BUKTI KORESPONDENSI PUBLIKASI JOURNAL TRENDS IN SCIENCES

JUDUL :

Predatory Capacity and Feeding Preference of Pesticide-Resistant Amblyseius deleoni Muma et Denmark (Mesostigmata: Phytoseiidae)

TRENDS IN SCIENCES 2021; 18(24): 1441

BAMBANG HERU BUDIANTO
ROKHMANI
EDI BASUKI

8

[Trends in Sciences formerly know as Walailak Journal of Science and Technology] Volume 18 (2021) Proofreading Request ID 1120

Trends in Sciences <journal.wu@gmail.com>
to me, Rokhmani, Edi

Wed, Dec

Dear Professor Bambang Heru Budianto,

Please inspect this proof version of your article and answer the following queries;

- Please inspect this copy-edited version of your article.
- We have highlighted the words and are waiting for you to confirm.

- Please give "Highlights" of the paper 3-5 bullet points (For example https://tis.wu.ac.th/index.php/tis/article/view/11).
- Please give "Graphical Abstract" (if available).
- Please sign the "TiS Consent to Publish and Copyright Transfer" in the attached file.
- Please promptly respond to these queries rapidly.
- If there are any minor changes, please let us know by the list of changes in the word file or the list of corrections in a reply mail.
- Please revise your paper in the attached file.
- Please return it as soon as you can, possibly within 48 hours.

PS. Walailak Journal of Science and Technology (WJST) has been renamed as Trends in Sciences (TiS). The TiS was launched in October 2021. Please visit our website at https://tis.wu.ac.th.

We apologize for any inconvenience this may cause you. Thank you in advance for your cooperation.

Yours sincerely,
Trends in Sciences
https://tis.wu.ac.th
2020 SJR (SCOPUS): 0.146

2 Attachments • Scanned by Gmail

TiS Consent to Pu...

11200-WJST Tem...

erly know ✕ +

google.com/mail/u/0/?tab=rm&ogbl#search/trends+in+sciences/FMfcgzGllVjtbtNxxvTCcfJCQQXblgzk

Search Free: Avira S... | Empire | The hot games | Big Farm | Funny pictures | Hot Game | Delta Search | Faktor Resiko Penya... | Go

erly know ✕ +

google.com/mail/u/0/?tab=rm&ogbl#search/trends+in+sciences/FMfcgzGllVjtbtNxxvTCcfJCQQXblgzk

Search Free: Avira S... | Empire | The hot games | Big Farm | Funny pictures | Hot Game | Delta Search | Faktor Resiko Penya... | Go

trends in sciences

Active

8

bambang.budianto 1 <bambang.budianto@unsoed.ac.id> Dec 17, 2021,

to Trends

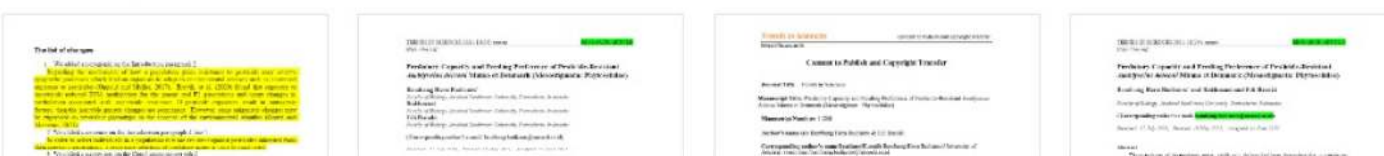
Dear Editorial Board
Trends in Sciences,

We have revised our article entitled Predatory Capacity and Feeding Preference of Pesticide-Resistant *Amblyseius deleoni* Muma et Denmark (Mesostigma
herewith we attached all documents you suggested us to complete.
We look forward to hearing good news from you.
Thank you for your cooperation

Yours sincerely,
Bambang Heru Budi Budianto,
Rokhmani,
Edi Basuki

•••

4 Attachments • Scanned by Gmail



https://doi.org/

Predatory Capacity and Feeding Preference of Pesticide-Resistant *Amblyseius deleoni* Muma et Denmark (Mesostigmata: Phytoseiidae)

Bambang Heru Budianto*
Faculty of Biology, Jenderal Soedirman University, Purwokerto, Indonesia
Rokhmani
Faculty of Biology, Jenderal Soedirman University, Purwokerto, Indonesia
Edi Basuki
Faculty of Biology, Jenderal Soedirman University, Purwokerto, Indonesia

(*Corresponding author's e-mail: bambang.budianto@unsoed.ac.id)

Received: 17 July 2020, Revised: 18 May 2021, Accepted: xx June 2021

Abstract

The population of the predatory mites *Amblyseius deleoni* had been decreasing due to continuous use of synthetic pesticides in tea plantations. Therefore, this study aimed to select pesticide-resistant individuals from a wild-type population of *A. deleoni* and to evaluate whether or not the resistant *A. deleoni* were still sensible as biological control agents. We exposed *A. deleoni* to (propargite), fungicide (copper oxide) and a neem seed extracts. We found that the propargite-resistant predatory mites consumed larvae and nymphs of *Tetranychus urticae* more than the control (wild type) ($P < 0.05$). There was no difference in the number of eggs and adults of *T. urticae* consumed ($P > 0.05$). The number of individuals of every stage of *T. urticae* consumed by copper-oxide resistant *A. deleoni* was the same ($P > 0.05$). In general, there were no changes in food preference in the resistant predators to the 3 of pesticides ($P > 0.05$). There were slight differences on the pattern of predatory capacity among the resistant predators to the three pesticides. Although the predators are resistant to the 3 pesticides, it took longer to consume their prey in comparison to the control. These findings suggested that pesticide-resistant *A. deleoni* were able to maintain their functions as a biocontrol agent.

