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Efficacy of some botanical insecticides against *Aphis gossypii* Glover (Hemiptera: Aphididae) on chrysanthemum

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Abstract

An evaluation of various botanical insecticides to control *Aphis gossypii* and its impact on aphid population dynamics on chrysanthemum plants was investigated. In order to control *A. gossypii* on chrysanthemum, the effectiveness of several botanical insecticides extracted from *Melia azedarach*, *Toona sinensis*, and *Chrysanthemum cinerariaefolium* was investigated in the current study. The research was carried out in the experimental field of the Indonesian Ornamental Crops Research Institute under plastic house conditions. Five concentrations, *i.e.*, 1.5, 2.0, 2.5, 3.0, and 3.5 g/L of three plant extracts, *M. azedarach*, *T. sinensis*, and *C. cinerariaefolium*, were sprayed on chrysanthemum cultivar White Fiji after 28 to 84 days after planting. *A. gossypii* had a dynamic population that changed according to the plant's developmental stages. In vegetative growth, the alate adult and nymphal stages were dominant, and the population of nymphs increased along the plant ages. The insect colonized young leaf surfaces in the terminal apices. During the reproductive stages, the population of alate adults diminished, and the distribution of the insect extended to mature, old leaves, flower buds, and bloomed flowers. The application of several botanical insecticides revealed various responses of aphid populations. Among the tested insecticides, *C. cinerariaefolium* extract at 3.0 and 3.5 g/L demonstrated the highest average percentage efficacy (76 and 72%) and was the most consistent in suppressing the population. The results of this study indicate the potential efficacy of botanical insecticides against *A. gossypii* suggesting a different approach to efficient and environment-friendly chrysanthemum pest management.

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Introduction

Chrysanthemum (*Dendrathema grandiflora* Tzvelev syn. *Chrysanthemum morifolium* [Ramat.] Kitam) in the form of cut flowers and potted plants is one of the top marketed ornamentals in the world (Zhang *et al*., 2020; El-Sayed and El-Ziat, 2021). In international trade, the commodity is ranked first among cut flowers and acquires approximately 35% of the world's market requests for cut flowers, which is second to roses (El-Sayed and El-Ziat, 2021). The Netherlands, Italy, Columbia, Spain, Germany, and the United States are the leading producers that supply more than 60% of the world trade for chrysanthemums (FloraCulture International, 2021). In Indonesia, chrysanthemum is usually grown commercially in the highlands, referring to environmental adaptation from their temperate origins (Sanjaya *et al*., 2015). Since 2006, chrysanthemums have replaced roses as the most popular fresh-cut flowers in the Indonesian floriculture market. It raised the floriculture contribution to the national gross domestic product by more than nine trillion Indonesian rupiahs (Kurniasih *et al*., 2016).

In the production process, however, several obstacles still constrain growers to produce high-quality and marketable cut flowers. One of the most common problems is pest and disease attacks, especially insect pests like aphids. *Aphis gossypii* Glover (Hemiptera: Aphididae) is one of the 15 aphid species that commonly inhabit

and attack chrysanthemums (Miller and Stoetzel, 1997; Bethke *et al*., 2003). The insect may attack all plant stages, and the colony can generally be found on the leaf and flower buds (Davies *et al*., 2004; Fu *et al*., 2018). In severe incidences, the attacks may cause organ malformation, and large colonies of aphids can significantly reduce plant vigor and kill the plant through mechanical injury (Zhang *et al*., 2020; Rahardjo *et al*., 2021). The aphids excreted a sticky substance called honeydew, which accumulated on the leaves and flowers. In the higher humidity of a greenhouse, honeydew provides an excellent substrate for the growth of black sooty mold. Large areas of mold covering the leaves can reduce photosynthesis and result in an unattractive plant with a much lower market value (Margaritopoulos *et al*., 2006; Zhang *et al*., 2020). The insect is also known as the vector of Cucumber Mosaic Virus and Chrysanthemum Stunt Viroid, now becoming a problem in several production centers (Fu *et al*., 2018).

Since a flower's physical quality is critical, growers tend to use various synthetic pesticides with inappropriate dosages and frequencies to reduce damage. These practices might cost 13-32% of the total production (Margaritopoulos *et al*., 2006) and make the business uncompetitive (Zhang *et al*., 2020; Rahardjo *et al*., 2021). The application of synthetic pesticides leaves toxic chemicals and hazardous residues in the plant, soil, and water that, in the long term, might accumulate and affect environmental quality and human health . Several reports have indicated that the use of long-term synthetic insecticides, such as phenyitrothion, pyrimiphos-methyl, carbosulfant, aldicarb, lambda-cyhalothrin, deltamethrin, and imidacloprid, to control *A. gossypii* has raised pest resurgence, the explosion of secondary pests, and new resistant aphid biotype populations (Koo *et al*., 2014; Wang *et al*., 2021).

