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Efficacy of *Melia azedarach* Extract Combined with Low-Dose Acetamiprid Against Cabbage Aphid in Urban Landscapes

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Abstract

Brevicoryne brassicae L., a major pest of brassica crops, is typically controlled with chemical pesticides; however, excessive use leads to resistance development, environmental contamination, and health hazards. Combining synthetic pesticides with botanical extracts offers a promising strategy to reduce pesticide doses while maintaining efficacy. This study evaluates the combined effect of acetamiprid and an ethanolic-water extract of *Melia azedarach* L. (Meliaceae) on cabbage aphids and their nymph production. Results showed that the treatment with acetamiprid (100 ppm) and *M. azedarach* ethanolic-water extract (2000 ppm) had the lowest population averages at all-time points: 2.75 ± 0.64 aphids on day 7, 10.60 ± 2.78 on day 14, and 29.5 ± 8.15 on day 21. The total nymph production over ten days was highest in the control treatment (33.67 ± 1.27), while the lowest average was observed in the acetamiprid (10 ppm) and *M. azedarach* (2000 ppm) treatment (26.98 ± 1.6). Furthermore, the total mortality percentages over ten days showed that the combination of acetamiprid (10 ppm) and *M. azedarach* (2000 ppm) caused the highest cumulative mortality (60.0 ± 4.21%), while the control group exhibited the lowest mortality (30.0 ± 4.47%). These findings indicate that combining pesticides with plant extracts may reduce the required pesticide dosage while maintaining effective pest control, although further research is needed to confirm these findings.

Keywords: Aphid management, Biopesticides, Insecticide synergy, Integrated pest management, Urban pest control.

INTRODUCTION

The cabbage aphid [*Brevicoryne brassicae* L. (Hemiptera: Aphididae)] is a major pest affecting plants in the Brassicaceae family. It is known for its ability to rapidly proliferate, causing significant damage through sap feeding and transmitting over 20 viral diseases (Pontoppidan *et al*., 2003). Ornamental cabbage (*Brassica oleracea* L. var. Acephala), a biennial plant from the Brassicaceae family, is commonly cultivated in urban green spaces during colder seasons due to its frost tolerance and attractive foliage (Ghasemi Ghehsareh, 2023). This aphid species, which lacks an alternative host, can complete both holocyclic and anholocyclic life cycles on cruciferous plants (Gabryś *et al*., 1997). During winter, *B. brassicae* often establishes dense colonies beneath the younger leaves of ornamental cabbage. These overwintering populations frequently serve as primary infestation sources for spring crops, potentially resulting in substantial early-season damage (Saeidi *et al*., 2012).

After feeding on host plants, cabbage aphid populations expand rapidly, causing leaves to curl inward and eventually discolor (Araya *et al*., 2023). Although, chemical insecticides are effective in managing this pest, their drawbacks include the development of pest resistance and the elimination of natural enemy populations, highlighting the necessity of alternative pest management approaches (Pedigo, 2014). Among the available alternatives, botanical pesticides are distinguished by their relatively low toxicity to non-target organisms, including fish, mammals, and pollinators (Isman, 2006). One promising botanical pesticide is Persian lilac, *Melia azedarach* L. (Meliaceae) extract, an eco-friendly solution with demonstrated insecticidal activity (Shafiei *et al*., 2018). For instance, a formulation containing 5% *M. azedarach* fruit extract combined with 0.2 % soap significantly reduced cabbage aphid populations in controlled studies (Nagappan, 2012). Studies have shown that increasing the concentration of *M. azedarach* extract significantly reduces the longevity of pests and helps maintain aphid populations below the economic injury threshold (Forouhar *et al.*, 2024). This botanical insecticide has demonstrated effectiveness against various aphid species, including cabbage aphids (*Brevicoryne brassicae*) and greenbug aphids (*Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), with effects that extend beyond the initial treatment to provide prolonged pest control (Pahlavan Yali and Mohammadi Anaii, 2017; Shafiei *et al*., 2018; Khan, 2021; McKenna *et al*., 2013; Forouhar *et al.*, 2024). Additionally, the extract was effective in reducing egg, larval, and pupal populations of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). It was also found to be safe for natural predators such as *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) (Kibrom *et al*., 2012; Rasouli *et al*., 2024).

