



## Effects of Several Botanical Insecticides Applied in Different Periods to Control Aphids (*Macrosiphoniella sanborni* Gillete) on Chrysanthemum

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### ABSTRACT

Pest management in the agricultural process has faced worldwide economic and ecological issues related to chemicals usage. Botanical insecticides have long been considered promising alternatives to control pests because botanicals reputedly pose a minor threat to the environment and human health. Some studies have evaluated several botanical insecticides to control aphids in chrysanthemum. The research was conducted under plastic house conditions at the Indonesian Ornamental Crops Research Institute from January to December 2018. Leaf extract of *T. sinensis*, *T. diversifolia* and *A. indica* at the concentration of 3 and 3.5 g/l, processed oil of *C. nardus* at the concentration of 2.5 and 5 g/l and synthetic insecticide Imidacloprid 1 g/l were sprayed in the morning or late afternoon. The results showed that aphid attacks severity and percentage of attacked plants were lower when the insecticides were applied in the late afternoon. Slightly softer than synthetic chemicals, the leaf extract of *T. sinensis* at 3 g/l suppressed the aphid development more effective for about 55.93% than other botanicals treatments. On reproductive properties, the leaf extract of *T. sinensis* at 3 and 3 g/l and *T. diversifolia* at 3 g/l induced a higher percentage of open flower for more than 65% than synthetic insecticides, Imidacloprid.

### INTRODUCTION

Chrysanthemum is one of the essential ornamental crops with high economic value all around the world. The plant is commonly planted under glass-or plastic house conditions. While in several places, growing under open conditions were also found using selected varieties. These conditions exhibited several species of becoming established on greenhouse and outdoor plantings, including aphids species. At least 15 aphids species are recognized to colonize on chrysanthemum, including *Macrosiphoniella sanborni* Gillete (Wang et al., 2014; Ali, 2017). The insects attacked the plant by piercing the cells and sucking the sap (Cichocka et al., 2015). The large colonies causing these mechanical injuries might reduce plant vigour and kill the plant (Saicharan et al., 2017). The insect also produced a sticky

substance called honeydew and might accumulate on the leaves and flowers. These substances were observed to be a suitable substrate for the growth of black sooty mould (Pringle et al., 2014). Large areas of mold covering the leave surfaces might reduce light interception to leaves, thus diminishing photosynthetic activities (Koch et al., 2016; Hussain, Razaq, Zaka, Shahzad, & Mahmood, 2015). The black mould also resulted in an unattractive plant with lower market value (Emam, 2016).

The application of synthetic insecticides was still considered to be the most common practise to reduce the damages. With inappropriate dosages and frequencies, such practices might spend 13-32% of total production cost and make the production process uncompetitive (Suhardi, 2009). The long term application of pesticides left toxic

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and hazardous residues and might be accumulated and affected the environmental quality and human health (Nicolopoulou-Stamati *et al.*, 2016) and might raise to new resistant aphids biotype population (Valizadeh *et al.*, 2013).

The concern for environmental safety and human health has demanded growers to seek alternative strategies in reducing the use of hazardous chemicals in production processes. Plant species can synthesize various secondary metabolites important in protecting against predators and microbial pathogens and the interaction of plants with other organisms. Many secondary metabolites have insecticidal, repellent as well as antifeedant activity. Furthermore, they cause reproduction retardation and act as insect growth regulators (Grdiša & Gršić, 2013). Active compounds of this botanical insecticide were less toxic to mammalian and would be naturally degraded in specific periods, thus safer to human health and the environment (El-Wakeil, 2013). Several reports also indicated that botanical insecticides had high specificity on targeted insect pests (Gholamzadeh-Chitgar & Pourmoradi, 2017; Zeb, Naeem, Khan, & Ahmad, 2016).

Several plants have been reported to have natural compounds that possessed as insecticide features. *Toona sinensis* L. (Meliaceae) with the common name of Chinese mahogany, Chinese toon or red toon, is native to eastern and south-eastern Asia from North Korea, China, Nepal, India, Myanmar, Thailand, Malaysia up to Indonesia (Adfa *et al.*, 2017). Extract fresh leaves contained a high amount of *sesquiterpenes*, including *copaene*,  $\beta$ -*caryophyllene*, *caryophyllene*,  $\beta$ -*eudesmene* and the compounds showed antimicrobial activity against *Staphylococcus aureus* (Wang *et al.*, 2014) and *Captotermes curvignathus* on woods (Adfa *et al.*, 2017). Several metabolite compounds like *limonoid*, *flavonoid*, *phytol*, *coumarins*, *norcyteine* derivatives, *surenon*, *surenin* and *surenolakton* extracted from leaf and seed were also reported effective to control leafminer (Rahardjo *et al.*, 2020) and aphid (Dedi Hutapea *et al.*, 2020) in chrysanthemum, red flour beetle *Tribolium castaneum* (Coleoptera, Tenebrionidae) (Parvin *et al.*, 2012), and *Bemisia tabaci* in tomato (Bezerra-Silva *et al.*, 2012).