One promising way to reduce the negative impact of synthetic insecticides is to replace them with botanical insecticides. Botanical insecticides contain biologically active plant extract components and are naturally degraded into harmless and non-toxic elements in a certain period; thus, they are considered safer for the environment and human health (Saleem *et al*., 2019; Shimira *et al*., 2021). Several authors were also convinced that the botanical insecticides did not distract the natural enemies and had high specificity, thus effective only on the targeted insect pest (Gouvea *et al*., 2019; Hutapea *et al*., 2019). The insecticide interferes with and inhibits metamorphic growth and reproductive phases, acting as a feeding deterrent/antifeedant, ovipositor restraint, and repellent of insect pests (Ahmad *et al*., 2019; Shimira *et al*., 2021).

The extracts of certain plants have the potential to be used as botanical insecticides. *Melia azedarach* L. (Meliaceae), with many common names such as chinaberry tree, pride of India, bead-tree, cape lilac, syringa berry tree, Persian lilac, Indian lilac, and white chedar, contains secondary metabolites such as toosendanine, margoside, kaempferol, resin, tannin, n-triacontane, β-sitosterol, and triterpene quinone that act as repellents and antifeedants, thus repressing pest populations (Baldin *et al*., 2020; Shaurub *et al*., 2022). Moreover, Nagappan (2012) reported that the application of 5% seed extract of *Melia azedarach* (Meliaceae) suppressed the aphid species *Brevicoryne brassicae* (Lin.) population up to 70% in cabbage while using leaf extract, the development of citrus leafminer *Phyllocnistis citrella* Stainton larvae was inhibited significantly (Mckenna *et al*., 2013).

Aside from *M. azedarach*, *Toona sinensis* with the common names of Chinese Mahogany, Chinese Toon, or Red Toon, also a member of Meliaceae, was also reported to be utilized as an insecticide on several crops. Several metabolite compounds such as limonoid, flavonoid, phytol, coumarins, and norcyteine derivatives contained in the plant were found to drive the insect away (Meng *et al*., 2016; Adfa *et al*., 2017). *T. sinensis* was also reported to have insecticidal and antifeedant compounds, surenon, surenin, and surenolakton, with high concentrations in the seed and leaf. Extracts from leaves and seeds were reported to be effective in controlling the chrysanthemum aphid *Macrosiphoniella sanborni* (Rahardjo *et al*., 2021), *Aphis gossypii* in gerbera (Hutapea *et al*., 2020), and *Bemisia tabaci* in tomatoes (Baldin *et al*., 2020).

Pyrethrins are known to be active biological components that have insecticidal features. Aside from pyrethrin, other compounds contained in pyrethrum are cinerin I and II, jasmolin I and II, and (E)-β-farnesene which also have insecticidal activity (Gallo *et al*., 2017; Li *et al*., 2019). This compound was found in *Chrysanthemum cinerariaefolium* (Asteraceae), which varied in concentration within the dried flower (Shimira *et al*., 2021; Wang *et al*., 2021). Pyrethrins interfere with the neural system of the insect by inhibiting impulse flows on the axon cell, resulting in an imbalance of preposition and orientation, and finally, the death of the insects (Xu *et al*., 2017; Li *et al*., 2019). The effectiveness of pyrethrum in controlling insect pests has been reported in chrysanthemum leafminer *Lyriomyza* spp. (Rahardjo *et al*., 2020) and thrips with a lethal concentration of 12.9 mg/ml (Shimira *et al*., 2021). Pyrethrins were also reported to have repellent characteristics for flies, mosquitoes, and some insects on pets (Gallo *et al*., 2017).

The present study aimed to evaluate some botanical insecticides extracted from *M. azedarach*, *T. sinensis*, and *C. cinerariaefolium* to control *A. gossypii* on chrysanthemum simultaneously. The study also focused on the population dynamics of aphids in relation to the application of these botanical insecticides. The result of the study was expected to become a basis for the improvement of an application on integrated pest management in the national chrysanthemum production system.

Materials and Methods

The research was conducted at the experimental field of the Indonesian Ornamental Crops Research Institute at 1100 meters above sea level, under plastic house conditions. A complete block experiment with three replications was designed to evaluate several botanical insecticide formulations on chrysanthemum cultivar White Fiji. The treatment was described as follows:

- *T. sinensis* leaf extract with concentrations of 1.5, 2.0, 2.5, 3.0, 3.5 g/L;
- *C. cinerariaefolium* flower extract with concentrations of 1.5, 2.0, 2.5, 3.0, 3.5 g/L;
- *M. azedarach* leaf extract with concentrations of 1.5, 2.0, 2.5, 3.0, 3.5 g/L;
- Registered botanical insecticide 'Neem Plus' from Indonesian Spice and Medicinal Crops Research Institute with a concentration of 2 ml/L;
- Untreated control (distilled water).

