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Research Article

Baseline Susceptibility of *Crotonothrips polyalthiae* (Thysanoptera: Phlaeothripidae) to Selected Insecticides in Laboratory

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Abstract

Background and objective: *Crotonothrips polyalthiae* Mound and Nasruddin (Thysanoptera: Phlaeothripidae) is a new but increasingly important pest of asoka tree in Indonesia. Nursery managements rely heavily on insecticides to control the insect, though no information available on the effective insecticides against the pest. Thus, the purpose of the current study was to determine the baseline toxicity and efficacy of 12 insecticides belonging to 8 IRAC's modes of action on *C. polyalthiae* in laboratory. **Methodology:** The *C. polyalthiae* individuals were collected from active leaf galls of mature ashoka trees that had never been exposed to insecticides. Twelve insecticides belonging to 8 IRAC modes of action (Imidacloprid, abamectin, diafenthiuron, λ-cyholothrin, spinosad, chlorpyrifos, fipronil, deltamethrin, profenofos, permethrin, clofentezin and dicofol) were evaluated for their toxicity against the thrips. Each insecticide was diluted in distilled water with 6 concentrations, including the control. The treatments were arranged in a completely randomized design with five replications. Leaf-dip method was used in the toxicity assay. **Results:** In general, the insecticides were more toxic against the adults than the larvae of *C. polyalthiae*. The LC_{50} values for the adults and the larvae ranged from 19.80-356.97 ppm and from 28.00-415.0 ppm, respectively. Imidacloprid, abamectin, diafenthiuron and λ-cyhalothrin were the most toxic against the adults, while diafenthiuron, imidacloprid, spinosad, abamectin, λ-cyhalothrin, fipronil and chlorpyrifos were the most toxic to the larvae. The results were confirmed by the results of the efficacy test in which only the recommended concentrations were used. **Conclusion:** Eight out of 12 insecticides tested: Six insecticides recommended for use on thrips of other crops (deltamethrin, abamectin, imidacloprid, fipronil, diafenthiuron and spinosad) and 2 insecticides not recommended for thrips control (λ-cyhalothrin and chlorpyrifos) were effective against the thrips. The study results also presented baseline susceptibility of the insecticides tested and the data could be used as a reference in monitoring future resistance development in the pest.

Key words: *Crotonothrips polyalthiae*, toxicity, baseline susceptibility, LC_{50}

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Polyalthia longifolia (Sonn.) (Annonaceae), usually called mast tree, false ashoka or asoka is originally from Sri Lanka and India and is currently found in many tropical countries[1,2]. The tree has been very popular because of its aesthetic and medicinal values and it is easy to grow without special care. In many countries, the plant is traditionally used to treat fever, skin diseases and hypertension[3,4]. It is also used as hepatoprotective, anti-inflammatory[5], antimicrobial[6] and antiulcer[7]. However, in Indonesia, the plant is mostly used as border and shade trees in parks, university campuses and city streets.

Crotonothrips polyalthiae Mound and Nasruddin (Thysanoptera: Phlaeothripidae) was encountered for the 1st time in 2010 in Makassar, Indonesia causing damages to *P. longifolia* by forming leaf galls. The galls were formed in a simple structure with leaf folding along the leaf midrib. All life stages of the thrips were spent inside of the gall. The thrips has white eggs, white banded red larvae, red pupae and black adults[8]. The insect does not kill mature plants but it causes misshapes of the plant canopy, reducing its aesthetic value. The tip of the canopy becomes irregular, otherwise, it is sharp towards the top of the tree. Thrips-infested plants experienced reduction in their height and stem circumference by 49 and 37%, respectively, in comparison with thrips-free plants that were regularly applied with insecticides[9].

Since, it was reported for the 1st time in 2012[8], *C. polyalthiae* has been found in most parts of the country, including Sumatera, Java and Sulawesi. Nursery keepers depend heavily on insecticide use to control the thrips and it is necessary to protect the plants until they are 6 months old. Unprotected young seedlings could die because the insect keeps attacking and killing the young leaves as soon as they are formed. Until the present, there is no information available about the efficacy of different insecticides against the thrips. The nursery grower's choice of insecticides is based on the insecticides recommended for the control of other thrips species on other plant hosts. This practice does not always fit with all pest problems, thus laboratory evaluation of available insecticides is necessary for effective control of this insect[10]. Thus, the objective of the current study was to determine the *in vitro* baseline susceptibility of *C. polyalthiae* to selected insecticides as future reference in assessing resistance of the insect to those insecticides. In addition, the *in vitro* efficacy of those insecticides against the thrips was also determined in this study and this information will be useful for growers in choosing effective insecticides for the insect control.