*Tithonia diversifolia* (Hemsl.) A. Gray, commonly known as tree marigold, Mexican tournesol, Mexican sunflower, Japanese sunflower,

or Nitobe chrysanthemum, was also reported for insect pest control in several plants. Several reports have indicated the use of leaf and flower extract of the plant to control *Callosobruchus maculatus* F. (Pangihutan *et al.*, 2016), *Chrysomya bezziana* in larva stage (Wardhana & Diana, 2014) and *P. xylostella* on *Brassica oleracea* (Mpumi *et al.*, 2020). Aside from *T. sinensis* and *T. diversifolia*, *Azadirachta indica* A. Juss, also known as neem, nimtree or Indian lilac, has also been used as a biopesticide to control several insect pests. The plant contained more than a dozen secondary metabolite analogues of azadirachtin that exhibited insecticidal activities such as antifeedant, ovicidal, larvicidal, insect growth regulatory, sperm production interruption and repellent activity (Chaudhary *et al.*, 2017). The successful application of *A. indica* as bio-insecticide was reported to control *C. maculatus* on cowpea seeds and *Sitophilus zeamais* on maize grains (Tofel *et al.*, 2017), *P. xylostella* in cabbage (Kolani *et al.*, 2016), *Spodoptera litura* (Prasannath, 2016; Pavela, 2009), and rice-moth *Corcyra cephalonica* Staint (Pathak & Tiwari, 2012). However, regarding these potential bio-insecticides, there was still limited information related to the insecticidal effects or specifically their toxicological levels on aphids in chrysanthemum.

Aside from types of botanical insecticide, time of application was also considered necessary on the effectiveness of an applied insecticide. Most insect species are poikilothermic creatures; thus, daily temperature affects the existence and position of the insect within the plants (Nowinszky & Puskás, 2013). A study conducted by Nurmasyah (2014) on applying citronellal extract to control *Helopeltis antonii* on cocoa revealed that the late-afternoon spraying gave different mortality on the insects than morning and mid-day applications. This research evaluated the efficacy of leaf extracts of *T. sinensis* and *T. diversifolia* and *A. indica* applied at different times to control aphids *Macrosiphoniella sanborni* Gillette in chrysanthemum considering the effects of botanical and time of application. The effectiveness of the plant extracts was relatively compared with fabricated lemongrass (*Cymbopogon nardus* L.) oils and synthetic pesticides that commonly used by chrysanthemum growers to control aphids, imidacloprid.

## MATERIALS AND METHODS

### Research Location and Experimental Design

The research was conducted under plastic house conditions at Segunung experimental station (1100 masl) of the Indonesian Ornamental Crops Research Institute (IOCRI) from January to December 2018. The chrysanthemum used in the study was cv. White Fiji collected from a commercial nursery. The leaves of *T. sinensis*, *T. diversifolia* and *A. indica* were gathered from wild plants grown surrounding the farmer fields in Cianjur, West Java. The processed lemon grass (*C. nardus* L.) oils were from the Indonesian Spice and Medicinal Crops Research Institute. A complete block experiment with three replications was designed to evaluate several botanical insecticide formulations on chrysanthemum (Table 1). The first factor was the time of application, i.e. morning (before 07.00 am) and late afternoon (02.00–03.00 pm). At the same time, the second factor dealt with a different source of botanical insecticides. The treatments

were applied based on the adjustment from Adfa *et al.* (2017), Green *et al.* (2017), Lawal *et al.* (2015) and Pumnuan *et al.* (2017) with the following description.

### Extraction of Botanical Insecticide Materials

The leaf extraction process of *T. sinensis*, *T. diversifolia* and *A. indica* was following the maceration method. The leaves of *T. sinensis*, *T. diversifolia* and *A. indica* were cut into pieces with the sizes of 1.5–2 cm. The cut materials were air dried under shaded conditions for about 14–18 days. The materials were blended into grated forms and sieved with 0.5 mm-filter. The filtered powder was then mixed with acetone as the solvent with the ratio of 1:10 (w/v) in an Erlenmeyer. The mixture was stirred for 2 hours and set aside for 24 hours. The extract solution was filtered and evaporated using rotary evaporator at 45°C and 227 mbar. The extracts in the form of pasta were stored at  $\pm 4^{\circ}\text{C}$  under dark conditions. The extracts were diluted with methyl alcohol (5:1 v/v) for the stock solution.