Aphid population monitoring

The aphid populations were developed naturally in chrysanthemums grown under plastic houses. The side walls of the plastic houses were open. The average temperatures in the plastic house during this experiment were 24°C and 17°C, day and night, respectively. Aphid monitoring was performed two weeks after planting to estimate the development of the aphid population by choosing ten plants per plot. The total number of apterous and alate adults and nymphs was counted from the young leaves, mature leaves, old leaves, and flower buds of chrysanthemum plants with the aid of a 5× lens. When aphids reached their economic threshold level (28 days after planting), the treatments were applied to the chrysanthe-

mum plots. Monitoring was performed a day before and two days after spraying. The reduction in aphid count before and after spraying was calculated using the following formula:

aphid population = population before treatment – population after treatment

Extraction of botanical insecticide materials

The extraction process of the plant materials of *T. sinensis*, *C .cinerariaefolium*, and *M. azedarach* followed the maceration method. The leaves of *T. sinensis* and *M. azedarach* and flowers of *C. cinerariaefolium* were cut into pieces with sizes of 1.5-2 cm. The cut materials were air-dried under shaded conditions for approximately 14-18 days. After drying, the materials were blended into grated forms and sieved with a 0.5 mm filter. The filtered powder was then mixed with acetone as a solvent at a ratio of 1:10 (w/v) in an Erlenmeyer flask. The mixture was stirred for 2 hours and set aside for 24 hours. The extract solution was filtered and evaporated using a rotary evaporator at 45°C and 227 bar. The extracts were stored at ±4°C under dark conditions. The extracts were diluted with methyl alcohol (5:1 v/v) to prepare a stock solution (Abizar and Prijono, 2010).

Land preparation, planting, and plant maintenance

The soil inside the plastic houses was tilled, and weeds were disposed of outside the plastic house. The soil was mixed with 30 tons/ha manure and 10 tons/ha bamboo humus; 52 planting beds of 1.2×1.2 m were constructed individually. The distances between planting beds were arranged in 50 cm. NThe planting bed was 25 cm in height, and the space between the planting beds was 60 cm. NPK (16:16:16) fertilizer at approximately 40 g/m^2 was mixed gently with the topsoil. The planting beds were then watered to maintain humidity. The long-day instrument was provided by the installment of 11-watt LED lamps that were arranged 1.5 m high above the planting bed, and the distance between lamps was 2×2 m.

The planting material used was rooted cutting after 18 days in the rooting process. The cuttings were planted with a density of 64 plants/bed and were poured with water to facilitate humidity and avoid plant stress. Water was supplied using a sprinkling system every 2-3 days until harvesting. The long day conditions started from the day of planting at night from 10.00 pm to 02.00 am (four hours) for 30 days. After 30 days, the long-day treatment was terminated, and the plants were grown to flower at neutral day length. Additional NPK fertilizers (16:16:16) were applied at 30 and 60 days after planting (DAP). A half dosage of pesticides (fungicides and bactericides) was used twice a week with foliar fertilizers to prevent disease attacks.

Application of botanical insecticides

The botanical extracts were diluted in water to obtain the required concentrations. The plants were sprayed with diluted botanical insecticides every week from two weeks after planting until the harvesting period. The volume of botanical insecticides was 0.5 L/m^2 when the plant was less than six weeks old and increased to 1 L/m^2 in line with the increase in plant age.

The observation of population dynamics included the incidence period of *A. gossypii* infestation. The distribution of *A. gossypii* population on the plant was determined by directly counting the aphid individuals on young (flush until 4th leaf from the apical), mature (5th to 9th leaf), and old leaves (>9th leaves) (Davies *et al*., 2004). The aphid population was recorded one day before the application of insecticide treatments, up to the harvesting period.

The effectiveness of insecticide treatments was determined based on the weekly attack severity and was calculated using the following formula (IRRI, 2002):

$$I = \frac{\Sigma n \times v}{Z \times N} \times 100\%$$

Where:
I = intensity of aphid attacks;
n = number of plant samples in the same category;
v = score value based on the attacked leaf area;
Z = highest score value;
N = number of observed plant samples.

The score values of the attacked leaf were described based on the criteria of: 0= no visual symptom, 1= the symptoms were detected at <20% leaf area, 3= the symptoms were at 20-40% leaf area, 5= the symptoms were at 40-60% leaf area, 7= the symptoms were at 60-80% leaf area, and 9= the symptoms were at 80-100% leaf area.