The anti-feeding properties of *M. azedarach* extract have been documented, particularly its significant reduction in the feeding activity of elm leaf beetle larvae, *Pyrrhalta luteola* (Muller) (Coleoptera: Chrysomelidae) when treated with ethanolic extracts (Valladares *et al.*, 1997). Acetamiprid has been used to control a variety of pests, including aphids (Li *et al*., 2024). High mortality rates of rose aphids *Macrosiphum rosae* (L.) (Hemiptera: Aphididae) have been observed with the application of acetamiprid (Jafari Nasab *et al*., 2015). Acetamiprid also demonstrated effective control of cabbage aphids, resulting in high mortality rates for both adults and nymphs five days' post-application (Abu-Duka and Mohammadali, 2021). The systemic action of acetamiprid enhances its efficacy, with studies showing its effectiveness against *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and its ability to reduce aphid fertility (Bacci *et al*., 2007). The combination of botanical and synthetic insecticides offers an effective approach to pest control, particularly when pests develop resistance to chemical pesticides (Dash *et al*., 2019).

By blending these two insecticide types, pest control efficacy can be enhanced while minimizing pesticide use. This reduction not only helps lower environmental pollution but also minimizes pesticide residues in agricultural products. Furthermore, this combination increases the overall toxicity and effectiveness of the insecticides, allowing for better management of pest populations (Isman, 2006; Maurya *et al*., 2012; Yi *et al*., 2012; Khan *et al*., 2013; War *et al*., 2014; Dash *et al*., 2019).

These synergistic effects lead to less reliance on high doses of individual insecticides, promoting more sustainable pest management strategies. In light of the need for effective cost management, reduced pesticide residues in urban environments, and the enhancement of the aesthetic appeal of urban green spaces, this study investigates the impact of sub-recommended concentrations of acetamiprid in combination with *M. azedarach* extract on the populations and reproductive performance of cabbage aphids.

These effects are evaluated both in urban landscape conditions and controlled laboratory environments. Such investigations are crucial for developing sustainable pest management strategies that mitigate the environmental impact of chemical pesticides, while preserving the functional and aesthetic qualities of urban green spaces (Isman, 2006; Mckenna *et al*., 2013; Pedigo, 2014).

MATERIALS AND METHODS

Treatments under study

Treatments in green spaces: The objective of this study was to evaluate the effect of sub-recommended concentrations of acetamiprid on controlling the population of cabbage aphids (*B. brassicae*) infesting ornamental cabbage. The experimental design included the following treatments:

•Treatment 1 (T1): A suspension of acetamiprid insecticide at a sub-lethal concentration of 10 ppm, corresponding to 2 ppm of active ingredient (Rajabi *et al*., 2023), utilizing a commercial formulation (20% SP) manufactured by Zhejiang Hisun Chemical Co., Ltd., China.

•Treatment 2 (T2): A suspension of acetamiprid insecticide at 100 ppm, corresponding to 20 ppm of active ingredient, using a commercial formulation (20% SP) by the same company.

•Treatment 3 (T3): A suspension of acetamiprid insecticide at 100 ppm, using a commercial formulation (20 % SP) from the same manufacturer, combined with an ethanolic-water extract of *M. azedarach* at 2000 ppm, prepared by the technology unit (SGZP) at Shahid Bahonar University of Kerman.

•Control (C): Water was employed as the untreated control.

The experimental treatments applied in the laboratory

•Treatment 1 (T1): Suspension of acetamiprid insecticide at a concentration of 10 ppm, in the form of a water-soluble powder (SP-20).

•Treatment 2 (T2): A combination of 10 ppm acetamiprid and an ethanolic-water extract of *M. azedarach* at a concentration of 2000 ppm.

•Treatment 3 (T3): Ethanolic-water extract of *M. azedarach* at a concentration of 2000 ppm.

•Control (C): Water as the control treatment.

Field experimentation in green space with population assessment

This study was conducted in the green space of Zarand city, Kerman province (coordinates: 30° 49′ 16″ N, 56° 34′ 41″ E, 1676 m a.s.l.) on ornamental cabbage plants, each infested with an average of 40 cabbage aphids, which (Shah *et al*., 2020) reported as being at the threshold of economic damage. Ten infested cabbage pots were randomly placed in 1 m² plots with 20 cm spacing between them. Spraying was done once using a 20 L backpack sprayer with fixed volume and uniform spraying pattern. Sampling of aphid populations occurred at 7, 14, and 21 days after spraying, with leaves collected from 10 randomly selected plants per treatment. The collected leaves were transported to the lab, and aphids (nymphs, wingless adults, and winged adults) were counted under a stereo microscope using a 1.5 × 1.5 cm template. The live aphid population was counted separately for each type and used for statistical analysis.