**Table 1.** Experimental design to evaluate several botanical insecticide formulations

No	Code	Leaf extract	Application time	Concentration (g/l)
1.	T11- <i>T. sinensis</i>	<i>T. sinensis</i>	morning	3
2.	T12- <i>T. sinensis</i>	<i>T. sinensis</i>	late afternoon	3
3.	T11- <i>T. sinensis</i>	<i>T. sinensis</i>	morning	3.5
4.	T12- <i>T. sinensis</i>	<i>T. sinensis</i>	late afternoon	3.5
5.	T21- <i>T. diversifolia</i>	<i>T. diversifolia</i>	morning	3
6.	T22- <i>T. diversifolia</i>	<i>T. diversifolia</i>	late afternoon	3
7.	T21- <i>T. diversifolia</i>	<i>T. diversifolia</i>	morning	3.5
8.	T22- <i>T. diversifolia</i>	<i>T. diversifolia</i>	late afternoon	3.5
9.	T31- <i>A. indica</i>	<i>A. indica</i>	morning	3
10.	T32- <i>A. indica</i>	<i>A. indica</i>	late afternoon	3
11.	T31- <i>A. indica</i>	<i>A. indica</i>	morning	3.5
12.	T32- <i>A. indica</i>	<i>A. indica</i>	late afternoon	3.5
13.	T41- <i>C. nardus</i>	<i>C. nardus</i>	morning	2.5
14.	T42- <i>C. nardus</i>	<i>C. nardus</i>	late afternoon	2.5
15.	T41- <i>C. nardus</i>	<i>C. nardus</i>	morning	5
16.	T42- <i>C. nardus</i>	<i>C. nardus</i>	late afternoon	5
17.	T51-Imidakloprid	Synthetic insecticide Imidakloprid	morning	1
18.	T51-Imidakloprid	Synthetic insecticide Imidakloprid	late afternoon	1
19.	Control-1	Distilled water spraying (2.5 l/m <sup>2</sup> )	morning	
20.	Control-2	Distilled water spraying (2.5 l/m <sup>2</sup> )	late afternoon	

### Land Preparation, Planting, and Plant Maintenance

The soil inside the plastic houses was tilled, and the weeds were disposed of outside from the plastic house. After the soils were mixed with 30 t/ha manures and 10 t/ha bamboo humus, sixty planting beds with 1 x 1.2 m was constructed individually. The planting bed had 25 cm in height, with the distance between planting beds was 50 cm, while the distance between replication was 60 cm. NPK (16:16:16) fertilizer for about 40 g/m<sup>2</sup> was mixed gently with the topsoil. The planting beds were then poured with water to keep the humidity. The instalment of 11 watts provided long day instrument LED lamps and these were arranged 1.5 m above the planting bed, and the distance between lights were 2 x 2 m.

The planting material used was rooted cutting after 18 days in the rooting process. The cutting was planted with the density of 64 plants per bed. After planted, the cuttings were poured with water to facilitate humidity and avoid plant stress. The water supply was given using a sprinkle system every 2-3 days until the harvesting period. The long day conditions were applied to start from planting for 4 h during night time from 10.00 pm to 02.00 am for 30 days. After 30 days, the long day treatment was terminated, and the plants were forced to flower in neutral day conditions. Additional fertilizers using NPK (16:16:16) were applied after 30 and 60 days of planting. A half dosage of pesticide (fungi and bactericides) was applied twice a week and foliar fertilizers to prevent disease attacks.

### Application of Botanical Insecticides

The extracts were diluted with water until the treatment concentrations were reached. The plants were then sprayed with diluted botanical insecticides every week from 2 weeks after planting until the harvesting period. The volume of botanical insecticides was 0.5 l/m<sup>2</sup> when the plant under 6 weeks old and increased up to 1 l/m<sup>2</sup> in line with the increase of plant ages.