Keywords: Predatory capacity, Feeding preference, *Amblyseius deleoni*, Pesticide-resistant

HIGHLIGHTS

- The effectiveness of *Amblyseius deleoni* as a natural predators have decreased due to continuous uses of synthetic pesticides to control pests and diseases on tea plantations.
- Continuous exposure to propargite, CuO and neem seed extracts induced the predatory mites *A. deleoni* to develop resistance.
- No significant changes in feeding preference and the predatory capacity of the mite *A. deleoni* on *T. urticae* after becoming resistant to pesticides.
- Exposures *A. deleoni* to pesticides may have induced heritable epigenetic changes permanently or just reversible phenotypes without changing the DNA sequence.

The list of changes

1. We added a paragraph on the Introduction paragraph 2.

Regarding the mechanism of how a population gains resistance to pesticide may involve epigenetic processes which lead an organism to adapt to environmental stresses such as continued exposure to pesticides (Oppold and Muller, 2017). Brevik, at al. (2020) found that exposure to insecticide reduced DNA methylation for the parent and F2 generations and many changes in methylation associated with insecticide resistance. If pesticide exposures result in mutagenic factors, then the heritable genetic changes are permanent. However, some epigenetic changes may be expressed as reversible phenotype in the absence of the environmental stimulus (Gowri and Monteiro, 2021).

2. We added a sentence on the Introduction paragraph 4 line 1.

In order to select individuals in a population that are resistant against pesticides inherited from their previous generations, a resistance selection of predatory mites is usually conducted.

3. We added a suggestion on the Conclusions paragraph 2.

Further studies need to be conducted especially to investigate whether the exposures *A. deleoni* to aforementioned pesticides induced epigenetic changes permanently or just reversible phenotypes.

Walailak Journal of Science and Technology

Responses the referee's comments

Manuscript ID: 11200

Title: Predation Capacity and Feeding Preference of Pesticide-Resistant *Amblyseius deleoni* Muma et Denmark (Mesostigmata : Phytoseiidae)

Reviewer D

No.	Referee's Comments	Responses (for author)
1.	Survival rate or population?	Yes we agree with you and we have already revised the article text.
2.	"I think it is not necessary to make the italic word of non-scientific name"	We have already revised those.
3.	In the subsection of Resistance selection (section of Materials and methods), please, check the number. "Exposures to copper oxide fungicide of 0, 10, 100, 1.000 and 10.000 ppm were performed on the parent samples, F1 to F4, while the concentrations of samples F5 to F12 were increased to 200.000 ppm."	The numbers are correct.
4.	Please present the fact	Yes we agree with you and we have already added the facts.
5.	Result and discussion	We have already added the discussion following our results.
6.	Note of the table 7, 8, 9, 10, 11, 12	We have already added the explanation of the notes.
7.	Please use the standard of references list.	We have adjusted the reference list to the standard of the journal.
8.	Guidelines	We have adjusted the the format of the draft in every detail to comply with the guidelines

Reviewer I

No.	Referee's Comments	Responses (for author)
1.	The English of this manuscript should be proofread by a professional editor.	The English has already been proofread.
2.		

WALAILAK JOURNAL
http://wjst.wu.ac.th

Research Article

Predation Capacity and Feeding Preference of Pesticide-Resistant *Amblyseius deleoni* Muma et Denmark (Mesostigmata: Phytoseiidae)

Running title

Predation Capacity and Feeding Preference

Abstract

The population of the predatory mites *Amblyseius deleoni* had been decreasing due to continuous use of synthetic pesticides in tea plantations. Therefore, this study aimed to select pesticide-resistant individuals from a wild-type population of *A. deleoni* and to evaluate whether or not the resistant *A. deleoni* were still sensible as biological control agents. We exposed *A. deleoni* to (propargite), fungicide (copper oxide) and a neem seed extracts. We found that the propargite-resistant predatory mites consumed larvae and nymphs of *Tetranychus urticae* more than the control (wild type) (P< 0,05). There was no difference in the number of eggs and adults of *T. urticae* consumed (P> 0,05) The number of individuals of every stage of *T. urticae* consumed by copper-oxide resistant *A. deleoni* was the same (P>0,05). In general, there were no changes in food preference in the resistant predators to the three of pesticides (P>0,05). There were slight differences on the pattern of predation capacity among the resistant predators to the three pesticides. Although the predators are resistant to the three pesticides, however, it took longer to consume their prey in comparison to the control. These findings suggested that pesticide-resistant *A. deleoni* were able to maintain their functions as a biocontrol agent.

Commented [1]: Survival rate or population?

Commented [2]: Please presented the fact

Keywords: Predation Capacity, Feeding Preference, *Amblyseius deleoni*, pesticide-resistant

Introduction

Naturally, the population of one of the most important pests in tea plantations in tropical areas, during dry and rainy seasons, the Scarlet mites *Brevipalpus phoenicis* is controlled by the predatory mites *Amblyseius deleoni* Muma and Denmark. However, the effectiveness of these natural predators seemed to have decreased due to continuous uses of synthetic pesticides to control pests and diseases on tea plantations (1). The predation behavior and capacity of *A. deleoni* could also change after becoming resistant to various pesticides used in tea plantations in Indonesia (2).

The resistance selection of predatory mites is principally an effort to select individuals in a population that are resistant against pesticides inherited from their previous generations. The character that phenotypically appears in a population will enhance their survival rate in a pesticide-polluted environment. Therefore, a repeating exposure to pesticides will favor the resistant or tolerant individuals than the susceptible ones. This selection process results in an increase in the frequency of pesticide-resistant genes, which in turn, will become dominant in a population (3). The speed of the increase of the frequency of resistant individuals in a population depends on how good the organisms adapt to a pesticide and the mode of action of the pesticides (4).

There have been successes in selecting a permethrin-resistant genus of *Amblyseius*. (5) managed to select resistant generations of *A. fallacis*, which include 55 generations (6). Furthermore, (7) and (8) found that pesticides resistance is inheritable to the descendants through selections. (9) observed that in *A. womersleyi,* the levels of resistance against permethrin decreased 20 months after the last exposure.

