Efficacy of Selected Plant Extracts to Control Leaf Miner (Lyriomyza spp.) in Chrysanthemum

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ABSTRACT

The use of eco-friendly methods in controlling pest and diseases has become an increasing concern in production system in many economically important crops, including ornamentals. The application of plant extract that has an insecticidal effect is considered as one promising alternative in reducing the negative effects of synthetic pesticides. The research was conducted to assess selected plant extracts in several concentrations against leaf miner (Lyriomyza spp.) in chrysanthemum. The experiment was carried out from January to December 2017 under plastic house conditions at the Indonesian Ornamental Crops Research Institute (IOCRI). The extracted organ of insecticidal plants regularly sprayed into chrysanthemum plants and compared with water as the controls. The results showed that the application of insecticidal plants extracts reduced attack intensity and incidence from water treatment (control). Certain treatment combinations, Chinese mahogany leaf extract at the concentration of 0.25%, C. pyrethrum petal 0.15 and 0.30%, and chinaberry leaf at 0.3, 0.35% even had 0.9-3.13% lesser attack incidences than commercial botanical insecticide Neem Plus. The respected treatments also suppressed more than 62% leaf miner attacks and induced the increase of marketable flowers.

INTRODUCTION

The use of biological agents to control important pests and diseases in agriculture has become wide world concern in the last few decades. Many authors have reported the potent and successful uses of biological agents, including botanical sources to reduce pest and disease attacks, thus diminish or even totally substitute chemical pesticides (Sharma et al., 2013). Biological control was not only attractive to food and industrial crops, yet to ornamental growers as well, including chrysanthemum that mostly grown under greenhouse condition. According to Marsh & Gallardo (2009), ornamental growers were attracted to use biological control due to several factors: (a) reduction of chemical exposure, labors and applicator, (b) issues related to pest resistant and environmental pollution, (c) attempt to reduce chemical residue on the product due to market/consumer preferences and (d) anticipation to government restriction on the use of chemicals. In several advanced countries, most growers currently used biological controls in their floriculture production system (Sanchez, Gallego, Gamez, & Cabello, 2014).

Many insect pests hosted in chrysanthemum and some of them have resulted production and flower quality degradation, especially during outbreak season. Leaf miner (Lyriomyza spp.) is considered as one of insect pests that might give significant economic losses in chrysanthemum. Many species have been identified causing leaf damage by their mining activity (Foba et al., 2015; Mujica & Kroschel, 2011) and some of them are polyphagous insects (Andersen, An, & Nordhus, 2008). The insects have been found to have a wide world distribution from temperate up to tropical
areas (Ismaeel, Hameed, Ahmed, Saif-Ur-Rehman, & Ahmed, 2016) with relatively very short life cycle (da Costa-Lima, das Chagas, & Parra, 2014; Mujica, Valencia, Ramírez, Prudencio, & Kroschel, 2009; Nonci & Muis, 2011).

The biological control of leaf miner using plant extracts have been reported in several crops like lime citrus against Phyllocnistis citrella Stainton (Jayanti & Verghese, 2007; Shareef et al., 2016), coffee against Leucoptera coffeella (Alves et al., 2013), castor plant against Liriomyza trifolii Burgess (Akashe, Gud, Shinde, & Deshpande, 2009) and horse chestnut tree against Cameraria ohridella (Tomczyk et al., 2007). Several potent plant extracts have also been found effective to act as repellent, developmental inhibitor or even deadly-contact substances to leaf miner. Our previous experiment regarding screening of potent plant extract to control leaf miner in chrysanthemum has revealed that leaf extracts of Chinese mahogany (Toona sinensis [A. Juss] M. Roem), chinaberry (Melia azedarach L.) and petal extracts of Chrysanthemum pyrethrum showed around 50% attack suppressions. Aside from screening the potent plant extracts, the effective concentration of these plant extracts were also still needed to investigate. Precise concentration, in one side allowed the applied substances effective to the targeted insect pest, yet on the other side, did not kill the beneficial insects (pollinator, predator and other natural enemies) and not toxic to the plants (El-Wakeil, 2013; Li, 2012; Ribeiro et al., 2017). Regarding to the said matter, the study was carried out to assess several botanical insecticides in various concentrations that gave effective control on leaf miner in chrysanthemum.