The parameters observed included attack severity, which were weekly observed and calculated based on the following equation.

$$I = \frac{\sum n \times v}{Z \times N} \times 100\% \dots\dots\dots 1)$$

Where :

- I = Intensity of aphids attacks,
- n = number of plant samples in the same category,
- v = score value based on the attacked leaf area,

Z = highest score value and

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 1<sup>st</sup> to 2<sup>nd</sup> leaves at the base.
- 2 = the symptoms were at 1<sup>st</sup> to 2<sup>nd</sup> leaves at the base and one leaf at the central part.
- 3 = the symptoms were at 1<sup>st</sup> to 2<sup>nd</sup> leaves at the base and 2 leaves at the central part.
- 4 = the symptoms were at 1<sup>st</sup> to 2<sup>nd</sup> leaves at the base, 1-2 leaves at the central part and 1-2 leaves at the terminal part

The number of attacked plants were observed every week, and the percentage of attacked plants were calculated based on (Hutapea, Rahardjo, & Marwoto, 2019) :

$$P = \frac{a}{b} \times 100\% \dots\dots\dots 2)$$

Where :

- P = percentage of attacked plants,
- a = number of attacked plants,
- c = number of observed plants.

The percentage of suppression was calculated using Hanudin, Nuryani, Yusuf, & Marwoto (2011) formula.

$$PS = \frac{C - T}{C} \times 100\% \dots\dots\dots 3)$$

Where:

- PS = Percentage of infection (%),
- C = aphids attacks intensity at control treatment,
- T = aphids attacks intensity on the respected treatment.

Agronomic properties, i.e. final plant height, percentage of fully-open flowering plants, flower diameter, and the number of marketable flowers, were observed during the harvesting period. All the data gathered were analyzed using ANOVA continued by HSD at a 95% level of confidence.

## RESULTS AND DISCUSSION

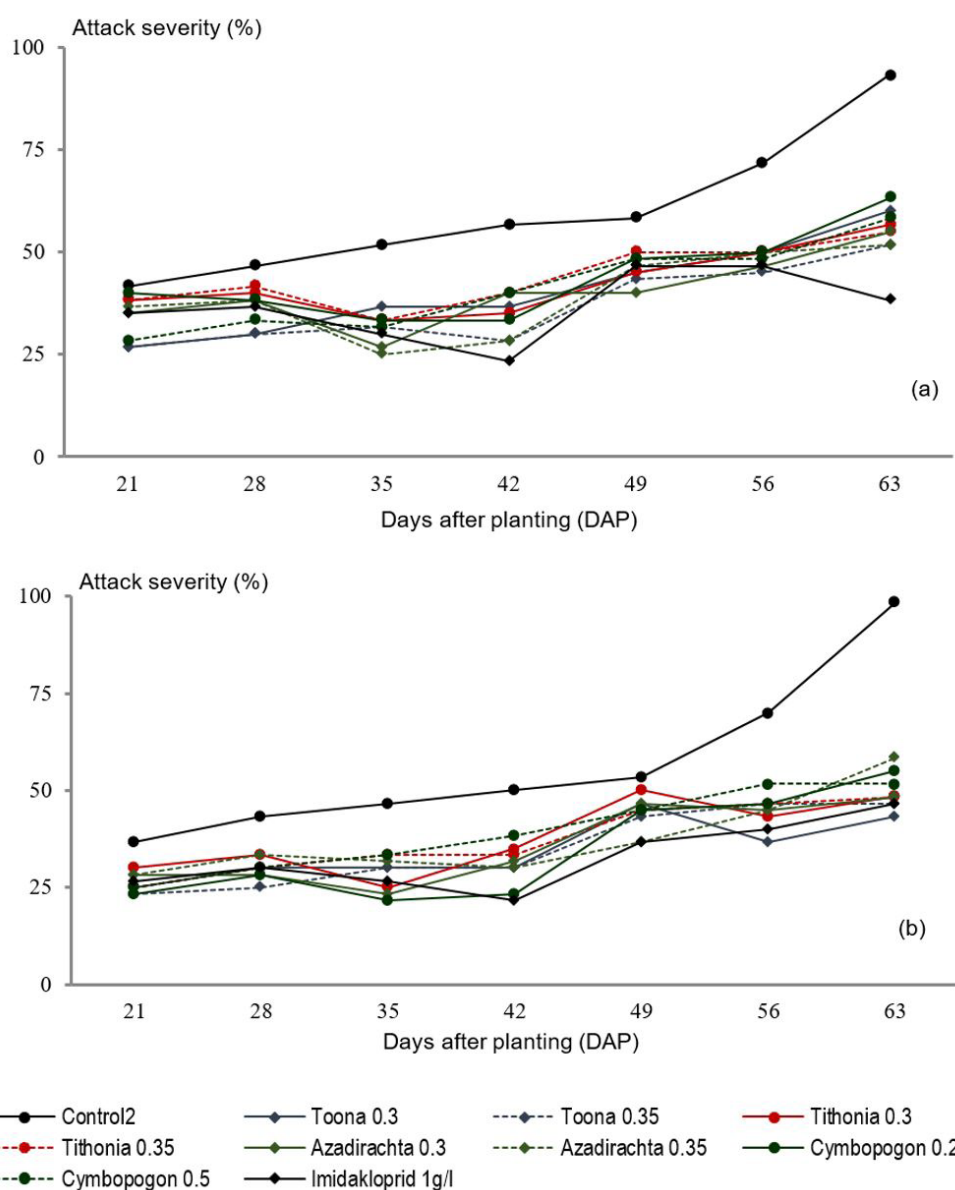
### Aphid Attack Severity and Percentage of Attacked Plants