Impact of various treatments on nymph production and female mortality under laboratory conditions

To establish an aphid colony, 40 petri dishes containing 0.7 % agar gel were prepared, with a 4 cm diameter cabbage leaf disc placed on the surface of the agar. Five aphids (from the underside of the leaves) were transferred onto each leaf disc. After allowing time for the aphids to acclimate, the petri dishes were placed in a controlled growth chamber with the following conditions: Temperature 25 ± 2 °C, humidity 50 ± 10%, 16 hours of light. The petri dishes were covered with fabric mesh and inspected daily at 08:00 AM. Aphids producing nymphs were collected during these inspections. For each experimental treatment, ten petri dishes with the same setup were used. On each leaf disc, five aphids of the same age, capable of producing nymphs, were placed, and a five-minute settling period was allowed before treatments were applied. The petri dishes were arranged in three rows on a laboratory bench, with no spacing between them, and sprayed using a laboratory reagent sprayer. After spraying, the petri dishes were covered with mesh and returned to the controlled growth chamber. After 24 hours, the first round of counting aphid nymphs and dead female aphids was performed under a stereomicroscope, and data were recorded. The counted nymphs and dead aphids were removed from the leaf discs, and this process continued for ten days. The aphid nymph and dead female aphid populations were used for statistical analysis.

Data analysis

To validate the underlying assumptions of the data, they were initially assessed for normality and homogeneity of variance using Bartlett's test (Köhler *et al*., 2012). As certain datasets violated the normality assumption essential for parametric analyses, the Box-Cox transformation was employed to stabilize variances and approximate a normal distribution. The resulting data were analyzed via one-way ANOVA using Statplus statistical software (version 4.9, 2007), with Fisher's LSD post-hoc test applied for mean comparisons.

RESULTS

Field experimentation in green space with population assessment

The experiment results indicated that seven days post-spraying (Fig. 1A), treatment C had the highest population average (46.87 ± 1.61), while the lowest was in T3 (2.11 ± 0.45), with significant differences among all treatments ($P < 0.0111$). In fig. 1B, T1 showed the highest average (24.66 ± 5.42) and T3 the lowest (0.66 ± 0.28). Treatments T2 and T3 differed significantly from C and T1 ($P < 0.0005$). Fig. 1C revealed the highest population average in treatment C (1.1 ± 0.31), whereas T1 (0.2 ± 0.13) and T2 (0.2 ± 0.2) had the lowest, with significant differences between C and the other treatments ($P < 0.0039$). Lastly, fig. 1D demonstrated that treatment C had the highest population average (63.62 ± 3.14) and T3 the lowest (2.75 ± 0.64), with significant differences across all treatments ($P < 0.0051$).