MATERIALS AND METHODS

The research was conducted from January to December 2017 under plastic house conditions at the Indonesian Ornamental Crops Research Institute (IOCRI) 1100 m above sea level (asl) at District of Cianjur, West Java, Indonesia. The chrysanthemum variety used in the study was cv. White Fiji. The research was arranged in a randomized complete block design with three replications. The first factor was the kind of plant extracts i.e. Chinese mahogany (Toona sinensis [Juss] M. Roem), chinaberry (Melia azedarach L.) and Chrysanthemum pyrethrum. While the second factor dealt with the concentrations of these respected plant extracts, i.e. 0.15, 0.2, 0.25, 0.3, 0.35 and 0.35%. The complete treatment combinations used in the study were leaf extracts of Chinese mahogany with the concentration of 0.15% (T1), 0.2% (T2), 0.25% (T3), 0.3% (T4), 0.35% (T5), petal extracts of Chrysanthemum pyrethrum with the concentration of 0.15% (T6), 0.2% (T7), 0.25% (T8), 0.3% (T9), 0.35% (T10), leaf extracts of chinaberry with the concentration of 0.15% (T11), 0.2% (T12), 0.25% (T13), 0.3% (T14), 0.35% (T15), Neem Plus, a commercial botanical insecticide produced by the Indonesian Spice and Medicinal Crops Research Institute with the concentration 0.2% served as the positive control (T16) and water, served as the negative control (T17). The experiment steps were described in the following procedures.

Preparation of Plants Extracts

The leaves of Chinese mahogany and chinaberry and the flower petals of Chrysanthemum pyrethrum were freshly collected and put into sealed plastic bags. The collected leaves and petals were naturally dried for several days. The extraction procedures of the respected leaves and petals were based on Karunaratne & Karunaratne (2012) method. The dried plant organs were grinded into powder forms and filtered with 1 mm mess-sieve. The plant powder were then mixed with acetone with the ratio of 1:10 (100 g plant powder : 1000 ml acetone) using erlenmeyer flask and stirred for 24 h. The mixture was then filtered using Buchner funnel and the acetone was vaporized room temperature. The extract solution was then diluted with water into the targeted concentration treatments. The extract solutions were then put into sealed-glass containers and stored at temperature ≤ 4°C.

Soil and Planting Bed Preparation

The soils inside the plastic house were tilled and the weeds were disposed away from the experimental site. For about 3 kg/m² of manures and 1.5 kg/m² bamboo liter were then gently mixed with the top soil. After allowing standing direct sunlight without water for 7 days, the planting beds were the constructed with the width of 1 m, 2 m in length and 25 cm in height for each treatment plot. The distance between treatments was arranged in 50 cm and between beds was 60 cm. The NPK fertilizer (16:16:16) were mixed the top soil of the planting beds with the dosage of 40 g/m². Water irrigations were then poured into the planting beds with the volume of 10 l/m² to facilitate humidity. Long day conditions were supplied with the installation of
11-watt LED lamps with the distance of 1.5 m above the planting beds and 2 m among the lamps points.

**Planting, Plant Maintenance and Treatment Applications**

The rooted cuttings were planted with the density of 64 plants per m². After all the cuttings were planted, water with the volume of 5 l/m² was given and continued every 2-3 days until the harvesting period. The long day condition was conducted by giving artificial lighting to the plants for 4 h every night from 10.00 pm to 02.00 am until 30 days after planting (DAP). After 30 DAP, the long day conditions were terminated and the plants were forced to flower by providing neutral day conditions. Additional fertilizers were given using NPK (16:16:16) with the dosage of 40 g/m² through top dressing at 15 and 30 DAP and once a week of Growmore based on recommended dosage through foliar application.

The plant extracts (T1-T15) were separately diluted with water and evenly sprayed into plant body with the volume of 1 l/m² during the morning period. These treatments were applied once a week starting 14 DAP. The treatment of Neem Plus (positive control) was applied once a week based on recommended dosage, while water (negative control) was sprayed with the same period, volume and frequency. All the treatments were terminated after 80 DAP.

The observed parameters included insect infestation (emergence and intensity) that were conducted every week from the first day of planting until one week before harvesting period. Level of treatment efficacy that was conducted through the observation of attack incidence and percentage of treatment suppression at 63 days after planting (DAP), growth performance and flower production. All the gathered data were analyzed using ANOVA and Least Significant Differences (LSD, α ≤ 5%).

**RESULTS AND DISCUSSION**

**Insect Infestations**

Insect attack was firstly observed at 14 days after planting (DAP) at several treatments plots. The initial symptoms were characterized as white spots in leaf surface resulted from the ovipositor piercing (Fig. 1a). After 28 days planting, further leaf damages were observed in all treatment plots (Fig. 1b) with various degrees of intensities. According to Chow & Heinz (2006), chrysanthemum leaf miner larvae fed on the cells within the host plant leaves and created silvery lines across the leaves. In low numbers, the damage was usually cosmetic, however severe infestations might reduce the photosynthetic capabilities of the plant or even leaf desiccation and drop. Environmental factors such as temperature, humidity and rain were reported to determine the dynamic lifecycle and development of the insects (Nonci & Muis, 2011).