Fig. 1a shows the aphid attacks severities from 21 to 63 DAP under various botanical and synthetic insecticides applied in the morning and late afternoon. When applied in the morning, aphid

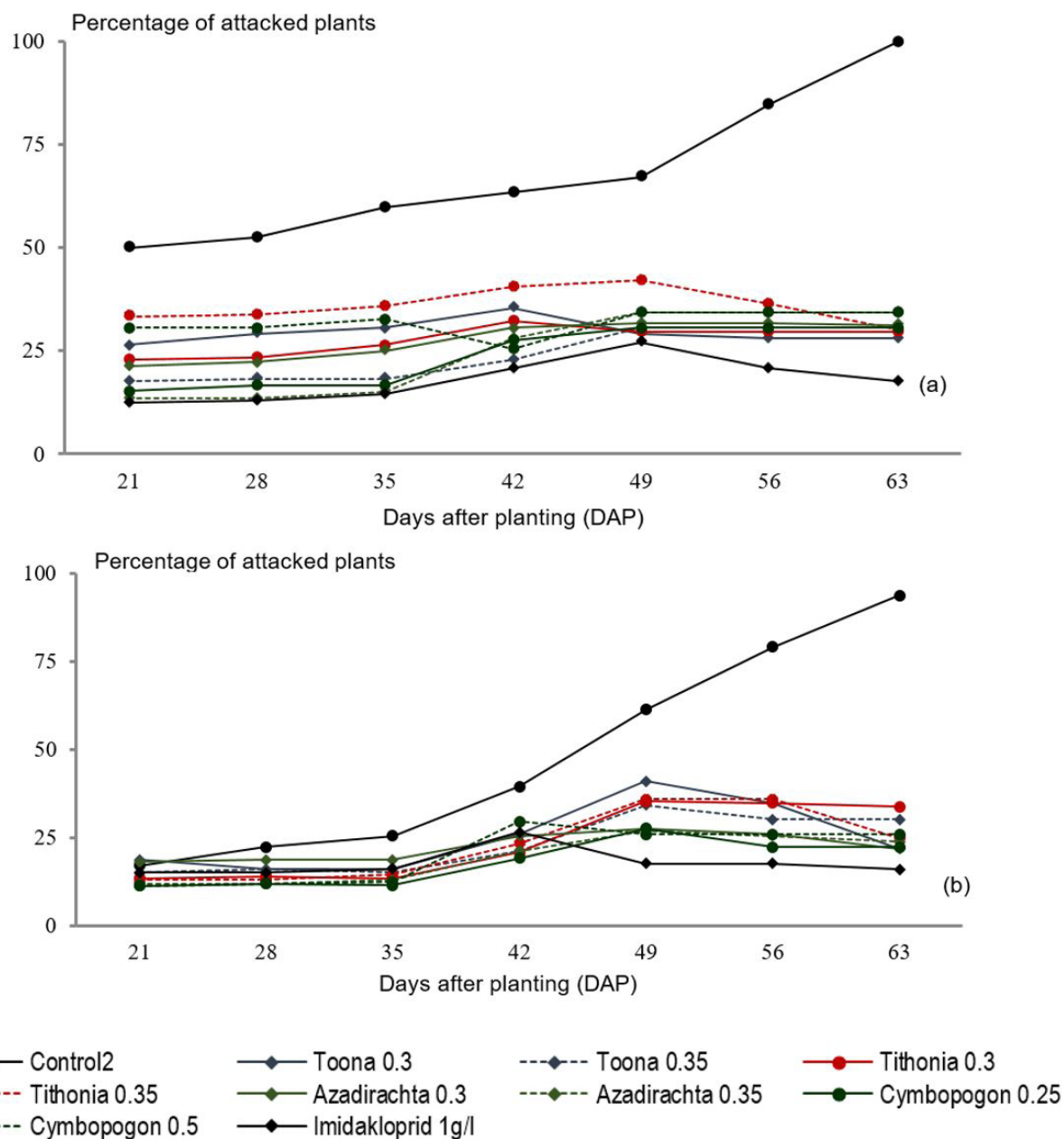


attacks were varied among the treated plants. At 21 days after planting (DAP), the attack intensities were still relatively low, ranging from 26.67 to 41.63%. The highest was detected on control, and the lowest was at *T. sinensis* leaf extract at the concentration of 0.3 and 0.35%. During 21 to 63 DAP, the aphid attack severities fluctuated, and the control plants were most consistently the highest among the treated plants. The plants treated by synthetic insecticide Imidacloprid 1 g/l showed the slightest aphid attack after 63 DAP. While under botanical insecticide

treatments, the lowest attacks severity was exhibited by leaf extracts of *T. sinensis* 0.3% and *A. indica* 0.35% treatments with the value of 51.67%. Attack severity on chrysanthemum treated by botanical and synthetic insecticides at late afternoon also showed similar trends (Fig. 1b). The plants sprayed with water (control plants) showed the highest in every observation period. At 63 DAP, leaf extract of *T. sinensis* 0.3% gave the lowest among botanical insecticide treatments, including synthetic insecticide Imidacloprid (1 g/l), which showed only 46.67%.



**Fig. 1.** Aphid attack severity on chrysanthemum plants treated by different botanical and synthetic insecticides applied (a) in the morning and (b) at late afternoon.



**Fig. 2.** Percentage of attacked plants treated by different botanical and synthetic insecticides applied (a) in the morning and (b) at late afternoon.

The lesser attack severity and percentage of attacked plants on insecticide-treated plants and sharp increase of attack severity at control on the early plant growth were related to the period before the treatment applications. Before the insecticides were applied, aphid has no restriction on feeding the abundant, newly planted young plants. The attacks incidences after 21 DAP were then actually caused by the existed adult aphid population (Pinto,

Barros, Torres, & Neves, 2013). They resulted in slight differences in the attack intensities among the treatments at these periods. After the insecticide treatments were applied, aphid population growth was somehow restricted due to the effects of botanical and synthetic insecticides. These insecticides might give deleterious effects like antifeedant, larvicidal, repellent activity or even direct killing to the insects and suppress the offspring's emergence (Zeb et