However, these pesticide-resistant predatory mites still need to be tested in terms of their predation capacity and behavior to make sure that their power is still sensible as biological control agents. The capacity of a predator is influenced by several factors, namely: the energy to kill the prey, the previous experience with a certain prey (10) (11), the density of its prey (12) (6) (6)and the preference (13) (14).

Several studies found different preferences of predatory *A. deleoni* to its prey. For example, (15) and (16) found that predatory *A. deleoni* prefers to prey *Brevipalpus phoenicis* (the scarlet mites) than other types of mites, while (17) found that *A. deleoni* prefers to prey *Eriophyidae* (gall mites) than other mites including *B. phoenicis*. The difference in preference indicates that *A. deleoni* may have the ability to select the most suitable prey in certain or different conditions. The feeding behavior of predatory mite *A. deleoni* may therefore change as a result of selection after being exposed to various pesticides used in tea plantations.

Considering that the modes of actions of *acaricides* vary depending on the contents, these may influence the behavior and capacity of predatory mites. For example, *propargite*, an *acaricide* that belongs to a phenoxy group, can cause damage in the respiratory system. The mode of action of this *acaricide* is "residual killing action" that inhibits cholinesterase in acetylcholine hydrolysis into choline and acetate (18). Contact Copper Oxide (C_uO) fungicide can impair the permeability of the cuticle and change the action site of its enzyme that reduces the affinity of Cu^{2+} (5). On the other hand, neem extract (*Azadirachta indica*) has diverse effects, because not only that it is consisted of *azadirachtin* as the major component, but its extract also contains salannin, nimbin, 6-desacetylnimbin, salannidolide, salanninolactone and intermediates ((18). The neem extracts especially azadirachtin acts as a strong antifeedant and "insect growth regulator" and can modify the behavior of target organisms ((19) (20).

Based on the above-mentioned mode of actions of pesticides, the toxicity of pesticide on predatory mites *A. deleoni* may differ from one pesticide to another. Therefore, the mites may respond differently, and may show different levels of tolerance or resistance, or may also change their feeding behavior. Accordingly, there is a possibility that the selection pressure from generation to generation will change the feeding preference and therefore the predation levels of *A. deleoni* needs to be further studied.

Currently, there is neither information regarding the resistance against pesticides nor the predation capacity and behavior of *A. deleoni* after becoming resistant to various pesticides. Therefore, this study aimed to select pesticide-resistant individuals from a wild-type population and to evaluate whether or not the selected *A. deleoni* population had changed their predation capacity and behavior after being exposed to acaricide (propargite), fungicide (Copper Oxide) and a neem seed extract. These efforts will improve the survival rate of the important biological control agent *Amblyseius deleoni* in pesticide-polluted plantations.

Materials and methods

Parental-mite Population

The parental of *A. deleoni* mites used in this study were taken from a wild type population from Gambung Village, West Java, Indonesia at the coordinate of 107º29′32″ – 107º31′11″ East Longitude and 07º07′18″ – 07º09′11″ South Latitude, with the altitude of 1.350 m above sea level. This area is an isolated tea plantation where no pesticides were used. These mites were tested for its resistance level by selecting the most vulnerable population indicated by the narrowest range of fiducial limit (FL). The selected mites were designated as the parent population with the resistance ratio equals to one.

Resistance Selection

The resistance selections were performed in the Entomology and Parasitology Laboratory of Faculty of Biology, Universitas Jenderal Soedirman, Indonesia. The wild-type predatory mites, *A. deleoni* were exposed to a gradient of concentrations of pesticides to obtain LC_{50} as the basis of selection. The concentrations of 0 ppm, 285 ppm, 570 ppm, 855 and 1140 ppm of *propargite acaricide* were applied to the wild-type samples, Filial 1 = F1/Generation 1 until F5/Generation 5 of predatory mites *A. deleoni*. Exposures to copper oxide fungicide of 0, 10, 100, 1.000 and 10.000 ppm were performed on the parent samples, F1 to F4, while the concentrations of samples F5 to F12 were increased to 200.000 ppm. The resistance selection of *A. deleoni* against neem seed extracts, with concentrations of 0%, 25%, 50%, 75% and 100%, were applied on the Parent to F8. Ten *A. deleoni* mites were exposed to each concentration of pesticides with 6 (six) replicates.

Preference and Predation-capacity Test

The resistant generations of predatory mites *A. deleoni*, resulted from the experiment were then tested for possible changes in their feeding behavior. The tests included free-choice feeding preference on several alternative prey. The resistant predatory mites were fed on tea pollens, red bean pollens, and *T. urticae* and *Tyrophagus* sp. for preference tests, with 6 replicates. The predation capacity tests of the resistant *A. deleoni* included feeding treatments on the eggs, larvae, nymphs and adults of *T . urticae*. Every treatment was repeated 6 (six) times.

Data Analysis

The data of resistance levels to pesticides were analyzed using a computer application Probit and Logit (POLO). This application was employed to analyze the lethal concentrations 50 (LC_{50}) with 95% Fiducial Limit (FL), the resistance ratio (RR), the slope and the standard errors (SE) and χ^2 at 0.05 error level. The resistant generations of predatory mites *A. deleoni*, resulted from the experiment were indicated by a non-overlapping range of a Fiducial Limit.

The data of feeding preferences and the predation capacity of the resistant generations of predatory mites *A. deleoni* were conducted using a Completely Randomized Design and analyzed statistically using Analysis of Variance, with error level of 0.05 and 0.01 and followed by Least Significant Difference tests with the same error level.

Results and discussion

Commented [3]: In result and discussion you should express your result, explain why the result and discussing with the relevant references

The results showed that LC_{50} of the wild-type *A. deleoni* against a gradient of concentrations of propargite of 483.46 ppm were ranging from 419.61 to 547.52 ppm (Table 1).