MATERIALS AND METHODS

Test insect: Active leaf galls were collected from mature plants that had never been sprayed with insecticides. The trees from which the galls were collected were more than 10 km away from the closest nurseries where intensive insecticide use was applied. This was to prevent from collecting thrips that had been previously exposed to insecticides. In order to obtain adult thrips of the same age, about 50 galls were placed inside of a jar (20 cm diameter and 25 cm height) after the adults were removed from the galls and only the larvae were kept inside of the galls. Newly emerged adults and second instar larvae were used in the assays.

Toxicity test: Twelve commercially formulated insecticides representing 8 IRAC's modes of action were tested in the trials (Table 1). The insecticides were purchased from local pesticide stores. Six concentrations, namely: 0, 0.25, 0.5, 1, 1.5 and 2X the recommended rate of each insecticide, diluted in distilled water were used for each insecticide. Each concentration had 5 replications, each with 20 larvae or adults of the *C. polyalthiae*. Young, tender and fully expanded asoka tree leaves were cut into small pieces (2×4 cm). The leaf pieces were then dipped into the insecticide solutions for 5 sec with gentle agitations before they were placed on a paper towel to air dry in room temperature for 30 min. Treated leaf pieces were individually placed into a clear glass vial (2.2 cm diameter and 4.5 cm height). Twenty adults or larvae were transferred into each vial using a fine camel hair brush. Five vials (replications) were used for each concentration treatment. The vials were kept in room temperature (27 ± 1°C) with a photoperiod of 12:12 (L:D). Adult or larval mortality in each vial was recorded 48 h after the initiation of the assay.

Efficacy test: The efficacy of 12 insecticide formulations (the same chemicals used in the toxicity test) with 8 modes of action[11] was also determined in this study. Each insecticide was diluted in distilled water at its respective concentration recommended by the manufacturer (Table 1). Young, tender and fully expanded asoka tree leaves were cut into small pieces (2×4 cm). The leaf pieces were then dipped into the insecticide solutions for 5 sec with gentle agitations before they were placed on a paper towel to air dry in room temperature for 30 min. Treated leaf pieces were individually placed into a clear glass vial (2.2 cm diameter and 4.5 cm height). Twenty adults or larvae were transferred into each vial using a fine camel hair brush. Five vials (replications) were used for each insecticide treatment. The vials were kept in room temperature (27±1°C) with a photoperiod of 12:12 (L:D). Adult or larval mortality in each vial was recorded 48 h after the initiation of the assay. The adults and larvae were counted as dead if they did not respond to gentle touch with a fine brush.

Statistical analysis: Insecticide toxicity expressed in LC_{50} values, 95% Fiducial Limits (FL) and slopes of the regression lines were estimated by probit analysis. Thrips mortality data for the insecticide efficacy trial were analyzed using a one-way ANOVA. If a significant difference was detected ($p<0.05$), treatment means were separated using Tukey's test[12].

RESULTS

Toxicity test: The toxicity of 12 commercial formulations of insecticides, belonging to 8 different IRAC's modes of action (Table 1) against the adults and larvae of *C. polyalthiae* was determined using leaf-dip testing method. The LC_{50} values of the tested insecticides ranged from 19.8-415.0 ppm after a 48 h exposure (Table 2). The insecticides were more toxic to the adults than the larvae in both toxicity and efficacy tests, as shown in Table 2 and Fig. 1, respectively. The LC_{50} values for the adults ranged from 19.8 (Imidacloprid) to 356.97 ppm (Dicofol), while for the larvae ranged from 28.00 (Diafenthiuron) to 415.0 ppm (Dicofol).