![Fig. 1. (A) Initial leaf miner attacks symptom characterized by with spots (indicated by black arrow) and (B) further damages caused by the insect mining activities](image-url)
In general, the leaf miner attack intensity on the plants treated with plant extracts is lower than control at 63 DAP (Fig. 2). None of the insecticide treatments performed stable in lowering the attack intensities from the early planting periods and there was no specific trend could be drawn between concentrations and type of botanical insecticides as far as these concerned. The lowest attacks intensities were detected on the plants treated with Chinese Mahogany leaf extracts 0.25% (10.83%), C. pyrethrum petal extracts 0.15% (11.07%) and Chinaberry leaf extracts 0.3% and 0.35% (11.90% and 11.43%, respectively). The attack intensities under these treatments even lower than those under commercial formulated botanical insecticide Neem Plus that reached 14.26% at 63 DAP. These conditions inferred that each botanical insecticide source had specific concentration ranges to give effective suppression (Barati, Golmohammadi, Ghajarie, Zarabi, & Mansouri, 2013; Puripattanavong, Songkram, Lomlim, & Amnuaikit, 2013) on leaf miner.

**Level of Treatment Efficacy**

Leaf miner attack incidence at 63 DAP and percentage of suppression among plant extract treatments are presented in Table 1. Leaf miner attack incidence varied among the treatments. It was almost similar to those at attack intensity, the attack incidences were found lower on the plants treated with Chinese mahogany leaf extract at the concentration of 0.25%, C. pyrethrum petal extract at the concentration of 0.15 and 0.30%, chinaberry leaf extracts at the concentration of 0.3, 0.35% and formulated botanical insecticide Neem Plus. These conditions indicated that Chinese mahogany leaf, C. pyrethrum petal and chinaberry leaf extracts with the respected concentrations could give maximum potential insecticidal properties against leaf miner and their efficacies were also comparable with the commercial formulated biopesticide Neem Plus.

![Fig 2. Leaf miner attack intensity during plant growth period under different concentrations of several botanical insecticides treatments](image-url)
Table 1. Leaf miner attack incidence at 63 DAP and percentage of suppression among botanical insecticide treatments

| Botanical insecticide treatments                        | Attack incidence at 63 DAP\(^\dagger\) (%) | Percentage of suppression (%) |
|--------------------------------------------------------|--------------------------------------------|--------------------------------|
| Chinese Mahogany leaf extracts at concentration of     |                                            |                                |
| - 0.15%                                                | 17.78 bcd                                  | 47.75                          |
| - 0.20%                                                | 18.3 bcd                                   | 46.22                          |
| - 0.25%                                                | 11.61 a                                    | 65.89                          |
| - 0.30%                                                | 19.35 d                                    | 43.16                          |
| - 0.35%                                                | 14.66 abcd                                 | 56.93                          |
| C. pyrethrum petal extracts at concentration of         |                                            |                                |
| - 0.15%                                                | 12.65 ab                                   | 62.83                          |
| - 0.20%                                                | 16.74 bcd                                  | 50.81                          |
| - 0.25%                                                | 16.07 abcd                                 | 52.78                          |
| - 0.30%                                                | 12.65 ab                                   | 62.83                          |
| - 0.35%                                                | 19.2 cd                                    | 43.59                          |
| Chinaberry leaf extracts at concentration of            |                                            |                                |
| - 0.15%                                                | 13.47 abc                                  | 60.43                          |
| - 0.20%                                                | 14.43 abc                                  | 57.59                          |
| - 0.25%                                                | 16.67 abcd                                 | 51.03                          |
| - 0.30%                                                | 10.34 a                                    | 69.61                          |
| - 0.35%                                                | 12.57 abc                                  | 63.05                          |
| Neem Plus (control +)                                  | 13.47 abc                                  | 60.43                          |
| Water (control -)                                      | 34.03 e                                    | 0.00                           |

Remarks: \(^\dagger\) Values in the same column followed by different letters differ significantly under Least Significant Different (LSD, \(\alpha = 5\%\))

Several botanical plant extract treatments showed higher percentage of suppression against leaf miner. Botanical insecticide treatments of Chinese mahogany leaf extract at the concentration of 0.25%, *C. pyrethrum* petal extract at the concentration of 0.15 and 0.30%, chinaberry leaf extracts at the concentration of 0.3, 0.35% showed higher percentage of suppression than formulated botanical insecticides. Several authors also reported the effectivity of Chinese mahogany leaf extract, *C. pyrethrum* petal extract and Chinaberry leaf extract in controlling many insect pests in several economically important crops though in different concentrations (Ahmadi, Amiri-Besheli, & Hosieni, 2012; Ateyyat & Abu-Darwish, 2009; El-Wakeil, 2013; Pavela, 2009; Shawan, Rashed, Mitu, & Jahan, 2018; Xavier et al., 2015; Zhong, Lv, & Qin, 2017). Greater suppression of these botanical extracts also indicated that these potential substances should furtherly be assessed for their efficacy, proper formulation and concentrations, and application in regards to be the alternative and complementary eco-friendly substances from the existing methods and/or commercial biopesticides (Rahardjo, Budiarto, & Marwoto, 2019) to control leafminer in chrysanthemum.