Table 1. Resistance tests of parent *Amblyseius deleoni* against propargite on the wild-type population

Generation	N	Range of concentrations (ppm)	**LC_{50}** (95% FL) (ppm)	Slope ± SE (ppm)
Wild type	300	0.00 – 1140	483.46 (419.61-547.52)	5.12±0.53

Table 1 also shows that the resistance levels of the parent population against propargite were more or less the same. The steep mortality data indicated that this range of concentrations was still tolerable by only a small portion of individual mites in the wild-type population. Therefore, the most vulnerable population was then designated as the parent population that presumably they had never been exposed to any pesticide. Thus, the results of the selection on the wild-type population was then used in the subsequent experiments as a standard parent (resistance ratio =1). Souza-Pimentel *et al.* (2017) found a similar result that the predatory mite *Neoseiulus californicus* was highly tolerant to the acaricide-insecticide than the recommended concentration for the use in citrus. This may be due to the fact both *Amblyseius deleoni and Neoseiulus californicus* are both from the same Family that they have character similarity.

The subsequent resistance selections of *A. deleoni* against propargite acaricide found that the resistance character was first noticeable in the fourth generation (F4), and then increased to reach the ratio of 7.36 times in the sixteenth generation (F16), compared to that of the parent population. The increasing resistance of *A. deleoni* against propargite from Parent (P) to the 16th generation (Table 2).

Table 2. The increasing resistance of *Amblyseius deleoni* against propargite from Parent generation (P) to 16th generation (F16).

Generation	**LC50** (95% FL) (ppm)	Resistance Ratio	Slope±SE (ppm)	χ^2(df=22)
P	483.46 (419.61-547.52)[a]	1	5.12±0.53	35.21
F1	517.57 (452.86-583.31)[a]	1.07	5.39±0.55	35.43
F2	566.96 (513.91-620.86)[a]	1.17	5.007±0.52	20.24
F3	596.14 (532.94-662.14)[a]	1.23	4.025±0.46	20.71
F4	799.17 (677.04-986.02)[b]	1.65	2.328±0.41	10.37
F5	1265.94 (1000.30-2006.56)[c]	2.62	2.121±0.44	5.83
F6	1704.94 (1517.08-2129.70)[c]	3.53	4.015±0.83	3.78
F7	2035.12 (1821.59-2211.90)[c]	4.21	4.689±1.27	6.41
F8	2546.38 (2648.48-2462.90)[d]	5.27	12.075±1.67	3.97
F9	2851.47 (2753.30-2981.23)[e]	5.89	11.223±1.78	5.17
F10	3086.59 (2999.20-3190.77)[f]	6.38	13.749±1.99	9.04
F11	3296.36 (3220.90-3376.48)[g]	6.82	17.706±2.26	10.14
F12	3340.01 (3258.10-3408.52)[g]	6.91	21.214±2.73	8.64
F13	3449.32 (3377.90-3516.20)[g]	7.13	21.586±2.63	11.34
F14	3507.13 (3428.30-3568.12)[g]	7.25	29.400±4.06	9.63
F15	3554.78 (3483.50-3613.61)[g]	7.35	29.257±3.78	11.48
F16	3557.53 (3478.70-3621.14)[g]	7.36	26.506±3.44	14.23

Note:

- The number (N) of individuals each generation was 300.
- FL (Fiducial Limit) is the upper and lower limits of the range of pesticide concentrations with confidence limit of 95%. The values of 95% FL followed by the same small letters, indicated that there is no significant difference, based on statistical tests using Probit and Logit (P>0.05).

These findings (Table 2) showed that the resistance character was inherited from their parent population. This result is in line with the study by (3) who studied compatibility of pesticides with the generalist predatory mite *Amblyseius largoensis*. Propargite is a selective acaricide because exposures to predatory mites *Phytoseiulus persimilis* resulted in lower mortality, thus, this pesticide is recommended to control the population of pest mites *T. urticae*, which is the prey of *P. persimilis* (11) (20).

In contrast to the results of exposure to propargite, the exposure of wild-type *A. deleoni* to 56% copper oxide (C_uO) in this study resulted in that the range of the fiducial limit of LC_{50} was narrower than that of the subsequent experiments (Table 3).

Table 3. Wild-type *Amblyseius deleoni* resistance tests against 56% Copper Oxide (**C_uO**)

Generation	N	Range of concentrations (ppm)	**LC50** (95% FL) (ppm)	Slope ± SE (ppm)
Wild type	300	0.00-10.00	60.63 (36.77-395.21)	1.033±0.121

Table 3 shows that the results of analysis of the exposures of the wild-type population against copper oxide range of Fiducial Limit (FL) was overlapping, with more or less similar slopes. Pimentel-Souza *et al.* (2017), mengemukakan bahwa pendedahan senyawa aktif fungisida mandipropamid, mefenoxam dan difenoconazol kurang toksik terhadap tungau predator *Phytoseiulus macropilis* (Banks) dan *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae). Perbedaan hasil penelitian ini diduga kuat lebih disebabkan oleh jenis senyawa aktif fungisida dibandingkan spesiesnya. Ke dua tungau predator tergolong ke dalam satu familia (Phytoseiidae) dengan tungau predator yang dipakai dalam penelitian ini. Based on the narrow range and overlapping values, the sample population from the test was used for subsequent selection tests of *A. deleoni* against copper oxide, and was then designated as a parent population. The results of the selection of *A. deleoni* against 56% copper oxide (C_uO) up to generation 12 are presented in Table 4.

Table 4. The increasing resistance of *Amblyseius deleoni* against 56% copper oxide (**C_uO**) from Parent generation (P) to 12th generation (F12).