Table 1: Insecticides and their active ingredients (a.i.) used in the laboratory bioassay against *C. polyalthiae*

Trade names	Active ingredients	IRAC group[a]	Rate[b] (mL L^{-1})	Manufacturer/supplier
Apollo 500SC	Clofentezin	10A	1.0	PT Royal Agro Indonesia
Decis 25EC	Deltametrin[c]	3A	1.0	PT Bayer Indonesia
Demolish 18EC	Abamectin	6	1.0	PT. Dharma Guna Wibawa
Delouse 200SL	Imidacloprid[c]	4A	1.0	PT. Dharma Guna Wibawa
Destar 50SC	Fipronil[c]	2B	2.0	PT Deltagro Mulia Sejati
Detacron 500EC	Profenofos	1B	2.0	PT Deltagro Mulia Sejati
Dicofan 460EC	Dicofol	UN	2.0	PT Royal Agro Indonesia
Dursban 200EC	Chlorpyrifos	1B	1.5	PT Dow AgroSciences Indonesia
Hamasid 25EC	Lambda-cyholothrin	3A	1.0	PT Deltagro Mulia Sejati
Pegassus 500SC	Diafenthiuron[c]	12A	2.0	PT Syngenta Indonesia
Prego 20EC	Permethrin	3A	2.0	PT Deltagro Mulia Sejati
Tracer 120SC	Spinosad[c]	5	2.0	PT Dow AgroSciences Indonesia

[a]Insecticide grouping based on IRAC mode of action classification scheme[11], UN: Unknown mode of action, [b]Commercial formulations and [c]Recommended for thrips control

Table 2: Toxicity of selected insecticides against adults and larvae of *C. polyalthiae in vitro*

Insecticides	Life stages	N[a]	Slope±SE	LC_{50}	95% FL
Imidacloprid[b]	Adult	500	1.81±0.79	19.80	14.01-47.43
	Larva	500	1.02±0.77	33.10	28.76-45.51
Abamectin[b]	Adult	500	2.67±1.89	21.77	17.42-29.19
	Larva	500	1.01±0.33	52.13	25.96-63.92
Diafenthiuron[b]	Adult	500	0.95±0.17	22.00	11.88-44.31
	Larva	500	0.85±0.56	28.001	12.00-55.01
λ-cyholothrin	Adult	500	1.57±0.52	30.96	21.80-43.41
	Larva	500	2.97±0.25	52.38	44.66-61.14
Spinosad[b]	Adult	500	3.53±1.56	39.01	34.55-48.07
	Larva	500	0.56±0.32	48.34	26.94-76.58
Chlorpyrifos	Adult	500	1.67±0.45	49.67	42.71-63.91
	Larva	500	2.00±0.48	64.11	47.63-76.31
Fipronil[b]	Adult	500	2.93±0.52	53.81	39.82-69.49
	Larva	500	2.04±0.51	61.13	51.29-76.09
Deltamethrin[b]	Adult	500	2.14±0.87	77.25	60.75-91.76
	Larva	500	1.97±0.37	135.6	102.35-161.22
Profenofos	Adult	500	1.34±0.44	126.93	116.17-144.64
	Larva	500	0.46±0.49	168.39	105.07-181.10
Permethrin	Adult	500	3.66±0.86	154.88	147.26-171.67
	Larva	500	2.38±0.74	175.56	169.04-192.10
Clofentezin	Adult	500	1.23±0.45	174.77	165.53-181.50
	Larva	500	1.10±0.12	195.00	177.43-220.26
Dicofol	Adult	500	1.59±0.37	356.97	344.01-384.13
	Larva	500	1.93±0.41	415.00	394.26-438.50

Lethal concentrations are in parts per million. Overlapping 95% fiducial limits indicate that their respective lethal concentrations are not significantly different, [a]Total number of thrips individuals tested for each insecticide and [b]Insecticides recommended for thrips control

