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(RESEARCH ARTICLE)

Analysis of biological organic extract of plants in suppressing aphid (*Myzus persicae*) infestations

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Abstract

Myzus persicae commonly known as the peach-potato aphid, poses a significant threat to agricultural productivity, particularly in potato cultivation, where infestations lead to substantial yield losses and quality degradation. In this study, we investigated the potential of botanical extracts from neem (*Azadirachta indica*), clove (*Syzygium aromaticum*), black pepper (*Piper nigrum*), and bay leaf (*Laurus nobilis*) in controlling *Myzus persicae* infestations. Experimental trials were conducted to assess the mortality effects of these botanical extracts on *Myzus persicae* populations. Results revealed that neem extract exhibited the highest mortality effect, with an impressive 97.7% mortality rate. Clove and black pepper extracts followed closely, demonstrating mortality rates of 92.2% and 77.7%, respectively. In contrast, bay leaf extract exhibited the least mortality effect, with only 48.8% mortality observed.

Keywords: *Myzus persicae*; Natural pesticides; Eco-friendly pest control; Botanical extracts; Toxicity; Mortality.

1. Introduction

Potato holds significant agricultural importance both in India and across Asia (Scott & Saurez, 2011). In Central India, the cultivation of potatoes occurs during the short winter season, typically from October to March. Within this agricultural context, diverse species of aphids assume a significant role as vectors for viral diseases affecting potato crops, consequently contributing to seed degeneration (Bhatnagar et. al., 2012).

In India, numerous researchers have previously conducted extensive studies on the appearance and population dynamics of aphids on potatoes, as well as their correlation with virus transmission. This exploration stands as a crucial prerequisite in enhancing our comprehension of aphids and their ecosystem (Bhatnagar et. al., 2012). Aphids, belonging to the family Aphididae within the order Hemiptera, are notable agricultural nuisances present worldwide. They cause considerable financial harm through their direct consumption of plant tissues, transmission of plant viruses, and secretion of honeydew (Kareem Ullah et al., 2023). A wealth of research has consistently emphasized the economic importance of aphids as agricultural pests (Emden & Harrington, 2017). The swift reproductive rate of aphids coupled with their capacity to transmit plant pathogens presents a formidable menace to crop yields on a global scale, thus demanding the implementation of efficient control strategies (Emden & Harrington, 2017). The transmission of over 110 plant viruses is attributed solely to *Myzus persicae* (Sulzer) (Singh & Agarwal, 2022). Wingless parthenogenetic adult females are oval-shaped, 1.2 to 2.1 mm long, displaying colours such as whitish green, pale yellow-green, grey-green, mid-green, dark green, pink, or red (CABI, 2021). The tobacco variant (nicotianae) ranges from bright yellow to nearly black. Genetic colour diversity exists, with some intensifying green or magenta pigmentation in colder climates. Immature stages have a noticeable sheen, while adults are less shiny. Winged variants feature a black dorsal patch on the abdomen (CABI, 2021). *M. persicae* has the potential to diminish the photosynthetic capacity of plants through the secretion of sugary honeydew, consequently impacting both the quality and quantity of crops (Frantz, et. al., 2004).

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Botanical pesticides, derived from natural sources like plants, animals, microbes, and specific minerals, are known for their low toxicity towards non-target organisms such as humans, animals, beneficial insects, and the environment (Adusei & Azupio, 2022)

2. Materials and methods

The experiment was conducted at 15°±5°C and 94 ± 5% humidity. The experimental design comprised 4 treatments, each replicated 5 times. The experimental setup involved the deployment of plastic containers as controlled environments, with oasis serving as the substrate for experimental conditions, and nylon synthetic fabric employed for precise filtration purposes.

2.1. Rearing of insect

Myzus persicae specimens were reared under controlled conditions using non-infested fresh daisy twigs as nutritive substrates to establish and maintain a standardized insect culture for experimental purposes. Plastic rearing containers, equipped with perforated cloth to ensure adequate ventilation are utilized. Uninfected fresh daisy twigs sourced from pesticide-free environments serve as nutritive substrates maintaining experimental integrity. *Myzus persicae* specimens are introduced onto these twigs with precision using a brush ensuring controlled inoculation. Environmental parameters were maintained. This includes maintaining a lower temperature range of 18-20°C and humidity levels sustained at 50-60% to simulate more temperate conditions conducive to experimental requirements. Additionally, a photoperiod of 16 hours simulates daylight conditions. Continuous twig replenishment ensures consistent aphid nutrition. After a period of 25-30 days, new instances of *M. persicae* were observed on the twigs indicating the completion of their developmental cycle. These aphids were carefully separated from the twigs to ensure accurate and controlled experimental conditions (Plate-1: Figure-5).