Generation	**LC_{50}** (95% Fiducial Limit) (ppm)	Resistance Ratio	Slope±SE (ppm)	χ^2(df=22)
P	60.63 (36.770-395.21)[a]	1	1.03±0.12	12.0394
F1	77.61 (47.76-121.54) [a]	1.28	1.03±0.12	17.8839
F2	125.97 (63.08-231.38)[a]	2.08	0.67±0.09	5.7035
F3	245.90 (137.40-431.75)[a]	4.06	0.74±0.09	6.4921
F4	907.03 (456.09-2066.97)[b]	14.96	0.58±0.08	7.3463
F5	3328.80 (2074.00-5583.12)[c]	54.91	1.11±0.13	28.756
F6	8043.28 (5869.26-11368.00)[d]	132.67	1.26±0.19	4.7088
F7	13929.21 (11840.20-16846.50)[e]	229.75	2.22±0.26	20.1566
F8	22712.15 (19612.4-26111.5)[f]	374.62	3.48±0.37	20.7412
F9	34762.00 (29952.70-40305.25)[g]	573.37	3.09±0.39	9.1361
F10	46261.10 (41166.80-51308.92)[h]	763.03	4.27±0.49	4.0731
F11	77223.90 (72009.50-82615.67)[i]	1273.73	6.81±0.78	5.8537
F12	84676.59 (77740.60-90690.30)[i]	1396.66	7.32±1.00	6.7008

Note:
- The number of individuals (N) in each generation was 300.
- FL (Fiducial Limit) is the upper and lower limits of the range of pesticide concentrations with confidence limit of 95%. The values of 95% FL followed by the same small letters, indicated that there is no significant difference, based on statistical tests using Probit and Logit (P>0.05)

The results of statistical analysis using Probit and Logit showed that the LC_{50} between the parent generations, F1, F2 and F3 were not significantly different (P>0.05, overlapping ranges of FL LC_{50}). Based on the comparison of $\chi 2$ values against $\chi 2$ in the statistical table, it is indicated that the populations of Parent to F3 were dominated by individuals that were susceptible to the pesticide, and proportions of the resistant individuals were very low (slope=1.033 in parent population). However, the exposure to this pesticide in the subsequent experiments resulted in the increasing proportion of resistant individuals, indicated by a significant difference among FL LC_{50} values of *A. deleoni* (P<0.05). Accordingly, the level of resistance (resistance ratio) increased by 14.96 times in generation F4 compared to that of the parent. The increasing proportion of the resistant individuals occurred until 10th generation (F10). Afterwards, the proportion of resistant individuals from F11-F12 only slightly increased.

The increasing ratio of resistance levels to reach 1396.66 times in 12th generation higher than that of the parent indicated that the predatory mite *A. deleoni* was highly tolerant to copper oxide fungicide. Thus, this type of fungicide is not harmful to the survival of these predatory mites. Hasil penelitian ini sama dengan hasil yang diperoleh oleh Pimentel-Souza (2017), meskipun senyawa aktif fungisida dan jenis tungau predator yang dipakai tidak sama. (21) found that penetration of copper oxide through the cuticle could cause the chemoreceptors organ to form a permeability barrier in the cuticle to be more selective. Furthermore, the cuticle of predatory mites contains many sterols that reduce its permeability to water and fungicides. However, continuous fungicide selection pressure on the predatory mites from generation to generation can cause the chemoreceptor organs to become thicker and more sensitive (10) (5).

The results of resistance tests on the wild-type population of *A. deleoni* against neem seed extract (Table 5).

Table 5. The results of resistance tests on the wild-type population of *A. deleoni* against neem seed extract

Generation	N	Range of concentration (ppm)	**LC_{50}** (95%FL) (ppm)	Slope ± SE (ppm)
Wild type	300	0.00-100	25.33 (21.68-28.34)	5.833±0.933

Based on the narrow and overlapping range of FL LC_{50} values, the wild-type population was then designated as the parent population, and was used in the subsequent selection tests of *A. deleoni* against neem seed extract (Table 6).

Table 6. Increasing resistance level of *Amblyseius deleoni* against neem extract from generation to generation

Generation	**LC_{50}** (95% FL) (ppm)	Resistance Ratio	Slope±SE (ppm)	χ^2(df=22)
P	24.67 (21.19-27.46)^a	1.00	6.163±1.063	6.7694
F1	27.62 (24.44-30.52) ^{a}	1.12	6.168±0.868	10.0704
F2	30.25 (26.46-33.69)^a	1.23	5.094±0.622	8.4714
F3	32.83 (29.16-36.29)^a	1.33	5.321±0.607	9.3795
F4	36.93 (33.46-40.35)^a	1.49	6.107±0.644	10.420
F5	45.86 (41.31-50.37)^b	1.86	5.787±0.576	25.986
F6	48.73 (44.23-53.21)^b	1.98	5.114±0.523	19.9165
F7	49.77 (44.49-55.08)^b	2.02	4.699±0.494	24.941
F8	49.85 (44.87-54.88)^b	2.02	4.849±0.502	23.296

Note:

- The number of individuals (N) in each generation was 300.
- FL (Fiducial Limit) is the upper and lower limits of the range of pesticide concentrations with confidence limit of 95%. The values of 95% FL followed by the same small letters, indicated that there is no significant difference, based on statistical tests using Probit and Logit (P>0.05)

Exposure to the neem seed extracts noticeably increased the proportion of the resistant individuals in F1, F2, F3 and F4 generations, to be more tolerant in the fifth (F5) generation, indicated by the non-overlapping range of FL LC_{50} and the decreasing values of the slopes (Table 6). The increase in tolerant levels of *A. deleoni* towards the neem seed extracts occurred gradually and finally reached 2.02 times in generations F7 and F8 compared to the tolerant level of the parent population. Accordingly, the proportion homogeneity of the tolerant individuals increased, indicated by the decrease in the slope value in the generation F8.

