Bioinsecticide Activity of Neem Oil and Tobacco Extract Mixture Against Citrus Aphids and Green Scale on Citrus

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Abstract. The aim of this research is to determine the effectiveness of a mixture of neem (*Azadirachta indica* A. Juss.) and tobacco (*Nicotiana tabacum* L.) as bioinsecticides on the mortality of aphids (*Toxoptera citricidus* [Kirkaldy, 1907]) and the green scale (*Coccus viridis* [Green, 1889]). Data were analyze by variance and by Duncan Multiple Range Test (DMRT) at 5 % level of significance. The results showed that the bioinsecticide caused mortality of *T. citricidus* more than 50 % starting from 24 h after application (HAP) and at the 168 HAP the mortality 96 % to 100 %, not significantly different from the imidacloprid synthetic insecticide and significantly different from the control. The application of the bioinsecticide was less effective against C. *viridis.* The mortality of *C. viridis* is significantly different from the insecticide imidacloprid. The application of a bioinsecticide mixture of neem and tobacco extracts was effective against the brown citrus aphid *T. citricidus*, but less effective against the green scale *C. viridis*.

Keyword: Botanical insecticides, citrus pest, environmental-friendly, natural pesticides, pest control

1 Introduction

The brown citrus aphid (*Toxoptera citricidus* [Kirkaldy,1907]), is a sap-sucking insect and is one of the world's most serious citrus pests [1, 2]. *T. citricidus* colonizes young leaves, stems, flowers, and growing points of plants. The main hosts of *T. citricidus* are oranges and citrus relatives [3]. *T. citricidus* can cause direct damage to citrus plants by attacking shoots, flower buds, and sometimes young fruit but the main impact of *T. citricidus* is the transmission of Citrus Tristeza Virus (CTV). Among the CTV vectors, the citrus aphid *T. citricidus* is the most efficient in transmission [4, 5].

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The other important pest in citrus apart from *T. citricidus* is the scale insect (*Coccus viridis* [Green, 1889]) [6, 7]. *C. viridis* is a pest that has a wide host range including vegetables, fruit, and ornamental plants [8]. *C. viridis* is an important pest on coffee plants, which is also a pest on citrus [9, 10]. *C. viridis* attacks twigs, leaves, and fruit on citrus plants. In the plant, this pest preferred to stay in the area away from direct sunlight, such as on the underside of the leaf surface. The leaves that are attacked by *C. viridis* will turn yellow, there are chlorotic spots then often cause the leaves to fall. Heavy attacks will cause twigs and dried branches and cracks occur in the twigs. If the attack of *C. viridis* occurs around the fruits, it will cause the fruit to fall. As a result of the attack can reduce the fruit quality. When the fruits are cleaned, the attack will create green or yellow spots on the rind [11, 12].

The impact of aphid and *C. viridis* attacks on citrus is considered to be detrimental, then it is necessary to control them with specially targeted, efficient, inexpensive, environmentally friendly techniques. However, in today's agricultural practice, the control technique that is still relying on synthetic pesticides. Continuous use and application of active ingredients can cause pest resistance, the emergence of secondary pests, and environmental damage caused by residues of the active ingredients [13]. In an effort to reduce these negative impacts, environmentally friendly control techniques are needed by utilizing toxic compounds derived from plants. Some plants that were extracted and used as ingredients for vegetable pesticides are neem [14–16] and tobacco [13, 17, 18].