al., 2016) reflected from the lesser attacks intensity after 21 DAP. Unlike in control plants, the population growth was not inhibited by the insecticides. Higher damages (higher percentage of attacked plants and attacks severity) on the control plants up to 63 DAP were then due to the unrestricted increase of insect populations.

The attacks severity and percentage of attacks among botanical and synthetic insecticide treatments were also different in application periods. Applied in the morning, the attacks severity and percentage of attacked plants were higher with the range of 25–63.3% and 12.5–42.18%, respectively (Fig. 1 and Fig. 2). The attacks severity and percentage of attacked plants were only 23.3–58% and 11.45–35.93% when botanical and synthetic insecticides were applied in the late afternoon. These conditions inferred that the late afternoon application had a more positive impact on lowering insect attacks. Nurmasyah (2014) also reported a similar phenomenon when treated cocoa plants with citronella extracts to control *Helopeltis antonii*. The lower attacks severity and percentage of attacks on late afternoon application were predicted to have a relation with the persistency of the active ingredient of the insecticides. When applied in the morning, the insecticide solutions were exposed to certain environmental conditions that induced the solution to evaporate, like direct sunlight and higher temperature. These conditions caused the insecticidal effects of the botanical insecticides to become less persistent (Isman & Grieneisen, 2014).

#### Percentage of Suppression Among Insecticide Treatments

The attacks intensity at 63 DAP and percentage of suppression among botanical and synthetic insecticide treatments were presented in Table 2. Among botanical and synthetic insecticide treatments, synthetic insecticide Imidacloprid 1 g/l seemed to be the most effective to control Aphid attacks. The application of this synthetic insecticide gave the lowest attack intensity and more suppression to the aphid insect when applied in the morning compared to late afternoon. This condition was predictably related to the characteristic of the chemical that has systemic and contacts toxic properties. Imidacloprid can be translaminar absorbed by plant tissues and acropetally transported to the whole plant body. While as a contact poison, the active compound has a neural

blockage effect and causes paralyzing and death of the insects (Li et al., 2018). These toxic properties required persistency and stability of the insecticides in a certain period, including high temperature during the application.