The compounds in the neem extract used in this research seemed to contribute similar selection pressures, but caused different impacts, depending on the target individuals and the concentration. This is in accordance with (8) and (5) that the neem seed extract contains various compounds including azadiradione, azadirone, nimbin and salannin, but only azadirachtin acts as a strong antifeedant. In addition, according to (4), different enzymes may catalyze various oxidative reactions of many compounds in the neem extracts. Supposedly, the resistance against neem extracts may continue and develop due to the genetic variations in a population and continuous selection pressures (20).

In this study, we have successfully developed resistant populations of predatory mite *A. deleoni* against propargite acaricide, 56% copper oxide (C_uO) and neem seed extracts. However, we need to test whether or not these pesticide exposures also changed the feeding behavior of the resistant populations of *A. deleoni*. For these purposes, several tests were conducted to study their feeding behaviors.

Regarding the feeding preference, the results showed that the propargite-resistant population of *A. deleoni* preferred T. urticae than other alternative prey. This preference was not significantly different from that of the control population (wild type), which had not been exposed to propargite (Table 7). A similar phenomenon was also found in resistant populations to 56% copper oxide (C_uO) (Tables 8) and to neem-seed extracts (Tables 9).

Table 7. The preference of propargite-resistant predatory mites *Amblyseius deleoni* on several alternative preys (data were transformed with √x+0.5)

Types of food	Number of individuals consumed ± SD/24 hours	
	A. deleoni (Control)	*A. deleoni* (propargite resistant)
Tetranychus urticae	2.78 ± 0.35 a A	3.28 ± 0.52 a A
Tea Pollen	2.84 ± 0.38 a A	2.73 ± 0.49 a A
Red bean Pollen	1.51 ± 0.25 b A	1.56 ± 0.69 b A
Tyrophagus sp.	1.86 ± 0.48 b A	1.12 ± 0.55 b A

Note: The numbers in the same column, followed by different letters indicate a significant difference with P = 0.05, while numbers in the same row followed by the same capital letters, indicates that there is no significant difference P = 0,05.

Table 8. Feeding preference of predatory mites *Amblyseius deleoni* resistant to 56% copper oxide (**C_uO**) on alternative foods, (the data was transformed with √x+0.5)

Types of food	Average number of individuals consumed ± SD/24 hours	
	A. deleoni (Control)	56% C_uO resistant *A. deleoni*
Tetranychus urticae	2.78 ± 0.35 a A	2.66 ± 0.52 a A
Tea Pollen	2.84 ± 0.38 a A	3.22 ± 0.37 b A
Red bean pollen	1.51 ± 0.25 b A	1.26 ± 0.32 c A
Tyrophagus sp.	1.86 ± 0.48 b A	1.58 ± 0.46 c A

Note: The numbers in the same column followed by different letters indicate a significant difference with P = 0.05. while numbers in the same row followed by the same capital letters, indicates that there is no significant difference P = 0,05.

Table 9. Feeding preference of predatory mites *Amblyseius deleoni* resistant to neem extract on alternative foods, (data was transformed with √x+0.5)

Types of food	Average number of individuals consumed ± SD/24 hours	
	A. deleoni (Control)	Neem extract resistant *A. deleoni*
Tetranychus urticae	2.78 ± 0.35 a A	2.88 ± 0.68 a A
Tea pollen	2.84 ± 0.38 a A	2.53 ± 0.54 ab A
Red bean pollen	1.51 ± 0.25 b A	2.09 ± 0.59 bc A
Tyrophagus sp.	1.86 ± 0.48 b A	1.40 ± 0.19 c A

Note: The numbers in the same column followed by different letters indicate a significant difference with P = 0.05, while numbers in the same row followed by the same capital letters, indicates that there is no significant difference P = 0,05.

The prey preference (Table 9) of the resistant population of *A. deleoni* on *T. urticae* might indicate that the selection pressure of propargite favored the individuals with more sensitive chemoreceptor organs. This finding is in accordance to (18) who found similar results on *Phytoseius sp.*. Correspondingly, since propargite has the residual killing action, thus the individuals with more sensitive chemoreceptor organs also possessed less permeable integuments so they were able to minimize the penetration of toxicants to reduce the toxic effects. The selective permeability barrier of the integument relates to the balance between hydrophilic character of the integument and the toxicants that are mostly lipophilic, so the toxicants will stay in or around the membrane of the chemoreceptor organs (3). (10) assumed that the reduction of the penetration of toxic substances through the integument involves enzymatic oxidative biotransformation. The increased sensitivity of chemoreceptor organs of propargite-resistant predatory mites may have improved the responsiveness of the mites to the existence and the movement of *T. urticae* than other alternative preys such as *Tyrophagus* sp. tea and red bean pollen (20) (22).

Regarding the predation capacity, we also found that *A. deleoni* did not change after being exposed to the selection pressures of propargite, 56% copper oxide (CuO) and neem extracts (Table 10, 11 and 12).

Table 10. Predation capacity of propargite-resistant *Amblyseius deleoni* on alternative foods (the data were transformed with √x+0.5)

Food (*T. urticae*)	Average number of individuals consumed ± SD/24 hours	
	A. deleoni (control)	*A. deleoni* (propargite resistant)
Egg	2.09 ± 0.21 a A	1.90 ± 0.11 a A
Larva	2.15 ± 0.18 a A	2.41 ± 0.07 b B
Nymph	1.18 ± 0.23 b A	1.51 ± 0.18 c B
Adult	1.02 ± 0.14 b A	0.94 ± 0.13 d A

Note: The numbers in the same column, followed by different letters indicate a significant difference with P = 0.05, while numbers in the same row followed by the same capital letters, indicates that there is no significant difference P = 0,05.