2.2. Preparation of powder and extracts

Four extract treatments were prepared from two leaf specimens, *Azadirachta indica* (Neem) and *Laurus nobilis* (Bay Leaf), as well as two spice specimens, *Syzygium aromaticum* (Clove) and *Piper nigrum* (Black Pepper). Neem leaves (*Azadirachta indica*) were collected from the campus of Isabella Thoburn College, Lucknow, while fruits of black pepper (*Piper nigrum*), flower buds of clove (*Syzygium aromaticum*), and leaves of bay laurel (*Laurus nobilis*) were obtained from domestic sources.

After air-drying for 5 days, Neem leaves were desiccated until crisp, while all specimens were then ground to a fine powder using an electric grinder. The resulting powders, each weighing 10 grams, underwent sieving for uniformity, yielding final samples weighing 8-10 grams for precise experimental conditions.

2.3. Preparation of extracts:

The prepared extracts were inspired by the research paper cited (Erdogan et. al., 2020), (Khalid et. al., 2020), (Madanat et. al., 2016) though few changes were made. The process involved mixing 10 g of fine powder of spices or leaves with 100 ml of water. Boiling the mixture for 30 minutes on medium-high flame with constant stirring, followed by cooling and filtration using Whatman filter paper no. 1 yielded an aqueous solution. To this solution 100 ml of ethanol was added. The resulting solution was kept in the dark for 48-72 hours before being used in spray bottles for it to ferment (Plate-1: Figure-4).

The Neem extract was combined with the other spice extracts in a 1:1 w/w ratio to create three distinct mixtures for demonstrating their joint efficacy against *Myzus persicae* thereby facilitating the assessment of potential synergistic effects.

2.4. Experimental setup

2.4.1. BIOASSAY 1: Analysis of toxic effect of plants extracts on Myzus persicae

Plastic containers 3mm in diameter and 56mm in height, underwent thorough cleaning and drying procedures to ensure sterility. Oasis, a hydrophilic foam was hydrated in water for 20-25 minutes to facilitate water absorption. Employing a smaller container with a 4mm diameter, Oasis was precisely cut to fit the container with a 1cm height to support the twigs. Uninfected twigs measuring 5cm in length were meticulously positioned within the Oasis substrate. 30 *Myzus persicae* aphids were introduced onto the twigs under controlled conditions. To maintain a pest and contaminant free environment, synthetic nylon fiber was used to cover the container opening secured with rubber bands. This methodology ensures a controlled and sterile experimental environment conducive to scientific investigation. Each

container was diligently labelled with the corresponding extract identifier to ensure precise investigation and treatment allocation, adhering to rigorous scientific protocols. The experiment involves counting the mortality rate by counting the number of dead *Myzus persicae* after 6hr, 12hr, 18hr, 24hr, 30hr, 36hr, 42hr, 48hr, 54hr, 60, 66hr, 72hr. In order to enhance result accuracy and facilitate comprehensive data analysis, each aphid-extract container was duplicated across five distinct sets. Additionally, to establish a comparative baseline a control group consisting of 30 untreated *Myzus persicae* within 5cm stems was also replicated across five separate sets, conducted concurrently (Plate-1: Figure-1).

$$\text{mortality\%} = \frac{\text{number of dead insects}}{\text{total number of insects}} \times 100$$

2.4.2. BIOASSAY 2: Analysis of mixed plants extract toxicity effect on Myzus persicae

Another aspect of the experiment involved investigating synergistic and antagonistic interactions by combining different extracts to assess their combined effects. Synergism, also known as potentiation, occurs when the combined effect of two or more extracts exceeds the sum of their individual effects. Antagonism, on the other hand occurs when the combined effect is less than the cumulative sum of their individual effects.