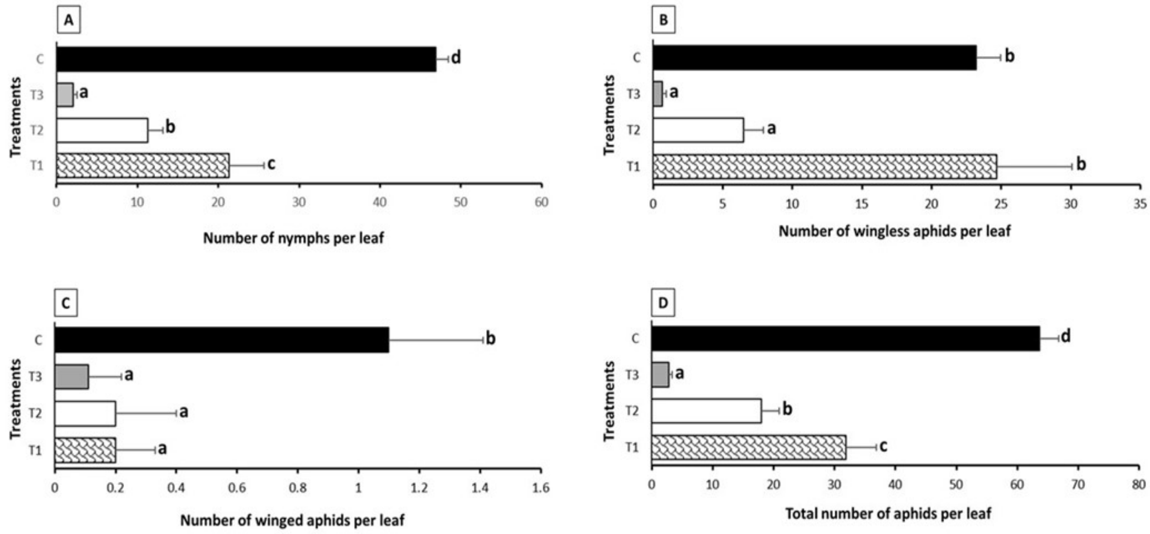

Fig. 1. The impact of acetamiprid at 10 ppm (T1), acetamiprid at 100 ppm (T2), acetamiprid (100 ppm) + *M. azedarach* (2000 ppm) (T3), and the control (C) on the population of various life stages of cabbage aphids, recorded 7 days post-treatment.

Fourteen days post-spraying, results showed that in fig. 2A, treatment C had the highest population average (49.5 ± 3.37) and T3 the lowest (6.8 ± 1.71), with significant differences observed between T3 and T2, C ($P < 0.0007$) as well as T2 and C ($P < 0.0196$). In fig. 2B, treatment C recorded the highest average (26.9 ± 3.04), and T3 the lowest (3.6 ± 1.12), with T3 significantly differing from T1, T2, and C ($P < 0.0095$). According to fig. 2C, treatment C exhibited the highest average (1.1 ± 0.3), while T3 had the lowest (0.12 ± 0.13), showing significant differences between C and all other treatments ($P < 0.0136$). In fig. 2D, treatment C achieved the highest population average (72.4 ± 4.0), with T3 recording the lowest (10.6 ± 2.78), differing significantly from T1, T2, and C ($P < 0.0001$).

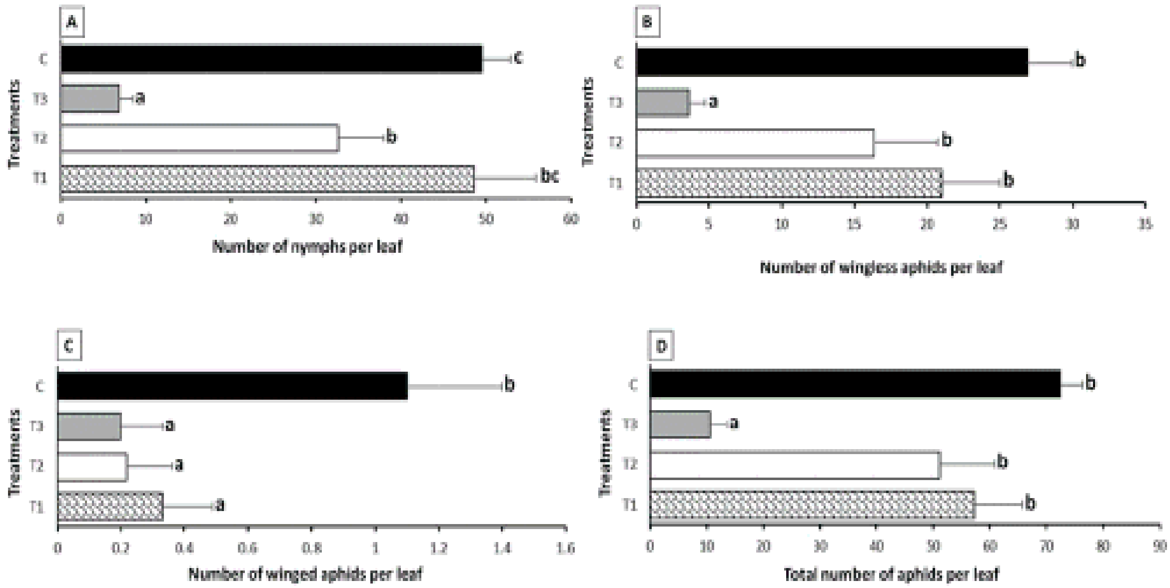

Fig. 2. The impact of acetamiprid at 10 ppm (T1), acetamiprid at 100 ppm (T2), acetamiprid (100 ppm) + *M. azedarach* (2000 ppm) (T3), and the control (C) on the population of various life stages of cabbage aphids, recorded 14 days post-treatment.