Table 11. Predation capacity of predatory mites *Amblyseius deleoni* resistant to 56% copper oxide (**CuO**) on alternative foods (the data were transformed with √x+0.5)

Food (various stages of *T. urticae*)	Average number of individuals consumed ± SD/24 hours	
	A. deleoni (control)	*A. deleoni* (**CuO** 56% resistant)
Egg	2.09 ± 0.21 a A	1.29 ± 0.31 a A
Larva	2.15 ± 0.18 a A	2.18 ± 0.26 a A
Nymph	1.18 ± 0.23 b A	1.29 ± 0.31 b A
Adult	1.02 ± 0.14 b A	1.02 ± 0.14 b A

Note: The numbers in the same column followed by different letters indicate a significant difference with P = 0.05, while numbers in the same row followed by the same capital letters, indicates that there is no significant difference P = 0,05.

Table 12. Predation capacity of predatory mites *Amblyseius deleoni* resistant to neem extracts on alternative foods (the data were transformed with √x+0.5).

Food (various stages of *T. urticae*)	Average number of individuals consumed ± SD/24 hours	
	A. deleoni (control)	*A. deleoni* (neem extract resistant)
Egg	2.09 ± 0.21 a A	2.25 ± 0.17 a A
Larva	2.15 ± 0.18 a A	2.06 ± 0.21 b A
Nymph	1.18 ± 0.23 b A	1.51 ± 0.18 c B
Adult	1.02 ± 0.14 b A	1.05 ± 0.20 d A

Note: The numbers in the same column followed by different letters indicate a significant difference with P = 0.05, while numbers in the same row followed by the same capital letters, indicates that there is no significant difference P = 0,05.

Tables 10, 11 and 12 show that although the selection pressures of propargite, 56% copper oxide (CuO) and neem seed extracts caused changes in the predation capacity, however, the positive-response stimulating power was not strong enough to significantly change the feeding behavior of predation level of predatory mites *A. deleoni*. These responses were more towards merely adaptive reactions under a selection pressure of the pesticides used in this study. Thus, the selection pressure of propargite, 56% copper oxide (C_uO) and neem seed extracts did not significantly modify the feeding preference of *A. deleoni* on *T. urticae.* Thus, these resistances appeared to be a phenomenon of adaptive reaction (8). Fenomena reaksi adaptif juga dikemukakan oleh Silva *et al.* (2013) yang mengemukakan bahwa berdasarkan pendekatan analisis toksisitas letal dan subletal, diketahui bahwa senyawa aktif neem bersifat selektif terhadap tungau predator *Proprioseiopsis neotropicus* (Acari: Phytoseiidae).

Conclusions

The selection pressures resulted in the predatory mites *A. deleoni* to develop resistance against propargite, 56% copper oxide (C_uO), and neem seed extracts, but did not affect the feeding preference and the predation capacity of the predatory mite *A. deleoni* on T. urticae

Acknowledgements

The authors would like to thank the Ministry of Research, Technology and Higher Education of Indonesia for the National Strategic Research Grant.

References

Commented [4]: Pelase using standart of refetence list

1. Budianto BH, Basuki E. Kemampuan predasi tungau predator. *JHPT* 2013;13(1):35–41.
2. Acari P, Minsik JP, Lee KJ. Sublethal effects of fenpyroximate and pyridaben on two predatory mite species , Neoseiulus womersleyi.xxx 2011;243–59.
3. Rocha R, Silva DA, Teodoro AV, Jesus MDE, Silva DES, Reis PR, et al. Compatibility of pesticides with the generalist predatory mite *Amblyseius largoensis* (Acari : Phytoseiidae). 2015;41(1):76–80.
4. Gahukar RT. Plant-derived products in crop protection : effects of various application methods on pests and diseases. Phytoparasitica [Internet]. 2016;379–91. Available from: http://dx.doi.org/10.1007/s12600-016-0524-3
5. Fountain MT, Medd N. Integrating pesticides and predatory mites in soft fruit crops. 2015;(December 2014):657–67.
6. Zhang X, Lv J, Hu Y, Wang B, Chen X, Xu X. Prey Preference and Life Table of Amblyseius orientalis on Bemisia tabaci and Tetranychus cinnabarinus. xxx2015;1–17.
7. Childers CC, Denmark HA. Phytoseiidae (Acari : Mesostigmata) within citrus orchards in Florida : species distribution , relative and seasonal abundance within trees , associated vines. 2011;331–71.
8. Afshar FR, Latifi M. Functional response and predation rate of. 2017;6(4):299–314.
9. Ghoosta Y, Pourmirza AA. Toxicity of Diazinon and Acetamipridon on Life Table Parameters of the Predatory Mite , Neoseiulus californicus (Acari : Phytoseiidae) when Fed on the European Red Mite Panonychus ulmi (Koch). 2016;26(1):15–9.
10. Calvo FJ, Knapp M, Houten YM Van, Belda E. Amblyseius swirskii : What made this predatory mite such a successful biocontrol agent ? 2015;419–33.
11. Hassan MF. Efficacy of Two Phytoseiid Predators and a Biopesticide Against Tetranychus cucurbitacearum (Sayed) (Acari : Tetranychidae) on Eggplant at Ismailia Governorate , Egypt. 2015;25(1):71–4.
12. Janssen A. Juvenile prey induce antipredator behaviour in adult predators. 2013;275–82.
13. Goleva I, Zebitz CPW. Suitability of different pollen as alternative food for the predatory mite

Amblyseius swirskii. 2013;259–83.