The experiment was done by using a mixture of neem extract with other extracts. Prepared different combinations of extracts in equal parts. The mixtures were:

Neem + black pepper; Neem + clove and Neem + bay leaf

Plastic containers were individually prepared, each containing a 5cm twig submerged in oasis substratum. Subsequently, 20 *Myzus persicae* were introduced into each container. A specific combination of extracts in a 1:1 v/v ratio was applied via spraying. Containers were carefully labelled to facilitate proper identification of the extract combinations. After 12 and 24 hours, the containers were examined, and the number of surviving and deceased *Myzus persicae* was recorded. The mortality percentage was computed using a designated formula. An analysis of the results was conducted to ascertain the efficacy of various spice combinations in repelling the insects (Plate-1: Figure-2).

$$mortality\% = \frac{number\ of\ dead\ insects}{total\ number\ of\ insects} \times 100$$

2.4.3. BIOASSAY 3: Analysis of repellent action of different plants extract on Myzus persicae

The repellency assay involved using a petri dish with filter paper divided into treated and untreated halves. Each half was subjected to extract sprayed. Ten adults *Myzus persicae* were introduced onto each filter paper, covered to prevent escape. After approximately 12 hours, the number of insects in treated and untreated areas was recorded. Three replications were conducted for each spice. Repellence percentage was calculated using the following formula (Plate-1: Figure-3).

$$repellency\% = \frac{(NC - NT)}{(NC + NT)} \times 100$$

Where, NC number of insects in control (untreated) area, And NT number of insects in treated area

2.5. Statistical analysis

The collected data were analysed in a completely randomized design to find significant difference between Neem, Black pepper, Clove and Bay leaf extract treatment. The analysis of variance (ANOVA) was applied. 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.

3. Result

During analysis it was found that the efficacy of specific extracts against *Myzus persicae*, a common agricultural pest, was evaluated. The study focused on assessing the mortality rates, combined effects and repellent actions of four distinct extracts. Through rigorous observation and analysis, it was determined that *Azadirachta indica* (neem) and *Syzygium aromaticum* (clove) exhibited the most notable insecticidal properties, demonstrating significant effectiveness against *Myzus persicae* in *Piper nigrum* (black pepper) displayed comparatively lower potency than clove. Conversely, *Laurus nobilis* was identified as the least efficacious among the extracts tested. These findings contribute to the understanding

of natural insecticidal agents and their potential applications in pest management strategies (Table1 and Figure6, in Table2 and Figure7 and in Table3 and Figure8).

The highest mortality rates observed within 72 hours post-application. Neem powder treatments exhibited the most substantial impact, achieving a mortality rate of 97.7%. Following closely behind, clove and black pepper treatments recorded mortality rates of 92.2% and 77.76% respectively. Conversely, other treatments displayed comparatively lower levels of mortality, with bay leaf treatment resulting in a 48.8% mortality rate (Neem>Clove>Black pepper>Bay leaves) in Table1 and Figure6. In a combined action study, mortality counts were assessed 24 hours post-treatment. Neem extract served as the standard, mixed in a 1:1 v/v ratio with various prepared extracts in Table2 and Figure7. Depict the outcomes, with the *Azadirachta indica* and *Syzygium aromaticum* mixture demonstrating the highest toxicity, resulting in a mortality rate of 64.43%. Following this, the combination of *Azadirachta indica* and *Piper nigrum* exhibited a mortality rate of 35.53%. Conversely, the combination of *Azadirachta indica* and *Laurus nobilis* displayed the lowest toxicity, with a mortality rate of 24.4%. (Neem + Clove > Neem + Black pepper> Neem + Bay leaves) (Table 2 and Figure 7). In the analysis of variance (ANOVA), it was observed that neem extract demonstrated the highest repellency efficacy, registering a significant percentage of 83.3%. Following neem, clove and black pepper exhibited repellency percentages of 66.6% and 56.6% respectively. Bay leaf demonstrated a repellency effect of 23.3% against *Myzus persicae*, as illustrated in Table3 and Figure8.

Figure 1 Experimental set up for mortality effect of prepared extract.