The efficacy of the applied insecticide was calculated using Abbott's (1925) formula:

$$IE = \frac{C - T}{C} \times 100\%$$

Where:
IE = efficacy of the tested insecticide;
C = aphid population or the intensity of aphid attack on control plants;
T = aphid population or the intensity of aphid attack on plants treated with the respective insecticide. All gathered data were analyzed using ANOVA, and mean comparisons were tested based on Duncan's Multiple Range test (α=5%). This formula is only applicable if the mortality in the control group is less than 20%.

Results and Discussion

Population dynamics of *A. gossypii* during plant growth

The *Aphis gossypii* population in all stadia was not found in the chrysanthemum until seven DAP. The appearance of the insect was detected after 21 DAP (Table 1), and the population increased along with the increment of plant age. The increase in *A. gossypii* population was related to abundant nourishment, *i.e.*, young leaves or other newly developed organs, along with plant growth. *A. gossypii* was commonly found in plant parts terminals where the flushes were located as the food source, though several factors, like plant genotypes (Fu *et al*., 2018), leaf area, age (Davies *et al*., 2004; Zhang *et al*., 2020), and environment also influenced the population dynamics (Margaritopoulos *et al*., 2006).

In the early vegetative growth (21 DAP), the population of alate adults and nymphs stadia were higher than the apterous ones in that alate adults were more dominant than nymphs (Table 1). Nymphs were predictably produced from the sexual and asexual reproduction of the adult population, and the population of nymphs increased after 28 DAP. Further developmental stages, however, have not been reached, as the non-alate adults were still absent during these periods, even after seven days. The appearance of apterous adults was detected after 35 DAP, and these were supposedly derived from further developmental phases of the existing nymphs. It was because chrysanthemum plants at 28 DAP were at the beginning of the vegetative phase, which provides an abundant food source for aphids. The ability to produce alate individuals allows aphids to spread to other plants when the quality of the food sources deteriorates. Adults of aphids may have wings or be wingless, depending on population density, feeding experience, and host plant quality (Elegbede *et al*., 2014). In tropical areas and protected cultivation, all aphids produce

Figure 1. Colonies of *A. gossypii* on chrysanthemum at the vegetative (A and B) and reproductive stages with black sooty molds (C and D).

nymphs from adult females within 7-10 days (Bethke *et al*., 2003; Rondon *et al*., 2005). The development of *A. gossypii* from nymphs to adults lasted 2 to 8 days, with the optimum environmental temperature ranging from 25 to 27°C and relative humidity of 70-75% (Elegbede *et al*., 2014; Talaga-Taquinas *et al*., 2020).

The population of nymphs and apterous adults sigmoidally increased with the shifted period from 21 to 84 DAP, whereas the population of alate adults tended to decrease. The population peaks of nymphs, apterous, and alates *of A. gossypii* in chrysanthemum occurred at 42, 63, and 21 DAP, respectively. These results indicate that the pressure of the colony influenced the composition of the aphid stages and dispersion to the other plants. The increase of nymphs and apterous adults had raised food and space competition among the colonies and resulted in the migration of the alate aphids. The alate population began to migrate to other plants at 21-28 DAP. The migration was dedicated to finding new food sources and or hosted plants and creating a new colony to prevent the species survival and generation (Bethke *et al*., 2003; Gouvea *et al*., 2019).

Distribution of *A. gossypii* within the plant structure

The distribution of *A. gossypii* population when the plant was still in the vegetative phase (42 DAP) was predominantly at the young leaves in terminal apices (Table 2). The colony occupied the whole leaf surface, where nymphal instars 1st, 2nd, and 3rd were merely found in the adaxial part (Figure 1). The young and newly

Table 1. Composition of different *A. gossypii* stages on chrysanthemum during several growth periods.

Plant ages (DAP)	Composition of *A. gossypii* stadia (%)		
	Nymphs	Alate	Apterous
21	32.00	68.00	0.00
28	80.00	20.00	0.00
35	77.30	16.33	6.37
42	89.75	0.00	10.25
49	79.33	4.67	16.00
56	54.67	2.84	42.49
63	39.16	0.51	60.33
70	80.25	1.75	18.00
77	85.62	1.60	12.78
84	78.25	2.00	19.75

DAP, days after planting.

Table 2. Distribution of *A. gossypii* in chrysanthemum based on plant developmental stages.