Twenty-one days post-spraying, fig. 3A showed the highest population average in treatment C (50.5 ± 2.83) and the lowest in T3 (20.3 ± 6.38), with significant differences between T3 and treatments C and T1 ($P < 0.0225$). In fig. 3B, treatment C had the highest average (26.9 ± 3.04) and T3 the lowest (9.2 ± 2.25), with T3 differing significantly from all treatments ($P < 0.0235$). Fig. 3C indicated that treatment C had the highest population average (1.1 ± 0.31), while T3 recorded zero, showing significant differences only between T3 and C ($P < 0.0007$). In fig. 3D, treatment C had the highest population average (78.5 ± 5.79) and T3 the lowest (29.5 ± 8.15), with significant differences between T3 and treatments C and T1 ($P < 0.0043$).

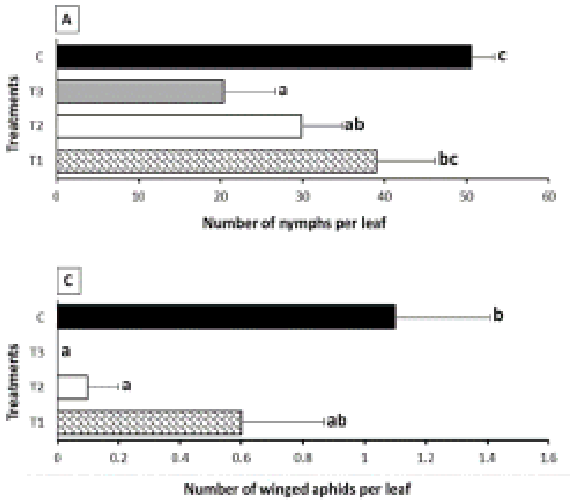

Fig. 3. The impact of acetamiprid at 10 ppm (T1), Acetamiprid at 100 ppm (T2), acetamiprid (100 ppm) + *M. azedarach* (2000 ppm) (T3), and the control (C) on the population of various life stages of cabbage aphids, recorded 21 days post-treatment.

Impact of various treatments on nymph production and female mortality under laboratory conditions

Fig. 4A shows the daily average nymph production per female. From the first to the fourth day, the highest averages were recorded in treatments C and T1, while the lowest were observed in T2 and T3. On the fifth and sixth days, treatments T1 and T3 showed the highest averages, with T2 having the lowest. On the seventh and eighth days, treatment T3 recorded the highest averages. On the ninth day, treatment C had the highest average, while treatment T1 had the lowest. On the tenth day, treatment T3 again had the highest average, with treatment T1 having the lowest. The total nymph production over the ten-day period, shown in fig. 4B, revealed that treatment C had the highest average (33.67 ± 1.27), while treatment T2 had the lowest average (26.98 ± 1.6). Statistically significant differences were found between treatment T2 and both C and T3 ($P < 0.0029$).

The mean daily mortality of ovipositing females is depicted in fig. 5A. Across the ten days, treatment T2 consistently exhibited the highest mortality rates, starting from 0.5 ± 0.16 on the first day and increasing to 1.1 ± 0.1 on the tenth day. In contrast, the control group (C) recorded the lowest mortality rates throughout, ranging from 0.0 on the first day to 0.5 ± 0.16 on the tenth day. Treatment T3 showed intermediate results, with values similar to C in the later days. The total mortality percentages over the ten-day period, as shown in fig. 5B, revealed that T2 had the highest cumulative mortality (60.0 ± 4.21%), significantly different from other treatments ($P < 0.0001$). The control group (C) had the lowest mortality (30.0 ± 4.47%), with no significant differences observed among other treatments compared to C.