This research observed different conditions on the percentage of suppressions on botanical insecticide treatments (Table 2). Most botanical insecticides showed better efficacy when applied in late afternoon than in the morning, except *A. indica* extracts with the range of attack intensity of 43–63.33% and the percentage of suppression of 32.14–58.93%. The highest rate of suppression was shown by 3 g/l *T. sinensis* leaf extract followed by 3.5 g/l of the same leaf extracts when applied in the late afternoon. Other treatments, i.e. *T. diversifolia* at 3 g/l and 3.5 g/l, *A. indica* 3 g/l and *C. nardus* at 3 g/l and 3.5 g/l were also following these trends degrees were different. Higher suppression of botanical insecticides on aphid attacks when applied in late afternoon merely pointed out that the persistency of the active ingredient of botanical insecticides on the plant surface was one important critical period to ensure the effectivity of the respected insecticides on the targeted insect pest. When applied in the late afternoon, the environmental temperature started to cool down. These conditions preserved the insecticides solution to persist longer on the plant surface and affect insect pests more effectively. This temperature decrement also avoided biodegradation of the respected botanical insecticides, resulting in the active compound's instability (Hartati, 2012).

#### Agronomical and Yield Properties of Chrysanthemum Treated Plants

The combination of different types of botanical insecticides, concentration and period of application gave different growth responses and flower qualities, as presented in Table 3. In terms of plant height, all the treated plants, including control, reached more than 75 cm, which was the minimum standard for cut flowers (Yusuf et al., 2019). Under botanical insecticides treatments, the plants were detected higher when applied in the morning, though negligible differences were found among the values. While the different situation was observed on the plants treated by synthetic insecticide Imidacloprid. The plants under this treatment gave longer stem when the insecticide was applied in the late afternoon, and the value was significantly

different from those when used in the morning. The highest plants was detected on the plants treated by *T. diversifolia* leaf extract at 3.5 g/l applied in the morning, followed by synthetic insecticides Imidacloprid used in the late afternoon. While the least was observed at Imidacloprid 1 g/l used in the morning followed by *C. nardus* oils at 2.5% applied in the late afternoon.

All synthetic and botanical insecticides, including control treatments, did not vary in flower diameter (Table 3). The synthetic and botanical insecticide treatments, however, affects the percentage of open flowers. According to Emam (2016), the adults and nymphs of *Macrosiphoniella sanborni* attack the plants by sucking the cell sap from flowers and making the petal's abnormality and the flower fails to optimally bloom. In line with the percentage of attacks severity (Fig. 1a), percentage of attack plants (Fig. 1b) and percentage of suppression (Table 2), the

percentage of open flowers were detected higher on insecticide treatments when applied in the late afternoon, except *T. diversifolia* and *A. indica* leaf extract treatments at 3.5 g/l. Among all treatments, *T. sinensis* leaf extract at 3 g/l gave the highest opened-flowers, followed by *T. diversifolia* extract at 3 g/l and *T. sinensis* extract at 3.5 g/l when applied in the late afternoon. These indicated that *T. sinensis* and *T. diversifolia* more effectively suppressed the aphid attacks on flower organs and reduced petal abnormality that might prevent flower opening. The effectivity of *T. sinensis* and *T. diversifolia* as a botanical insecticide was also reported by Kurniawan, Yulani, & Rachmadiarti (2013) to control *Plutella xylostella* on green mustard, *Spodoptera litura* on soybean (Noviana et al., 2012), brown planthopper (*Nilaparvata lugens*) on rice (Subandi, Chaidir, & Nurjanah, 2016) and *Callosobruchus maculatus* on storage of seeds (Pangihutan et al., 2016).