Leaf extract of neem (*Azadirachta indica* A. Juss.) was reported to be able to control approximately 127 pests and was able to act as a fungicide, bactericide, nematicide, and molluscicide [19]. Study by [15] stated that neem seed extract was the most toxic among other plant extracts, namely Eucalyptus, sweet orange seed extract and lime sour orange leaf extract against mealybugs. It was also stated that neem seed botanical insecticides play an important role in reducing the toxic hazard of synthetic chemical insecticides. Neem has the potential to be an effective control for mealybugs and hemipterous pest groups [15]. In addition, in the application of mosquito coils, neem leaf extract was able to suppress mosquito bites up to 84.5 % to 85 % [20]. Not only neem, many sources of plant material can be used as bioinsecticides to control insect pests [21]. But the other plant extract used as insect control was also tobacco (*Nicotiana tabacum* L.) [17]. It was reported that tobacco extract was effective to control insect pests, namely (*Nilaparvata lugens* [Stal, 1854]), (*Spodoptera litura* [Fabricius, 1775]) [22], citrus psyllid (*Diaphorina citri* [Shigure Kuwayama, 1908]) and Colorado Potato beetle (*Leptinotarsa decemlineata* L.) [17, 18].

Neem and tobacco plant extracts have been widely used in various tests on insect mortality, but have not been tested against these two citrus pests. In this study, the bioinsecticides of neem and tobacco extracts will be tested which are combined into bioinsecticides and compared with synthetic pesticides with the active ingredient of imidacloprid against *T. citricidus*, and *C. viridis*.

2 Material and methods

The research was carried out from February to May 2022 at the Entomology Laboratory and the screen house of the Indonesian Citrus and Subtropical Fruits Research Institute — ICSFRI (*Balai Penelitian Tanaman Jeruk dan Buah Subtropika* —Balitjestro), Tlekung, Batu, East Java, Indonesia, altitude: 892.4 m a.s.l, and coordinates: S 7°54'12.042" to E 112°32'5.4996

2.1 Preparation of *Toxoptera citricidus*

Aphids that will be used as test material were carried out by mass rearing. The host plants for mass rearing were Tangerine citrus or Japansche Citrus (JC) rootstock. Mass rearing of the population of *T. citricidus* was carried out by taking imago from citrus plants in the field around ICSFRI . The next step was imago *T. citricidus* infested on the shoots of the host plant. After giving the offspring and the population is full in the shoots, the adult was transferred to other shoots, and so on until the population is sufficient for treatment. The aphids used for treatment were the 2nd to 3rd nymphs stage.

2.2 Preparation of *Coccus viridis*

C. viridis which was used for breeding was taken from citrus plants around the ICSFRI area. Twigs and leaves of citrus plants that were attacked by *C. viridis* were cut and the cut ends were placed into water to keep the twigs fresh and *C. viridis* to be active. The crawlers that came out of the moving and walking from female *C. viridis* were picked up using a fine brush and transferred to the leaves at the top close to the leaf bones. Tangerine citrus plants used for *C. viridis* mas rearing. If the *C. viridis* attached to the twig does not move, the twig is attached to the citrus plant used for breeding so that *C. viridis* moves on its own. After *C. viridis* reproduce on citrus plants, and the number was sufficient, *C. viridis* was ready to be used for treatment.

2.3 Preparation of neem and tobacco extract

The extract was made from two kinds of plant materials, i.e. tobacco leaf and neem seed. The dried plants were processed powder using made powder by grinder then processed into extracts by maceration. The solvent used for maceration was acetone. The extract was made by soaking the powder in a solvent with a ratio of 1:2 (v v^{-1} for 24 h. Further process of the solution that has been soaked is filtered and evaporated with a rotary evaporator at a temperature of 40 ℃ until a thick liquid extract was obtained and the solvent has all can be evaporated. To make the formulation of a mixture of bioinsecticides from neem and tobacco extracts, each ingredient was provided with volumes of 0.5: 0.5: 0.5: 8.5 (v) respectively for neem extract, a tobacco extract, emulsifier, distilled water. The mixed formulation is then diluted according to the test concentration, which was determined.

2.4 Bioassay of mix neem–tobacco bioinsecticides

The bioassay was carried out on Tangerine citrus plants as hosts for *T. citricidus* and *C. viridis* as well as the experimental arena. The treatments tested were concentrations of bioinsecticide mixture of neem and tobacco extracts 0.2 %; 0.3 %; 0.4 %; and 0.5 %, the comparison treatment was imidacloprid insecticide and water control. The treatment was repeated four times. Plant shoots were used as replicates.