Growth stages	Plant age (DAP)	*A. gossypii* distribution (%)				
		Young leaves	Mature leaves	Old leaves	Flower buds	Further flower developmental stages
Vegetative	21	100	-	-	-	-
	28	100	-	-	-	-
	35	100	-	-	-	-
	42	100	-	-	-	-
	49	95.17	4.83	-	-	-
	56	87.40	12.60	-	-	-
Reproductive	63	57.18	23.69	1.14	17.74	0.25
	70	39.40	27.05	1.73	26.92	4.90
	77	20.21	16.75	0.13	12.46	50.45
	84	14.60	19.40	0.15	18.50	47.35

Remarks: young leaves included the flushes up to the forth developed leaves from the apical; mature leaves included the fifth to the ninth developed leaves from the apical; old leaves included the leaves positioned after the ninth leaves from the apical. DAP, days after planting.

developed plant organs usually contain high concentrations of proteins and amino acids needed for the insect. The aphids can suck cytoplasmic cells of young and succulent tissues causing the death of the tissues. In addition, *A. gossypii* detects food scarcity when chrysanthemum plants form flower buds (84 DAP). When the plant shifted into reproductive stages, the new leaf development was terminated and resulted in tissue hardening. These conditions induced a lower nutrient content for insects. After the availability of the food source subsided, the alate adults migrated to other plants (Davies *et al*., 2004; Yassin, 2020).

The distribution of *Aphis gossypii* at chrysanthemum reproductive stages was more varied not only on young leaves but also on mature and old leaves, flower buds (Figure 1), and developed flowers. When the plants were in the vegetative stage, the aphid colonies were predominantly found in young leaves and apical flushes. In the reproductive phase (after 63 to 84 DAP), the aphid colony was shifted at flower buds and further flower developmental stages (Table 2). At bloomed flowers, aphids were merely found in the inner floret rows and calyx. The shift in the distribution of aphid attacks on chrysanthemum plants is closely related to the physiological growth of the plant. Under this condition, the number of young leaves that are more suitable for developing aphids becomes insubstantial. Consequently, aphids alter the allocation of physiological resources of chrysanthemums from the apical growth zone to bud and flower organs and mobilize amino acids to meet their necessary nutrients. The attacked flower appeared to be opaquely paler in color than the wilted florets. According to Miller and Stoetzel (1997) and Zhang *et al*. (2020), the common symptoms of aphid attacks might vary in different plants and plant parts. In the leaves and petals, blotched-necrotic, chlorotic and curly-leaf/petal margin with the appearance of honeydew were found in several hosted plants. In severe infestations, the plant vigor might be affected by reducing vase life.

Infestation rate of *A. gossypii* and effectiveness of botanical insecticides

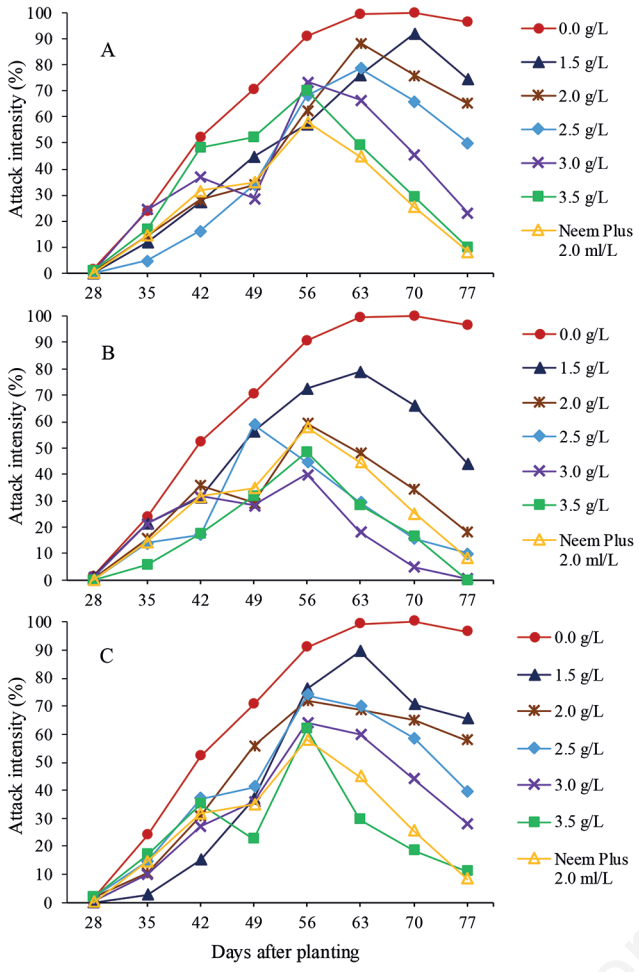
The intensity of *A. gossypii* infestation through various observation periods under different botanical insecticide treatments is presented in Table 3. The population of aphids at control treatments was consistently the highest throughout the plant ages and significantly different from the botanical insecticide treatment at 35 to 77 DAP. At the same time, botanical insecticide treatment was varied in every observation period. In 35 DAP, the infestation intensity was still low (<35%) and started to increase in all treated plants at various degrees from 28 to 56 DAP (Figure 2). Under *T. sinensis* treatments, the plants sprayed with 3.5 g/L extracts showed a decrease in infestation intensity at 42 DAP and 49 DAP but sharply increased up to 56 DAP. Similar phenomena were observed on the plants sprayed with *C. cinerariaefolium* at 2.0 and 3.0 g/L and *M. azedarach* at 3.0 g/L. In contrast to the Neem Plus insecticide, the attack intensity tended to increase up to 56 DAP but continuously decreased until 77 DAP. In most botanical insecticide treatments, the highest pest attacks were detected after 56 DAP and started to sharply diminish at 63-77 DAP.