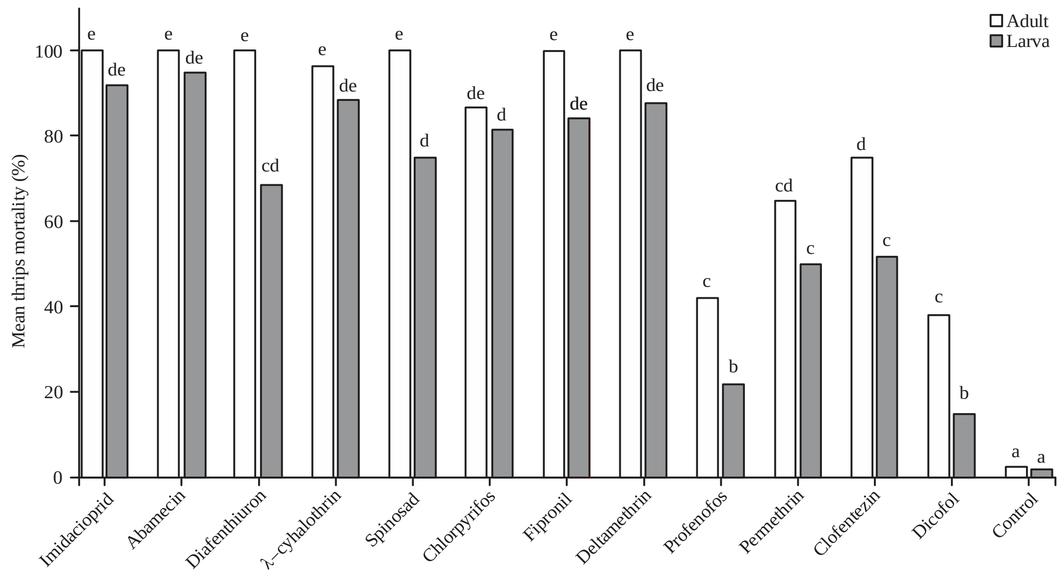

Fig. 1: Mean percent mortality of adults and larvae of *C. polyalthiae* after being exposed to insecticides for 48 h in a leaf-dip bioassay at a recommended rate for each insecticide. Significant differences (Tukey's test, $p<0.05$) are indicated by different letters in trips mortality after 48 h exposure

Results indicated that the toxicity of insecticides tested can be divided into several groups based on their LC_{50} values and fiducial limits (Table 2). For the adults, the most toxic insecticides were imidacloprid, abamectin, diafenthiuron and λ-cyhalothrin with LC_{50} values ranged from 19.8-30.96 ppm, followed by spinosad, chlorpyrifos, fipronil and deltamethrin with LC_{50} values ranged from 39.01-77.25 ppm. The next group of insecticides were profenofos, permethrin and clofentezin with LC_{50} values ranged from 126.93-174.77 ppm and dicofol was the least effective to *C. polyalthiae* with LC_{50} value of 356.97 ppm. However, for the larvae, the most toxic insecticides were diafenthiuron, imidacloprid, spinosad, abamectin, λ-cyhalothrin, fipronil and chlorpyrifos with LC_{50} values ranged from 28.00-64.11 ppm, followed by deltamehtrin, profenofos, permethrin and clofentezin with LC_{50} values ranged from 135.6-195.00 ppm. Dicofol was the least effective to *C. polyalthiae* with LC_{50} value of 415.00 ppm.

Efficacy test: When the insecticides were applied at the recommended dosages, thrips mortality rates varied amongst those insecticides tested, but the mortality rates were significantly higher in the insecticide treatments than the mortality in the untreated control (Fig. 1). Similar to the toxicity assay results, there was a tendency that the adults were more susceptible to the insecticides than the larvae are. Five insecticides, diafenthiuron, spinosad, profenofos, clofentezin and dicofol were significantly more effective against the adults than the larvae. However, mortality rates of the adults and larvae were not significantly different for the other 7 insecticides tested: Imidacloprid, abamectin, λ-cyhalothrin, spinosad, chlorpyrifos, fipronil and deltamethrin. Profenofos and dicofol was the least effective for controlling the thrips adults and larvae (Fig. 1).

DISCUSSION

Crotonothrips polyalthiae is an important pest of the ashoka tree that can cause substantial losses to nurseries. In addition, though the insect does not kill mature trees but it can severely reduce their aesthetic value[9]. Nursery operators rely heavily on insecticide to control the insect. This predisposes the thrips to develop resistant populations in the future[13]. This is the 1st report of baseline sensitivity of *C. polyalthiae* to 12 insecticides, encompassing 8 IRAC's modes of action that are locally available to growers. Since, the test insects were collected from mature trees that had never been sprayed with insecticides, the results of the current study can be used as a reference in monitoring tolerance development in the insect against those insecticides in the future.

