.00±0.57
3	*S. aromaticum*	20	1.33±0.33	3.66±0.33	5.66±0.33	8.33±0.33	11.66±0.33	14.00±0.57
4	*P. nigrum*	20	0.66±0.33	1.66±0.33	3.66±0.33	7.00±0.57	9.33±0.33	12.33±0.33
5	*L. nobalis*	20	0.33±0.33	0.33±0.33	2.33±0.33	3.66±0.33	6.33±0.33	7.66±0.33

Three out of five replications were considered; Test significance is $p<0.05$

Table 2 Mean Residual effect of different extracts extract on *Phenacoccus solenopsis*

S.N	Extract	No. of *Phenacoccus solenopsis* (N)	Mortality ±S.E					
			12HR	24HR	36HR	48HR	60HR	72HR
1	Control	20	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.33± 0.33
2	*A, indica*	20	1.00±0.00	2.33±0.33	3.66±0.33	5.66±0.33	7.66±0.33	10.33±0.33
3	*S. aromaticum*	20	0.66±0.33	1.66±0.33	3.66±0.33	4.66±0.33	6.33±0.33	8.66±0.33
4	*P. nigrum*	20	0.00±0.00	0.66±0.33	1.00±0.57	2.66±0.33	4.00±0.57	5.66±0.33
5	*L. nobalis*	20	0.00±0.00	0.00±0.00	0.00±0.00	0.33±0.33	1.00±0.57	2.33±0.33

• Three of the five replications were considered; • Test significance is p<0.05

Table 3 Contact effect of different extracts on *Phenacoccus solenopsis*

S. N	Extract	No. of *Phenacoccus solenopsis* (N)	Mortality (Mean±S.E)					
			12HR	24HR	36HR	48HR	60HR	72HR
1.	Control	20	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.33±0.33
2.	*A, indica*	20	1.66±0.33	3.66±0.33	6.00±0.57	8.33±0.33	10.66±0.33	13.00±0.57
3.	*S. aromaticum*	20	1.00±0.00	2.33±0.33	4.00±0.57	6.33±0.66	9.00±0.57	11.33±0.33
4.	*P. nigrum*	20	0.66±0.33	2.00±0.66	3.33±0.88	5.00±0.57	6.00±0.57	8.66±0.33
5.	*L. nobalis*	20	0.66±0.33	0.66±0.33	1.00±0.33	2.66±0.33	3.66±033	4.66±0.33