In the treatment for aphids, citrus plants that were at the sprouting stage are prepared as treatment arenas. Aphids were infested on the shoots of citrus plants as many as 20 individuals $shoot^{-1}$. Applications were made by spraying the solution directly on the test insects attached to the shoots for each test concentration. Tests for green scale *C. viridis* were carried out on plants that already contained scale lice with a minimum number of 20 on leaves and twigs on one twig from the results of propagation. The same methods was applied to that on *C. viridis* treatment.

Mortality data were collected by observing the number of dead and life-test insects. Observations of insect mortality were carried out at 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, and 168 h after application. The data from the observations were then calculated according to [23] corrected mortality percent if there were insect mortality based on Equation (1)

$$CM = \frac{X - Y}{X} \times 100\% \quad (1)$$

CM is the corrected mortality, X is the sittlife insect in the control, and Y is the sittlife insect in the treatment. If there is no death in the control treatment, then it is calculated by Equation (2)

$$P0 = \frac{r}{n} \times 100\% \quad (2)$$

P0 is insect mortality, r is the number of dead insects, and n is the initial number of insects.

3. Result and discussion

The application of imidacloprid and a mixture of neem and tobacco biopesticide had a significant effect, as indicated by the results on the mortality rate of the brown aphid *T. citricidus* (Table 1). In the first observation at 24 h after application (HAA) it was found that the application of imidacloprid and a mixture of neem and tobacco at concentrations of 0.4 % and 0.5 % caused the death rate of the brown aphid *T. citricidus* to at more than 50 %. At the last observation on the 7 d (168 HAA), the application of a mixture of neem and tobacco caused the death rate of the brown aphid *T. citricidus* to be at 100 %. [24–26] explained that neem and tobacco extracts both have compounds that are toxic to insects. Thus, the two extracts are widely used as botanical pesticides which are effective in controlling insect pest populations, one of which is aphids.

Reported by Siswoyo, *et al.*, [27] that neem extract was able to control *Theobroma cacao* L. pests in cocoa plantations. Explained by [14] that there are compounds in neem that play an active role in killing insects, azadirachtin compounds. Manjunath, *et al.*, [28] also explained that azadirachtin was antifeedant. Reported by [29] that the use of tobacco extract was able to reduce the attack rate and population of aphids on potato plantations. Sharma [30] stated that in addition to nicotine, tobacco extract also contains several compounds that are toxic to insect pests, such as saponins, terpenoids, and flavonoids. Furthermor reported by [30] that the nano formulation of tobacco botanical insecticide could suppress the population of citrus aphids (*Aphis gossypii* [Glover, 1877]).

The results of this study also showed that the application of the active ingredient imidacloprid and a mixture of neem and tobacco had a significant effect on the mortality rate of *C. viridis* (Table 2). The sixth observation (144 HAA) showed that the application of imidacloprid caused the mortality rate of *C. viridis* at 55 %. During the observations, the application of imidacloprid and a mixture of neem and tobacco 100 % mortality rate of *C. viridis*. The ineffectiveness of the application of neem mixture with tobacco might be due to the solution does not come into contact with the tick body directly or is consumed by *C. viridis. C. viridis* has thick scales and can protect the body from insecticides.