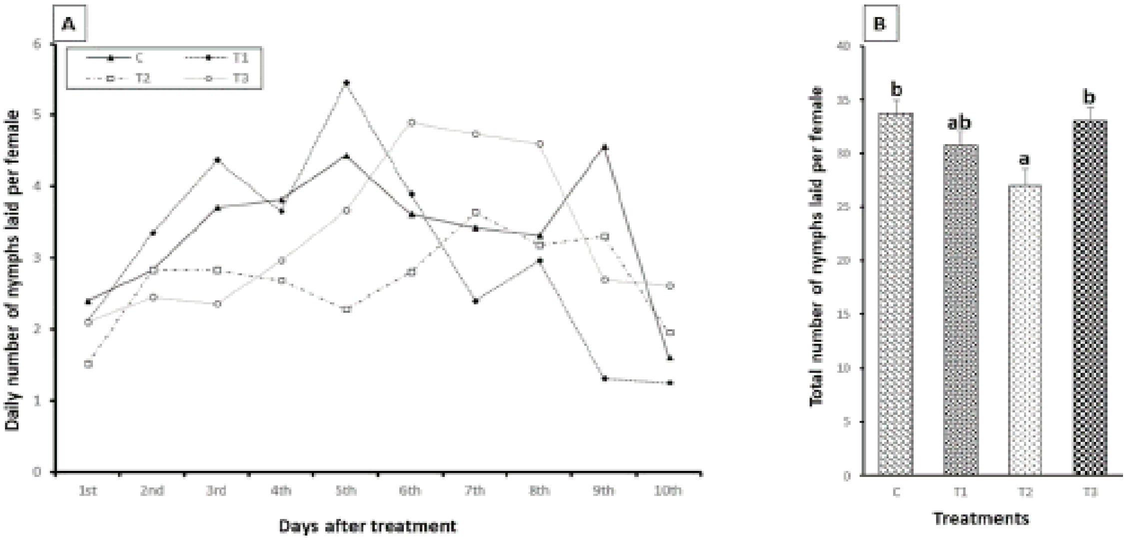

Fig. 4. The effect of acetamiprid 10 ppm (T1), a combination of acetamiprid 10 ppm and *Melia azedarach* L. extract 2000 ppm (T2), *M. azedarach* extract 2000 ppm (T3), and control (C) on the mean daily number of nymphs produced per female (A) and the total mean number of nymphs produced per female over a ten-day period (B).

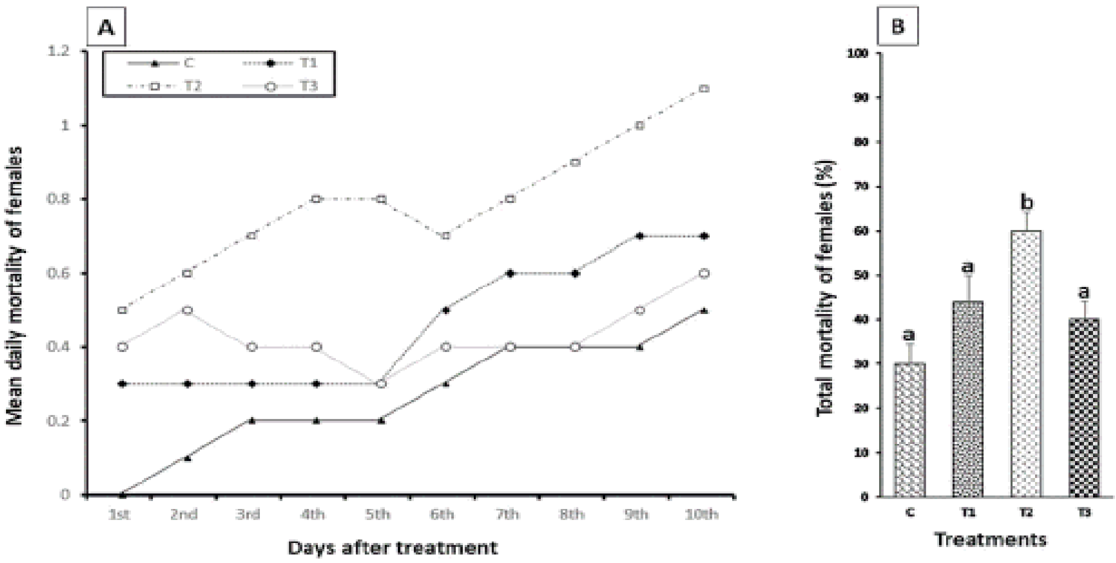

Fig. 5. The effect of acetamiprid (10 ppm, T1), a combination of acetamiprid (10 ppm) and *Melia azedarach* extract (2000 ppm, T2), *M. azedarach* extract (2000 ppm, T3), and Control (C) on the mean daily mortality of females (A) and the total female mortality percentage over a ten-day period (B).