• Three out of five replications are considered; • Test significance is p<0.05

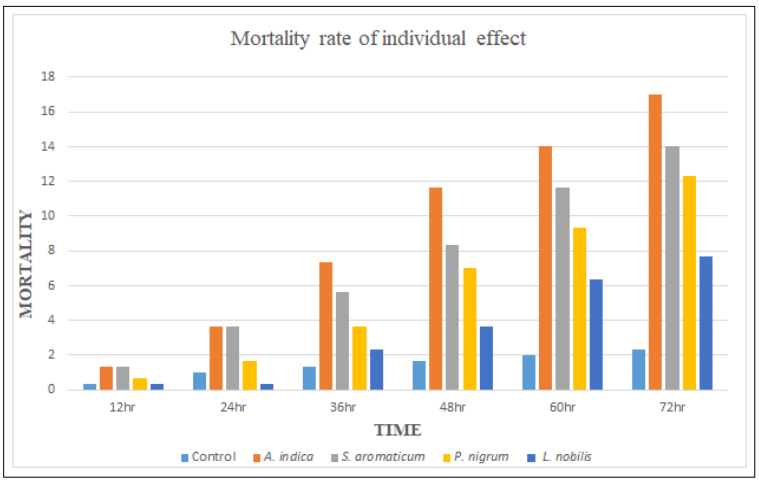

Figure 7 Mean mortality effect of different extracts on *Phenacoccus solenopsis*

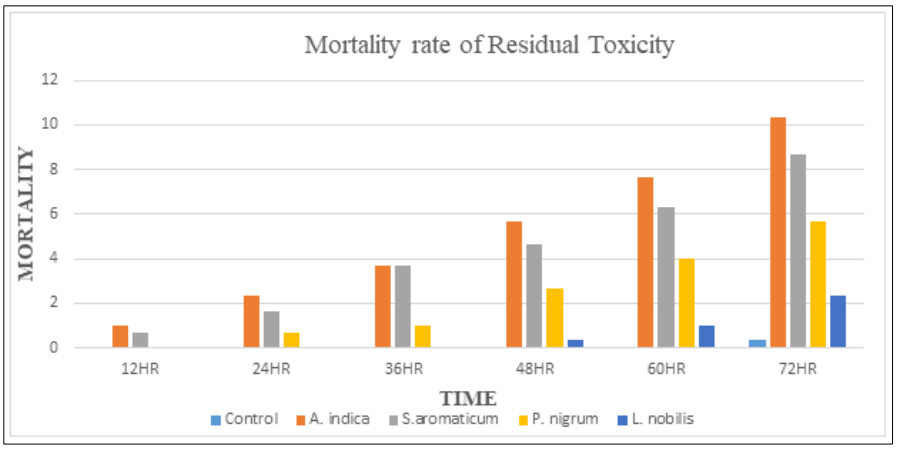

Figure 8 Mean Residual effect of different extracts on *Phenacoccus solenopsis*

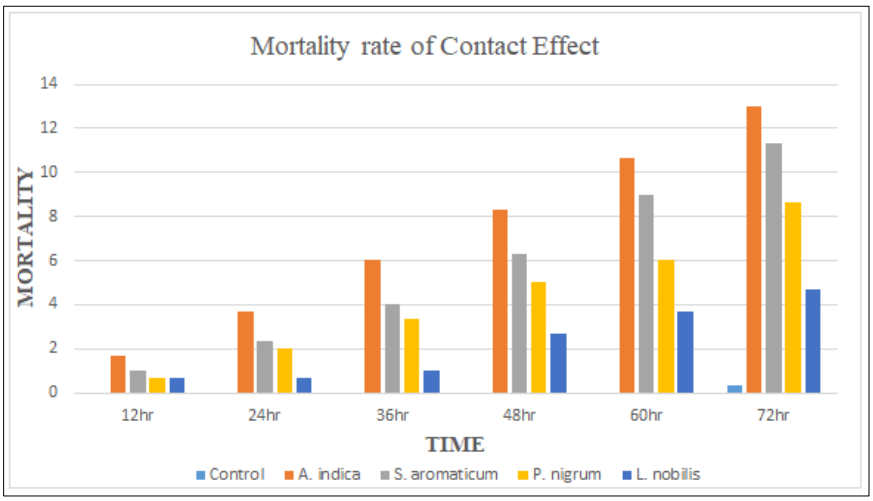

Figure 9 Mean of contact effect of different extracts on *Phenacoccus solenopsis*

4. Discussion

Neem (*A. indica*) and clove (*S. aromaticum*) extract exhibited notable insecticidal effect within 12 hours following treatment. A parallel study suggests the mortality rates of neem extract alone can reach approx. 85% with 72hour (Prishanthini & Vinobaba, 2014) which stands true for this experiment as the mortality rate of replica treated with Neem extract reaches 86.6%. Another study suggested that high concentrations of neem extract can result into 100.0% mortality rates within a week (Badshah et al., 2017). The research suggests that utilizing neem oil at a 5% concentration can lead to a 70% mortality rate via residual effect (Mahmoon-ur-Rashid et al., 2012). It was found that Eugenol in clove oil extract in high concentration can result into 77% mortality (Avila et al., 2022). In terms of lethality, the results indicate that black pepper shows significant insecticidal efficacy with an LC99 of 41% against common house pest. The bay leaf extract exhibited limited effectiveness, with mortality rates of less than 50% recorded within the shortest exposure duration.

5. Conclusion

Botanical extracts have been studied for their potential to control mealybugs, which are common pests of many plants, including crops and ornamentals. Neem (*Azadirachta indica*) is well-known for its insecticidal properties and has been extensively studied for its effects on various pests, including mealybugs. Neem contains compounds such as azadirachtin, nimbin, and salannin, which act as antifeedants. When mealybugs come into contact with neem extracts or neem-derived products, they may stop feeding on the treated plant tissues. This can lead to starvation and reduced

population growth. The above research clearly demonstrate that all the treatments showed notable mortality effect under 72 hours with neem having the maximum insecticidal efficacy against *P. solenopsis*. The hierarchy of effectiveness, ranked from greatest to least mortality, is Neem > Clove > Black pepper > Bay leaf. These discoveries enrich our comprehension of natural insecticidal substances and their potential utility in strategies for pest control.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have no any conflict of interest for publishing this article.

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