**Table 2.** Aphid attacks intensity at 63 DAP and suppression percentage of various botanical and synthetic insecticide treatments on chrysanthemum

Leaf extract	Concentration (g/l)	Time of application <sup>*)</sup>	Attack intensity at 63 DAP <sup>**) (%)</sup>		Percentage of suppression (%)
T11- <i>T. sinensis</i>	3	M	60.00	a	35.71
T12- <i>T. sinensis</i>	3	A	43.33	b	55.93
T11- <i>T. sinensis</i>	3.5	M	51.67	a	44.64
T12- <i>T. sinensis</i>	3.5	A	46.67	a	52.54
T21- <i>T. diversifolia</i>	3	M	56.67	a	39.28
T22- <i>T. diversifolia</i>	3	A	48.33	b	50.85
T21- <i>T. diversifolia</i>	3.5	M	55.00	a	41.07
T22- <i>T. diversifolia</i>	3.5	A	48.33	a	50.85
T31- <i>A. indica</i>	3	M	55.00	a	41.07
T32- <i>A. indica</i>	3	A	48.33	a	50.85
T31- <i>A. indica</i>	3.5	M	51.67	a	41.66
T32- <i>A. indica</i>	3.5	A	58.33	a	40.68
T41- <i>C. nardus</i>	2.5	M	63.33	a	32.14
T42- <i>C. nardus</i>	2.5	A	55.00	b	44.07
T41- <i>C. nardus</i>	5	M	58.33	a	37.50
T42- <i>C. nardus</i>	5	A	51.67	a	47.45
T51-Imidacloprid	1	M	38.33	a	58.93
T51-Imidacloprid	1	A	46.67	b	52.54
Control-1		M	93.33	a	
Control-2		A	98.33	a	

Remarks: <sup>\*)</sup> M = morning, A = late afternoon; <sup>\*\*) Values followed by different letters in the same insecticide treatment within the same column differ significantly under paired T- test ( $P \leq 95\%$ )</sup>



**Table 3.** Final plant height, flower diameter and percentage of open flowers of chrysanthemum treated by synthetic and various botanical insecticides

Leaf extract	Concentration (g/l)	Time of application <sup>*)</sup>	Plant height <sup>**) (cm)</sup>	Flower diameter <sup>**) (cm)</sup>	Percentage of open flowers <sup>**) (%)</sup>
T11- <i>T. sinensis</i>	3	M	84.89 bc	9.07 a	23.96 a
T12- <i>T. sinensis</i>	3	A	79.17 ab	9.93 a	71.88 c
T11- <i>T. sinensis</i>	3.5	M	85.94 bc	9.46 a	33.85 ab
T12- <i>T. sinensis</i>	3.5	A	82.50 abc	10.00 a	66.15 bc
T21- <i>T. diversifolia</i>	3	M	85.89 bc	9.08 a	38.02 ab
T22- <i>T. diversifolia</i>	3	A	78.84 ab	9.21 a	67.19 bc
T21- <i>T. diversifolia</i>	3.5	M	88.96 c	9.38 a	60.94 bc
T22- <i>T. diversifolia</i>	3.5	A	85.33 bc	9.88 a	60.42 bc
T31- <i>A. indica</i>	3	M	81.25 abc	9.44 a	42.19 ab
T32- <i>A. indica</i>	3	A	81.31 abc	9.42 a	54.17 bc
T31- <i>A. indica</i>	3.5	M	84.83 bc	8.38 a	50.52 bc
T32- <i>A. indica</i>	3.5	A	83.69 bc	9.56 a	43.23 ab
T41- <i>C. nardus</i>	2.5	M	82.93 abc	9.02 a	26.04 a
T42- <i>C. nardus</i>	2.5	A	76.22 a	9.23 a	41.15 ab
T41- <i>C. nardus</i>	5	M	80.67 ab	9.04 a	33.33 ab
T42- <i>C. nardus</i>	5	A	79.22 ab	8.93 a	39.06 ab
T51-Imidakloprid	1	M	75.42 a	9.52 a	45.83 ab
T51-Imidakloprid	1	A	87.56 c	9.70 a	49.48 bc
Control-1		M	83.29 bc	8.89 a	46.88 abc
Control-2		A	84.79 bc	8.82 a	58.85 bc

Remarks: <sup>\*)</sup> M = morning, A = late afternoon; <sup>\*\*) Values in the same column followed by different letters differ significantly under HSD ( $P \leq 95\%$ )</sup>

## CONCLUSION

Insecticidal effects of several botanical insecticides sprayed in different application periods were investigated. In general, lower attack severity and percentage of the attacked plant were detected when botanical insecticides were sprayed in the late afternoon than in the morning. Leaf extract of *T. sinensis* in the concentration of 3 and 3.5 g/l applied in the late afternoon gave higher suppression on aphid development than other tested botanical insecticides. When used in the late afternoon, 3 and 3.5 g/l, the leaf extract of *T. sinensis* and 3 g/l of *T. diversifolia* also induced a higher percentage of open flower for more than 65% than a synthetic pesticide, Imidacloprid that produced less than 50% opened flowers.

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