**Plant Growth Performance and Flower Production**

The effects of plants extracts on plant height, number of marketable flowers and flower diameter are presented in Table 2. The values of plant height and number of marketable flowers varied, while flower diameter was seemed not affected by plant extract treatments. In general, the application of botanical insecticide improved plant growth and flower yield than control through the increase of plant height and number of marketable though the trends were not in accordance with the increased concentrations in any plant extracts. Better growth performance and marketable flowers on chrysanthemum under botanical insecticide treatments were predictably related to the growth improvement due to greater suppression on leaf miner attacks. Greater suppression referred to lesser damages on leaf tissues, thus increased potential leaf area for photosynthesis to support plant growth and flower development (Jones, Parrella, & Hodel, 1986), including the number of marketable flower, though the effects were less visible on flower diameter. The growth improvement and yield as results of botanical pesticide application were also reported in chrysanthemum (Rahardjo, Budiarto, & Marwoto, 2019), citrus (Shinde et al., 2017), tomato (de Evert, López, & de López, 2015), coffee (Alves et al., 2013), other economically important crops.
Table 2. Effects of botanical insecticide treatments on plant height, number of marketable flowers and flower diameter in chrysanthemum

| Botanical insecticide treatments | Plant height *(cm)* | Number of marketable flowers (stalks) | Flower diameter *(cm)* |
|---------------------------------|---------------------|---------------------------------------|------------------------|
| Chinese Mahogany leaf extracts at concentration of |                     |                                       |                        |
| - 0.15%                         | 93.27 b             | 102.4 ab                              | 5.24 a                 |
| - 0.20%                         | 92.30 ab            | 105.1 ab                              | 5.31 a                 |
| - 0.25%                         | 94.36 b             | 107.4 b                               | 5.47 a                 |
| - 0.30%                         | 91.92 ab            | 103.5 ab                              | 5.35 a                 |
| - 0.35%                         | 88.7 ab             | 105.7 b                               | 5.57 a                 |
| C. *pyrethrum* petal extracts at concentration of |                     |                                       |                        |
| - 0.15%                         | 90.67 ab            | 108.2 b                               | 5.22 a                 |
| - 0.20%                         | 91.31 ab            | 105.3 ab                              | 5.53 a                 |
| - 0.25%                         | 87.99 ab            | 106.4 b                               | 5.36 a                 |
| - 0.30%                         | 85.10 a             | 110.3 b                               | 5.24 a                 |
| - 0.35%                         | 91.07 ab            | 104.5 ab                              | 5.45 a                 |
| Chinaberry leaf extracts at concentration of |                     |                                       |                        |
| - 0.15%                         | 90.79 ab            | 105.4 ab                              | 5.48 a                 |
| - 0.20%                         | 88.48 ab            | 105.2 ab                              | 5.36 a                 |
| - 0.25%                         | 89.06 ab            | 104.6 ab                              | 5.11 a                 |
| - 0.30%                         | 91.15 ab            | 107.7 b                               | 5.27 a                 |
| - 0.35%                         | 84.38 a             | 109.3 b                               | 5.66 a                 |
| Neem Plus (control +)           | 87.25 ab            | 102.5 ab                              | 5.25 a                 |
| Water (control -)               | 84.48 a             | 95.7 a                                | 5.17 a                 |

Remarks: *Values in the same column followed by different letters differ significantly under Least Significant Different (LSD, α ≤ 5%)

CONCLUSION

The application of plant extracts of China Mahogany leaves, *C. pyrethrum* petals, Chinaberry leaves reduced leafminer attacks on chrysanthemum viewed from less attack intensity and incidence. There were no specific trends related to the attack intensity and incidence with the concentrations of extracts in any botanical insecticide types. The plants treated with Chinese mahogany leaf extract at the concentration of 0.25%, *C. pyrethrum* petal extract at the concentration of 0.15 and 0.30%, chinaberry leaf extracts at the concentration of 0.3, 0.35% showed 0.9-3.13% lesser attack incidences than the formulated botanical insecticide Neem Plus and 21.46-23.69% than the control. The respected treatments also performed more than 62% suppression than control and induced the increase of marketable flowers.

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