The results of both toxicity and efficacy tests showed a general trend that adult thrips were more susceptible to insecticides than the larvae. The results are in line with the previous finding that the adults are the preferred target when using insecticides because they are more sensitive to insecticide[14].

Six of the 12 insecticides tested (Deltamethrin, abamectin, imidacloprid, fipronil, diafenthiuron and spinosad) are recommended for thrips control on other crops, such as chili and tomato. Those insecticides were also toxic and effective in controlling *C. polyalthiae* in this study. In addition, results of this study showed 2 other insecticides, λ-cyhalothrin and chlorpyrifos were also toxic and effective to control the thrips, though they are not currently recommended for thrips control in Indonesia. These two insecticides should be considered for thrips control recommendation. Lambda-cyhalothrin and chlorpyrifos are recommended for thrips control on soybean in Indiana, USA[15]. Before they can be recommended, λ-cyhalothrin and chlorpyrifos must go through field efficacy trials with promising results. The addition of the 2 insecticides to the currently recommended insecticides for thrips control increases the number of insecticides that farmers can choose from, which is good for insecticide resistance management efforts.

Given the expanding distribution, high fecundity, shorter life cycle and the grower's sole reliance on the chemical control[9], it is very probable that *C. polyalthiae* will develop resistance to the insecticides. Past experience indicated that the Western flower thrips, *Frankliniella occidentalis* Pergande was effectively controlled by using abamectin, cyfluthrin and chlorpyrifos in protecting ornamental plants in greenhouses in Michigan, USA[16]. However, the insect has been reported developing resistance to numerous insecticides, including abamectin[17,18], chlorpyrifos, fipronil, spinosad[19] and deltamethrin[20]. The results of this study can be useful in preventing or delaying the development of resistance by following the recomemendation of using the effective insecticides in rotation based on their active ingredients[21]. In order to lessen insecticide selection pressure on the thrips population, the insecticide application should be based on the action threshold[22]. Therefore, further study should be conducted to determine the population level at which an insecticide application is warranted.

The current study results indicated that spinetoram was the most effective in suppressing the adult and larva populations of *C. polyalthiae*. This is in agreement with the fact that this chemical has been recommended in thrips management on many important crops, because it was very effective against the pest[23]. However, heavy use of the chemical triggered resistance in *F. occidentalis* on pepper[24]. Thus, spinetoram must be used cautiously in the management of *C. polyalthiae* in order to preserve its effectiveness. Inclusion of a failed insecticide in an insecticide rotation program could impair the effectiveness of the whole program[25].

CONCLUSION

Results indicated that eight of the insecticides tried were effective against *C. polyalthiae*. Six of them (deltamethrin, abamectin, imidacloprid, fipronil, diafenthiuron and spinosad) are registered for thrips control on other crops, while chlorpyrifos and λ-cyhalothrin are not currently registered for thrips control. These insecticides could be incorporated into an insecticide resistant management strategy to prevent or delay the development of insecticide resistance. The strategy employs alternating or rotating insecticides belonging to different modes of action to ease the insecticide selection pressure on pest population. The same end can also be achieved by using co-formulation mixtures or tank mixes containing chemicals with different modes of action and different modes of resistance. However, before this strategy is applied, it needs to be studied further in field trials. This study, results also presents baseline toxicity of 12 insecticides from different modes of action. The data can be used as future reference for monitoring development of resistance in *C. polyalthiae* against those insecticides.

SIGNIFICANCE STATEMENTS

Nursery growers are heavily dependent solely upon insecticides to control *C. polyalthiae*, an important pest on seedlings of ashoka tree. This practice predisposes the thrips to develop resistance encouraging growers to use more insecticide by increasing the dosages and the frequency of applications. In order to prevent or at least delay the thrips resistance development, effective insecticides should be used in rotation and tank-mixing of several effective insecticides with different modes of action. The results of this study provide information about the tested insecticides (with different modes of action) that are effective in controlling the thrips. Those effective insecticides can be rotated or mixed by the growers in protecting their plants from *C. polyalthiae*. In addition, the results also provide baseline susceptibility of the thrips against several insecticides with different modes of action. This result can be used as reference in monitoring resistance of the insect in the future. Thus, the results of this study have public health policy implication because they can be used to reduce the amount of insecticide for the thrips control.

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