DISCUSSION

Based on the results of the present study conducted in urban green spaces, it was observed that seven days post-spraying, treatments (T1), (T2), and (T3) significantly reduced aphid populations compared to the control treatment (C). This finding is consistent with the results of Abu-Duka and Mohammadali (2021), who reported a 99.91% mortality rate for adult aphids and a 99.94 % mortality rate for cabbage aphid nymphs five days after spraying with acetamiprid at a concentration of 500 ppm. The lower mortality observed in the present study relative to the aforementioned research could be attributed to differences in the concentrations used. Bacci *et al*. (2007) suggested that the systemic action of acetamiprid facilitates rapid distribution throughout the plant, leading to interference with nicotinic receptors, which contributes to aphid mortality. The results from the initial counting showed that treatment (T3) exhibited superior performance compared to treatments (T1) and (T2), with significant differences between these treatments. This suggests that the combination of acetamiprid and *M. azedarach* extract can significantly reduce cabbage aphid populations. In the second and third counting periods, treatment (T3) effectively kept aphid populations below the economic threshold, while the other treatments did not demonstrate significant efficacy in controlling aphid numbers. The results of this study are similar to the findings of Dash *et al.* (2019). Their study showed that the combination of imidacloprid 17.8% SL at 17.5 ml a.i./ha with multineem (300 ppm) at 1.25 l/ha effectively reduced the populations of white-backed plant hopper and brown plant hopper, making it a recommended approach for their control in rice. Additionally, the combined use of imidacloprid and *Ocimum basilicum* L. (Lamiaceae) extract, as highlighted by Maurya *et al*. (2012), demonstrated synergistic effects and high larvicidal activity against *Anopheles stephensi* Liston (Diptera: Culicidae), while being safe for non-target aquatic organisms. Plant-derived chemicals can induce alterations in feeding behavior and digestive enzyme activities, as demonstrated in the study by War *et al.* (2014). Their findings revealed that the combination of neem oil and endosulfan significantly enhanced antifeedant activity and led to significant changes in the activities of digestive and detoxifying enzymes in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae. This suggests that such a combination could help reduce the required dosage of endosulfan. In addition, plant-based compounds may have a better effect on resistant insects, as highlighted in the study by Khan *et al.* (2013). Their research demonstrated that insecticide mixtures, particularly pyrethroids combined with other compounds, can enhance toxicity in resistant house fly populations, providing a potential strategy for resistance management. The synergistic effects of plant compounds vary depending on the substances combined and their ratios, as noted in Yi *et al*. (2012). Their study found that the Destruxins (Des) to Rotenone (Rot) 1:9 ratio exhibited the strongest synergistic effect against *Aphis gossypii* Glover (Hemiptera: Aphididae) showing high toxicity in both laboratory and greenhouse tests. The highest total nymph production was recorded in the control treatment, while the lowest production was observed in the combination of acetamiprid and *M. azedarach*. Furthermore, the highest cumulative mortality was noted in the acetamiprid and *M. azedarach* mixture compared to the control group. The results of this study suggest that changes in aphid nymph production may be attributed to the combined effect of acetamiprid and the plant extract on the feeding behavior of aphids, leading to starvation and consequently impacting nymph production. This phenomenon was explored in the study by Xu *et al*. (2019), which demonstrated that periods of starvation affect ovarian development and reproduction in *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae). Under starvation conditions, the number of offspring produced decreased, while the number and volume of mature embryos increased. Additionally, lifespan and fecundity were reduced in starved aphids, but these aphids contained more mature embryos than those with shorter starvation periods. The use of this combination could effectively manage pest populations by reducing both nymphs and reproductive females, offering an efficient strategy for pest control. The research by Shonga and Getu (2021) demonstrated that plant-based insecticides, particularly a mixture of garlic, onion, and pepper, along with synthetic insecticides such as imidacloprid, significantly reduced cabbage aphid populations on Ethiopian kale, while posing minimal harm to coccinellid predators, thus providing a promising alternative for integrated pest management (IPM).

CONCLUSION

This study found that the combination of acetamiprid and *M. azedarach* extract effectively reduced aphid populations in urban green spaces. The mixture reduced nymph production and increased aphid mortality, keeping populations below the economic threshold in subsequent periods. These results support the use of combined chemical and plant-based insecticides as a promising strategy for integrated pest management (IPM) in controlling aphid populations.

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