The patterns of attack intensity were in accordance with the population dynamics of aphid insects (Table 4). The population increased in number from 28 to 49 DAP and reached the peak after 56 to 63 DAP, then decreased after that. The increase in population is related to the emergence of a new individual derived from the initial adult population (Zhang *et al*., 2020). Provided by abundant nourishment, the aphid population increased sharply during a certain period. It was due to a more extended period of immature stages, and the population increased with subsequently incoming new individuals (Moreno-Delafuente *et al*., 2021). Under botanical insecticide treatments, this increase in population was somehow suppressed and significantly different from control treatments that continued to grow and were higher up to 63-70 DAP (Figure 2 and Table 4). The

Table 3. Percentage of *A. gossypii* attacks and botanical insecticide efficacy at various observation periods under different botanical insecticide treatments.

Treatments	Percentage of *A. gossypii* attacks and insecticide efficacy (%)*/**														Average IE
	35	IE	42	IE	49	IE	56	IE	63	IE	70	IE	77	IE	
T. sinensis 1.5 g/L	2.60 d	92.11	15.10 c	74.07	36.98 bc	56.28	76.04 ab	22.75	92.92 ab	7.08	89.67 ab	10.33	68.84 abc	31.16	41.97
T. sinensis 2.0 g/L	10.42 bcd	68.45	30.73 abc	47.25	55.73 abc	34.11	71.88 ab	26.98	72.08 abcd	27.92	65.00 abc	35	59.15 abcd	40.85	40.08
T. sinensis 2.5 g/L	14.58 abcd	55.84	36.98 abc	38.52	41.15 bc	51.35	73.96 ab	26.87	69.90 abcd	30.1	68.42 abc	31.58	39.48 cdef	60.52	42.11
T. sinensis 3.0 g/L	11.90 abcd	63.97	30.42 abc	48.78	44.27 bc	47.66	71.98 ab	30. 29	66.98 abcd	33.02	37.65 def	64.35	21.38 fg	80.63	56.40
T. sinensis 3.5 g/L	10.52 bcd	68.14	22.90 bc	62.69	41.06 bc	52.45	61.98 bc	37.04	39.69 ef	62.31	20.31 gh	83.69	7.81 h	92.19	65.50
C. cinerariaefolium 1.5 g/L	21.35 abc	35.33	31.25 abc	47.35	66.25 ab	21.67	72.40 ab	28.45	78.81 abcd	21.19	66.00 abc	34	43.96 bcde	57.04	35.00
C. cinerariaefolium 2.0 g/L	13.87 abcd	59.01	39.27 abc	35.58	47.34 bc	47.03	69.38 ab	29.52	56.73 bcde	43.27	41.88 cde	58.13	21.42 ef	78.58	50.16
C. cinerariaefolium 2.5 g/L	11.98 abcd	63.71	22.78 bc	62.9	54.17 abc	37.96	44.65 bc	59.65	22.40 def	78.6	12.29 efg	87.71	4.04 fg	95.96	69.50
C. cinerariaefolium 3.0 g/L	12.35 abcd	64.59	28.31 abc	54.39	33.75 c	61.1	41.06 d	65.29	12.83 g	91.17	1.88 i	99.13	0.00 i	100	76.52
C. cinerariaefolium 3.5 g/L	5.73 cd	82.65	17.71 c	69.6	45.83 bc	54.81	48.33 cd	55.9	32.26 f	67.74	15.86 h	84.14	4.75 h	95.25	72.87
M. azedarach 1.5 g/L	11.98 bcd	63.72	26.08 bc	55.22	54.79 abc	35.22	70.63abc	28.25	86.35 abc	13.65	91.88 ab	8.13	74.58 ab	25.42	32.80
M. azedarach 2.0 g/L	14.58 abcd	55.84	28.13 abc	51.72	50.19 abc	40.67	62.50 bc	36.51	86.98 abc	13.02	74.04 ab	25.96	65.42 abc	34.58	36.90
M. azedarach 2.5 g/L	11.98 abcd	63.72	34.38 abc	40.99	60.63 ab	28.33	83.13 ab	15.55	79.13 abcd	20.88	59.65 bcd	40.35	39.99 cdef	60.01	38.55
M. azedarach 3.0 g/L	24.48 ab	25.87	40.31 ab	30.79	57.65 abc	31.85	83.44 ab	15.24	76.56 abcd	23.44	57.60 bcd	42.4	36.56 def	63.44	33.29
M. azedarach 3.5 g/L	16.67 abc	49.53	35.50 abc	39.06	51.56 abc	39.04	64.98 bc	33.98	54.95 cde	45.05	24.17 fgh	75.83	10.98 gh	89.02	53.07
Neem Plus 2 ml/L	14.58 abcd	55.84	28.44 abc	57.18	34.90 bc	64.74	58.02 bc	51.06	41.46 ef	68.54	25.40 h	78.6	10.10 h	95.9	67.41
Control	33.02 a	0	58.25 a	0	84.58 a	0	98.44 a	0	100 a	0	100 a	0	100 a	0	0
CV (%)	12.32		8.82		6.25		3.82		5.4		5.7		7.4		