Figure 2 Experimental set up for joint effect of prepared extracts

Figure 3 Experimental setup for repellency

Figure 4 Prepared extracts

Figure 5 Rearing of aphids

Table 1 Mean mortality effect of different extracts on *Myzus persicae*

S. No.	Extract	No. of *Myzus persicae* (N)	Mortality (Mean±S.E)											
			6hr	12hr	18hr	24hr	30hr	36hr	42hr	48hr	54hr	60hr	66hr	72hr
1.	Control	30	0.33± 0.33	0.33± 0.33	1.00± 0.00	1.33± 0.33	1.33± 0.33	1.33± 0.33	1.66± 0.33	1.66± 0.33	1.66± 0.33	2.00± 0.33	2.33± 0.33	2.33± 0.33
2.	Neem	30	2.33± 0.33	4.33± 0.33	6.33± 0.33	8.33± 0.33	12.66± 0.33	14.66± 0.66	17.33± 0.33	19.33± 0.33	21.66± 0.88	25.00± 0.57	26.00± 0.57	29.33± 0.33
3.	Black pepper	30	1.66± 0.33	2.33± 0.66	5.33± 0.33	7.66± 0.33	8.66± 0.33	10.00± 0.57	11.33± 0.88	12.66± 0.66	14.66± 0.66	16.33± 0.88	18.33± 1.20	23.33± 0.57
4.	Clove	30	2.00± 0.57	4.33± 0.88	6.00± 0.57	7.00± 0.57	8.33± 0.33	12.66± 0.33	14.33± 0.33	16.66± 0.33	18.33± 0.33	21.33± 0.88	24.33± 0.33	27.66± 0.33
5.	Bay leaf	30	0.33± 0.33	1.33± 0.33	2.66± 0.33	4.33± 0.33	6.00± 0.57	7.33± 0.33	8.66± 0.33	10.00± 0.57	11.33± 0.33	12.66± 0.88	13.33± 0.33	14.66± 0.88

Data represented in the table are the mean of three out of five replications; Test significance is $\boldsymbol{P<0.5}$

Table 2 Mean mortality effect of different extracts mixed with Neem extract on *Myzus persicae*

S.No	Extract	Number of aphids	Mortality (Mean ±S.E)		Type of action
			12hr	24hr	
1	Control	30	1.33±0.33	1.66±0.33	
2	Neem + black pepper	30	7.33± 0.66	10.66± 0.33	Synergistic
3	Neem +clove	30	10.66± 0.66	19.33± 0.88	Synergistic
4	Neem+ bay leaf	30	4.66± 0.33	7.33± 0.33	Synergistic

Three of the five replicas were considered; Test significance is $\boldsymbol{p > 0.05}$

Table 3 Repellency effect of different extracts on *Myzus persicae*

S. No	Plants used for Extract	Number of aphids	Number of aphids on untreated area			Number of aphids on treated area			Mean±S.E
			A	B	C	a	b	c	
1	Neem	10	9	8	8	1	2	2	8.33±0.33
2	Black pepper	10	5	6	6	5	4	4	5.66±0.33
3	Clove	10	7	7	6	3	3	4	6.66±0.33
4	Bay leaf	10	2	2	3	8	8	7	2.33±0.33

Three out of five replications are considered; Test significance is$\boldsymbol{P < 0.05}$