Table 1. Effectivity of bioinsecticide mix of neem and tobacco extract against *T. citricidus*

Treatments	% of mortality						
	24 HAA	**48 HAA**	**72 HAA**	**96 HAA**	**120 HAA**	**144 HAA**	**168 HAA**
Mix neem–tobacco 0.2 %	52 b	60 b	70 b	85 b	90 b	74 b	198 b
Mix neem–tobacco 0.3 %	51 b	63 bc	81 b	84 b	89 b	97 b	196 b
Mix neem–tobacco 0.4 %	83 b	88 cd	81 b	98 b	99 b	98 b	100 b
Mix neem–tobacco 0.5 %	85 b	91 d1	91 b	92 b	96 b	99 b	100 b
Imidakloprid	83 b	83 cd	88 b	98 b	98 b	99 b	199 b
Control	10 a	0 a	10 a	10 a	11 a	14 a	116 a

Note: Number followed by the same letter in the same column was not significantly different at 5 % DMRT.
HAA : Hour After Application

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Table 2. Effectivity of bioinsecticide mix of neem and tobacco extract against green scale *C. viridis*

Treatments	% of mortality						
	24 HAA	**48 HAA**	**72 HAA**	**96 HAA**	**120 HAA**	**144 HAA**	**168 HAA**
Mix neem–tobacco 0.2 %	5 cd	10 ab1	12 ab	12 ab	15 bc	16 bc	5 cd
Mix neem–tobacco 0.3 %	2 ab	15 abc	7 ab	18 ab	19 ab	12 ab	13 ab
Mix neem–tobacco 0.4 %	6 cd	12 bcd	18 bc	22 bc	18 bc	27 cd	28 c
Mix neem–tobacco 0.5 %	8 d1	16 d	25 c	28 c1	32 cd	41 d1	43 d
Imidakloprid	1 a1	13 ab1	13 abc	33 c1	39 d1	55 e1	59 e
Control	0 a1	0 a1	0 a1	0 a	0 a	1 a	11 a

Note: The number followed by the same letter in the same column was not significantly different at 5 % DMRT
HAA : Hour After Application

Based on probit analysis, lethal concentrations of 50 % and 90 % (LC50 and LC90) in *T. citricidus* and *C. viridis* showed different values. The results of the LC50 equation for the two pests tested were obtained were = 3.215 + 2.743 X for *T. citricidus*, and y = –3.066 + 2.087 X for *C. viridis* (Table 3). This means that each increase in concentration of 0.1 mL L^{-1} of a mixture of neem and tobacco extracts caused an increase inof mortality of 2.74 % for *T. citricidus*, and 2.08 % for *C. viridis*. Then sequentially, the LC50 values of the two pests tested, were 0.002 mL L^{-1} for *T. citricidus*, and 0.003 mL L^{-1} for *C. viridis*. The LC90 value of the mixture of neem and tobacco extracts against the two pests tested was lower than the use of neem and tobacco extracts against other insects. Reported by [32] that the LC50 value of neem extract against the butterfly (*Doleschallia polibete* [Cramer,1779]) was 50 mL L^{-1}.

In another study, [33] tobacco waste at a concentration of 33.333 % (333.330 mg L^{-1}) was able to kill 50 % of ants. Results of this study can be used as a reference in controlling citrus pests, especially citrus aphids T. citricidus. For green scale *C. viridis*, it is necessary to review the possibility of higher doses of bioinsecticides. At least, the use of bioinsecticides can reduce the negative impact on consumers and the environment [34].

Table 3. The lethal concentration of mixmixed neem and tobacco application against *T. citricidus* and *C. viridis* at 7 HAA

Pest	Regresi Equation	Sandart Error	LC 50 (mL L^{-1})	LC 90 (mL L^{-1})
Brown citrus aphids *T. citricidus*	Y = 3.215 + 2.743 X	0.957	0.002	0.003
Green scale *C. viridis*	Y = –3.066 + 2.087 X	7.000	0.003	0.004

4 Conclusion

The application of a mixture of neem and tobacco extracts was effective against the brown aphid *T. citricidus*, but was not effective against the scale aphid *C. viridis*. Bioinsecticide mixture of neem and tobacco extracts at levels of 0.4 % and 0.5 % was effective caused the mortality of brown citrus aphid *T. citricidus* comparable with the imidacloprid insecticides.

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