*Percentage of *A. gossypii* attacks values were transformed using $\sqrt{(x+0.5)}$; **Values in the same column followed by different letter differ significantly under Duncan Multiple Range test (α=5%). IE, insecticide efficacy; CV, coefficient of variation.



emergence of new individuals, the unfavorable conditions due to botanical insecticides, and the reduction in nourishment due to the increase of plant tissue ages are all factors that induced internal competition within the population. Within this situation, the immature stages tended to hasten the biological development into winged forms of the adult (Davies *et al*., 2004; Wang *et al*., 2021). Such transformation enabled the insect to transfer or migrate to find new feed sources (other host plants) and establish new colonies. The acceleration of biological transformation and the migration of the alate adults impacted the reduction of the population that started at 56 to 63 DAP (Table 4).

The botanical insecticides studied had different effectiveness in suppressing *A. gossypii* attacks. Each botanical insecticide treatment's attack intensity fluctuated during every observation period (Figure 2). Only flower extract of *C. cinerariaefolium* at the concentration of 3.0 and 3.5 g/L was consistent in suppressing the damages, viewed from the attack intensities of less than 50% throughout the observation periods. The efficacies were also consistent, above 50% in every observation period, with an average percentage efficacy of 76 and 72%, respectively (Table 3). These conditions inferred that aphid insects were merely susceptible to pyrethrin as an active ingredient in *the C. cinerariaefolium* extract. Pyrethrin, also known as a knockdown insecticide, is a contact poison that quickly penetrates the nervous system and causes paralysis of the insect. The compound also induced autophagy in the non-nervous system, interfered with particular protein pathways, and reduced insect cells' viability (Xu *et al*., 2017; El-Sayed and El-Ziat, 2021). Moreover, active

Figure 2. Intensity of *A. gossypii* attacks on chrysanthemum plants treated with botanical insecticides of *M. azedarach* (A), *C. cinerariaefolium* (B), and *T. sinensis* extracts (C).

Table 4. Population of *A. gossypii* at various observation periods under different botanical insecticide treatments.