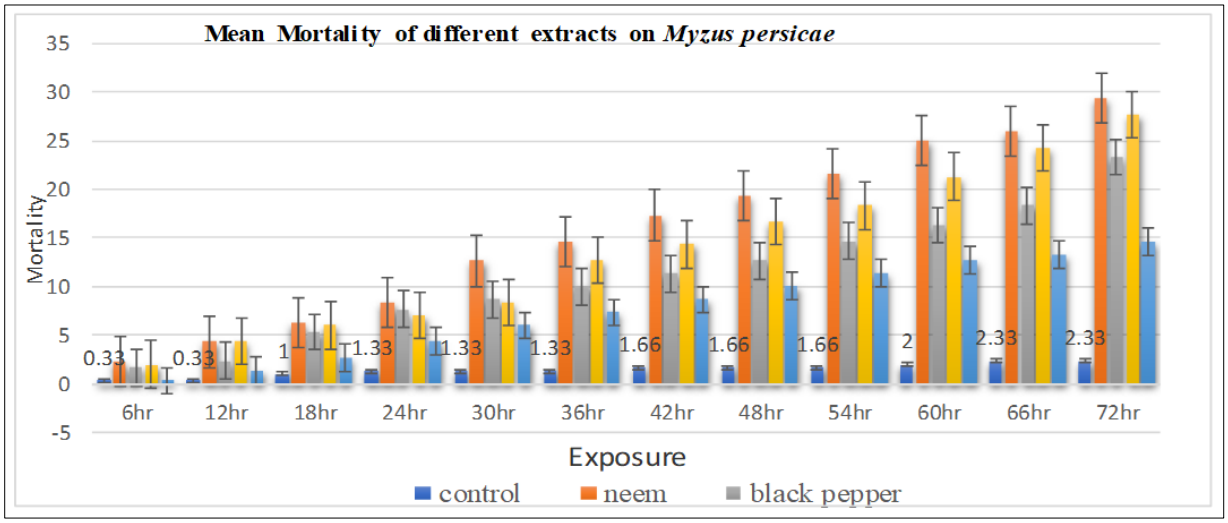

Figure 6 Mean mortality effect of different extracts on *Myzus persicae*

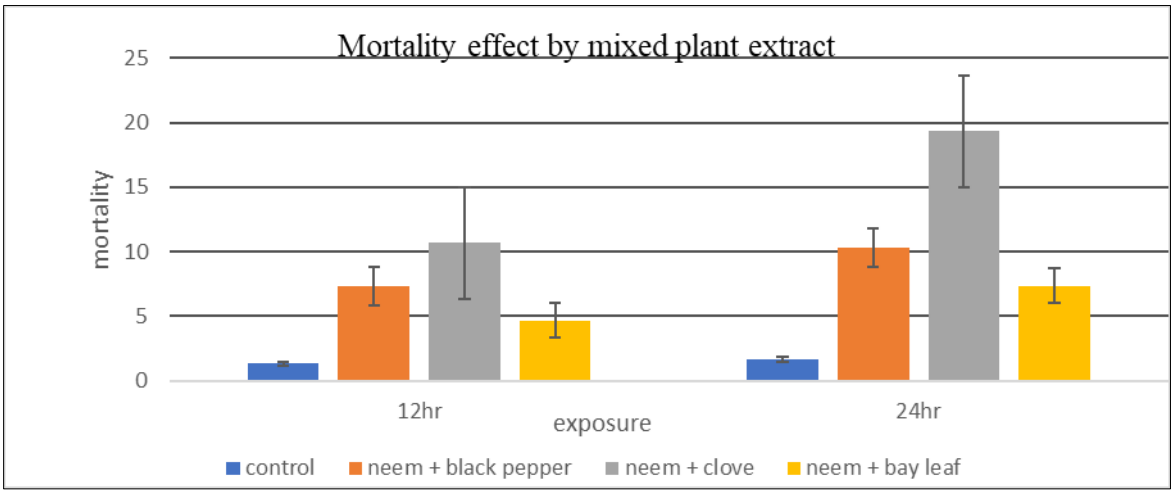

Figure 7 Mean mortality effect of different extracts mixed with Neem extract on *Myzus persicae*