Treatments	Population of *A. gossypii* per plant at … DAP*/**							
	28	35	42	49	56	63	70	77
T. sinensis 1.5 g/L	2.54±0.59 b	12.03±3.05 de	33.22±3.0 def	82.88±4.15 cd	136.13±18.37 b	130.27±4.99 b	116.72±2.35 b	113.04±3.34 b
T. sinensis 2.0 g/L	5.37±0.38 ab	9.00±1.51 e	20.00±2.0 fg	67.81±9.37 def	89.09±20.65 cdefg	91.58±8.10 cdef	86.85±3.66 bcd	82.08±14.42 bc
T. sinensis 2.5 g/L	6.17±2.05 ab	12.12±2.21 de	14.03±2.44 g	31.31±2.20 g	118.67±5.26 bc	105.37±3.13 bcd	73.86±8.44 cd	44.32±4.29 ef
T. sinensis 3.0 g/L	7.71±2.24 ab	16.68±2.65 de	43.33±4.90 de	73.17±4.58 cde	75.20±12.06 efg	101.43±9.07 bcde	69.74±6.23 de	43.56±2.00 efg
T. sinensis 3.5 g/L	7.28±1.70 ab	19.46±2.43 bcde	31.16±1.73 def	65.19±3.32 def	85.14±3.19 defg	70.87±1.34 cdef	56.68±2.04 de	36.97±5.44 fg
C. cinerariaefolium 1.5 g/L	6.85±1.85 ab	13.56±1.78 de	27.32±2.11 efg	63.13±2.85 def	106.30±7.16 bcde	103.91±4.60 bcde	87.44±7.72 bcd	66.85±5.93 cde
C. cinerariaefolium 2.0 g/L	4.52±1.27 ab	13.49±3.04 de	37.62±6.01 def	47.97±4.09 fg	96.91±2.10 cdef	57.52±9.16 gh	30.27±3.67 fg	11.59±2.84 hi
C. cinerariaefolium 2.5 g/L	6.93±1.81 ab	16.80±1.31 cde	47.23±7.41 cde	62.33±7.03 def	59.37±12.6 1 g	40.36±11.78 h	25.27±6.02 fg	12.30±2.15 hi
C. cinerariaefolium 3.0 g/L	9.51±1.82 a	30.26±3.02 abc	53.25±9.35 cd	74.61±4.89 cde	78.54±7.93 defg	48.12±2.55 gh	14.73±3.83 g	2.89±2.36 j
C. cinerariaefolium 3.5 g/L	8.02±0.95 ab	18.58±2.36 bcde	55.01±10.89 cd	56.14±10.46 ef	72.78±5.36 fg	56.52±2.84 gh	28.10±2.80 fg	6.97±2.12 ij
M. azedarach 1.5 g/L	6.71±1.64 ab	17.97±6.03 de	83.69±13.15 b	122.83±8.72 b	142.45±6.40 b	127.60±6.80 bc	108.01±7.61 bc	86.33±7.66 bc
M. azedarach 2.0 g/L	5.61±0.76 ab	22.23±5.17 bcd	48.25±7.34 cde	95.45±4.86 bc	108.23±10.07 bcd	93.52±5.15 bcdef	79.24±4.64 cd	59.76±8.43 cdef
M. azedarach 2.5 g/L	5.19±1.39 ab	15.26±3.06 de	45.21±9.16 de	82.25±14.72 cd	105.27±9.97 bcdef	95.33±2.93 bcdef	88.56±3.52 bcd	75.87±5.49 cd
M. azedarach 3.0 g/L	5.01±1.42 ab	23.37±3.92 bcd	52.80±11.84 cd	74.66±7.46 cde	96.29±6.68 cdef	101.35±16.85 bcde	82.42±16.52 cd	50.38±10.75 def
M. azedarach 3.5 g/L	7.62±2.49 ab	32.35±6.92 ab	74.66± 9.48 bc	95.15±6.60 bc	106.55±6.67 bcde	87.41±3.23 def	76.75±8.99 cd	37.14±2.70 fg
Neem Plus 2 ml/L	9.52±0.76 a	22.37±2.74 bcd	47.69±4.85 cde	77.31±5.11 cde	86.63±9.41 cdefg	66.92±11.40 fg	44.97±10.48 ef	15.76±2.92 gh
Control	6.43±1.69 ab	42.96±7.50 a	132.59±15.79 a	172.43±6.38 a	246.42±19.33 a	212.93±8.28 a	204.24±15.17 a	187.07±7.75 a
CV (%)	25.01	16.62	13.85	9.14	8.91	9.88	11.78	15.03

*Average population values were transformed using √(x+0.5); **Values in the same column followed by different letters differ significantly under Duncan Multiple Range Test (α=5%). DAP, days after planting; CV, coefficient of variation.

compounds, such as cinerin I and II, jasmolin I and II, and (E)-β-farnesene, contributed to the decline in the population of *A. gossypii*. These compounds have a repellent characteristic that was reported to be effective in controlling aphids (Li *et al*., 2019; Wang *et al*., 2021).

Aside from *C. cinerariaefolium* extract of 3 g/L, *T. sinensis* and *M. azedarach* extract with a concentration of 3.5 g/L and positive control registered bio-insecticide 'Neem Plus' also had an average efficacy of more than 50%. However, the efficacy of these treatments was not consistent during the observation period. *M. azedarach* showed efficacy above 50% at 49, 56 and 63 DAP. *T. sinensis* had the same at 56 DAP, while Neem Plus showed a percentage of pest attacks above 50% at 56 DAP. Rahardjo *et al*., (2021) and Shaurub *et al*., (2022) stated that a percentage of efficacy above 50% was substantial for botanical insecticides in representing their effectiveness. The level reflected the target specificity, as also reported by Xu *et al*., (2017) and Saleem *et al*., (2019) when screening botanical insecticides to control spider mites.

Conclusions

The population dynamics of *A. gossypii* and the effectiveness of several botanical insecticides in pest control were studied. In the early vegetative growth, the alate adult and nymph stages were dominant, and the population of nymphs increased along the plant ages. Most of the colonies were found occupying the whole surfaces of young leaves in the terminal apices. When the plant reached the reproductive stage, the population of alate adults began to diminish. The distribution of aphids spread to mature, old leaves, flower buds, and colored flowers, causing blotched-necrotic, chlorotic and curly margins of the petal.

The application of several botanical insecticides revealed various responses in *A. gossypii* populations. *C. cinerariaefolium* extract at the concentration of 3.0 and 3.5 g/L was the most consistent in suppressing the infestation intensity of less than 50% in every observation period and had the highest average percentage efficacy of 76 and 72%, respectively. Botanical insecticides have the potential to be used in sustainable pest management for aphid control in chrysanthemum production.

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