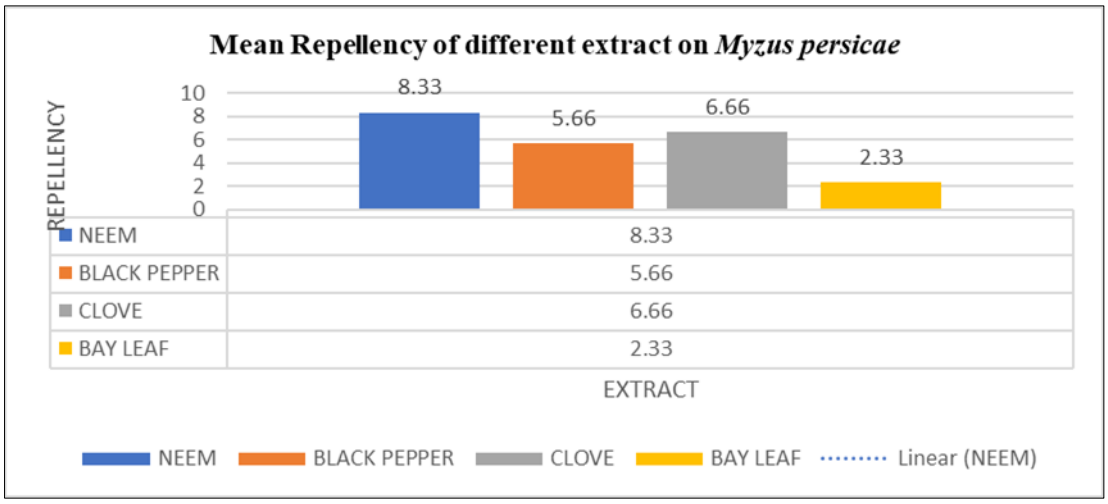

Figure 8 Mean of repellency effect of different extracts on *Myzus persicae*

4. Discussion

Parallel work found out that, during the nymphal period of Aphid, mortality rates reached 100.0% at the highest concentration, indicating significant and potentially lethal effects when treated with neem bio-pesticide (Santos et. al., 2004), which stands true for this experiment as the mortality rate of replica treated with Neem extract reaches 97.76% (Table1 and Figure6). Another study showed, Neem seed oil (NSO) at a 1.0% concentration induced mortality ranging from 94% to 100% in second instar nymphs of the green peach aphid, *Myzus persicae* (Sulzer) (Murray, 1994). Another showed that the Hydroethanolic extract derived from neem leaves exhibited mortality rates exceeding 95% on *Myzus persicae*, indicating significant efficacy against this species (Déla, et. al., 2014). Neem leaf extract contains saponins, meliantriol, and azadirachtin, acting as vegetable insecticides against aphids. Saponins are stomach and contact poisons, meliantriol works as a repellent, while azadirachtin inhibits aphids' hormone function, crucial for their development. (Tobing, et. al., 2023), which stands true with this experiment (Table1 and Figure6).

The estimated lethal concentration (LC95) of clove essential oil, designed to terminate 95% of aphids, in the work of (Pedro, et al., 2020), which stands true for this experiment as the mortality rate of replica treated with Neem extract reaches 97.7% (Table1 and Figure6). Neem oil exhibited the highest nymph mortality rates at 96.67%, 100%, and 100% in concentirations of 1%, 3%, and 5% respectively, after 72 hours. Clove oil followed with 80.00% mortality of *Myzus persicae* seen in (Sonowal, et al., 2023). The methanol extracts from *Azadirachta indica* leaves, at concentrations of both 5% and 10%, provided complete protection (100%) to lentil and chickpea seeds from aphids by (Bhuiyah, et al., 2003). Therefore, this study indicates that neem exhibits the highest mortality rates, followed by clove, attributed to the presence of Azadirachta and eugenol respectively. Our findings align closely with (Haddi, et al., 2015) studies indicating that clove oil possesses insecticidal toxicity against *Sitophilus zeamais* Motschulsky, attributed to eugenol and the sesquiterpene β-caryophyllene. (Table1 and Figure6)

Another study of (Ahmed, et. al., 2021) states that, Black pepper essential oil has been identified as a potent insecticide, demonstrating significant efficacy with an impressive 80% mortality rate upon contact application. The bay leaf extract demonstrated minimal efficacy, with less than 50 percent mortality recorded during the shortest exposure time by (Kaur, et. al., 2018) (Table1 and Figure6).

In a recent study, the efficacy of neem against aphids was examined, revealing a repellency rate of 68.9%. This finding underscores the potential of neem as a natural and effective means of aphid control in agricultural settings. In another recent study (Hartati, 2020), the synergistic effect of a blend containing clove and neem extracts was assessed, resulting in an observed repellency rate of more than 30.33% against pest. The combined utilization of biopesticides, specifically *A. indica* and *E. globulus*, at a concentration of 50%, has proven highly efficacious in combating wheat aphids (Baig & Yousaf, 2021). Furthermore, the synergistic potency of these botanicals surpasses their individual effectiveness when applied alone (Table2 and Figure7). In the recent scientific investigation, (Adhikari, et. al., 2020) empirical data indicated that neem exhibits an 83% repellent efficacy, thereby providing further validation for the results obtained in the conducted experiment. In (Lacotte, et. al., 2023) it was demonstrated that the repellent efficacy of clove, black pepper and bay leaves is measured at 40%, 35% and 23.3% respectively, thus providing empirical validation for the outcomes of the conducted experiment (Table3 and Figure8).

5. Conclusion

In conclusion, the conducted experiment confirmed the high effectiveness of neem against *Myzus persicae*, with a repellency rate exceeding that of clove and black pepper extracts. However, while clove and black pepper demonstrated notable repellent properties, bay leaf extract emerged as the least effective bio-pesticide in combating aphids. These findings underscore the potential of neem as a natural and potent solution for aphid control, highlighting the importance of continued research into botanical alternatives for pest management in agriculture.

Compliance with ethical standards

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Disclosure of conflict of interest

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

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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