14. Suidasidae A. Life table parameters and capture success ratio studies of Typhlodromips swirskii (Acari : Phytoseiidae) to the factitious prey Suidasia medanensis. 2013;69–78.
15. Puchalska EK, Kozak M. Phytoseiidae) as potential biocontrol agents against spider mites (Acari : Tetranychidae) inhabiting willows : laboratory studies on predator development. Exp Appl Acarol. 2016;68(1):39–53.
16. Parsa S, Hazzi NA, Chen Q, Lu F, Vanessa B, Campo H, et al. Potential geographic distribution of two invasive cassava green mites. 2015;195–204.
17. Mutisya DL, Khamala CPM, Kariuki CW. Management of cassava green mite Mononychellus progresivus (Acari : Tetranychidae) in different agro-ecological zones of Kenya Management of cassava green mite Mononychellus progresivus (Acari : Tetranychidae) in different agro-ecological zones of Ken. 2015;(January).
18. Fiedler Ż, Sosnowska D. Side effects of fungicides and insecticides on predatory mites , in laboratory conditions. 2014;54(4).
19. Hewitt LC, Shipp L, Buitenhuis R. Seasonal climatic variations influence the efficacy of predatory mites used for control of western flower thrips in greenhouse ornamental crops. 2015;435–50.
20. Mohamed FSA, Hussein E, Darwish ZEA, Amer SAA, Salama AB, El-desouky ME. Influence of some Extracts from Three Lamiaceae Plants on Toxicity , Repellency and some Biological Aspects of Tetranychus urticae Koch (Acari : Tetranychidae). 2015;25(1):255–61.
21. Buitenhuis R, Murphy G, Shipp L, Scott-dupree C. Amblyseius swirskii in greenhouse production systems : a floricultural perspective. 2015;451–64.
22. Michalska K. The effect of predation risk on spermatophore deposition rate of the eriophyoid mite , Aculops allotrichus. Exp Appl Acarol. 2016;68(2):145–54.

WALAILAK JOURNAL
http://wjst.wu.ac.th https://doi.org/

Research Article

Predation Capacity and Feeding Preference of Pesticide-Resistant *Amblyseius deleoni* Muma et Denmark (Mesostigmata: Phytoseiidae)

Bambang Heru Budianto[1], Rokhmani and Edi Basuki[2]

[1,2]Faculty of Biology, Jenderal Soedirman University, Purwokerto, Indonesia

(*Corresponding author's e-mail: bambang.budianto@unsoed.ac.id)

Received:, Revised:, May 17th 2021 Accepted:

Running title

Predation Capacity and Feeding Preference

Abstract

The population of the predatory mites *Amblyseius deleoni* had been decreasing due to continuous use of synthetic pesticides in tea plantations. Therefore, this study aimed to select pesticide-resistant individuals from a wild-type population of *A. deleoni* and to evaluate whether or not the resistant *A. deleoni* were still sensible as biological control agents. We exposed *A. deleoni* to (propargite), fungicide (copper oxide) and a neem seed extracts. We found that the propargite-resistant predatory mites consumed larvae and nymphs of *Tetranychus urticae* more than the control (wild type) ($P< 0,05$). There was no difference in the number of eggs and adults of *T. urticae* consumed ($P> 0,05$) The number of individuals of every stage of *T. urticae* consumed by copper-oxide resistant *A. deleoni* was the same ($P>0,05$). In general, there were no changes in food preference in the resistant predators to the three of pesticides ($P>0,05$). There were slight differences on the pattern of predation capacity among the resistant predators to the three pesticides. Although the predators are resistant to the three pesticides, however, it took longer to consume their prey in comparison to the control. These findings suggested that pesticide-resistant *A. deleoni* were able to maintain their functions as a biocontrol agent.

Keywords : Predation Capacity, Feeding Preference, *Amblyseius deleoni*, pesticide-resistant

Commented [1]: Survival rate or population?

Commented [2]: Please presented the fact

Introduction

Naturally, the population of one of the most important pests in tea plantations in tropical areas, during dry and rainy seasons, the Scarlet mites *Brevipalpus phoenicis* is controlled by the predatory mites *Amblyseius deleoni* Muma and Denmark. However, the effectiveness of these natural predators seemed to have decreased due to continuous uses of synthetic pesticides to control pests and diseases on tea plantations [1]. The predation behavior and capacity of *A. deleoni* could also change after becoming resistant to various pesticides used in tea plantations in Indonesia [2].

The resistance selection of predatory mites is principally an effort to select individuals in a population that are resistant against pesticides inherited from their previous generations. The character that phenotypically appears in a population will enhance their survival rate in a pesticide-polluted environment. Therefore, a repeating exposure to pesticides will favor the resistant or tolerant individuals than the susceptible ones. This selection process results in an increase in the frequency of pesticide-resistant genes, which in turn, will become dominant in a population [3]. The speed of the increase of the frequency of resistant individuals in a population depends on how good the organisms adapt to a pesticide and the mode of action of the pesticides [4].

There have been successes in selecting a permethrin-resistant genus of *Amblyseius*. [5] managed to select resistant generations of *A. fallacis*, which include 55 generations [6]. Furthermore, [7] and [8] found that pesticides resistance is inheritable to the descendants through selections. [9] observed that in *A. womersleyi,* the levels of resistance against permethrin decreased 20 months after the last exposure.

However, these pesticide-resistant predatory mites still need to be tested in terms of their predation capacity and behavior to make sure that their power is still sensible as biological control agents. The capacity of a predator is influenced by several factors, namely: the energy to kill the prey, the previous experience with a certain prey [10-11], the density of its prey [12] [6] and the preference [13-14].

Several studies found different preferences of predatory *A. deleoni* to its prey. For example, [15] and [16] found that predatory *A. deleoni* prefers to prey *Brevipalpus phoenicis* (the scarlet mites) than other types of mites, while [17] found that *A. deleoni* prefers to prey *Eriophyidae* (gall mites) than other mites including *B. phoenicis*. The difference in preference indicates that *A. deleoni* may have the ability to select the most suitable prey in certain or different conditions. The feeding behavior of predatory mite *A. deleoni* may therefore change as a result of selection after being exposed to various pesticides used in tea plantations.

Considering that the modes of actions of acaricides vary depending on the contents, these may influence the behavior and capacity of predatory mites. For example, *propargite*, an acaricide that belongs to a phenoxy group, can cause damage in the respiratory system. The mode of action of this acaricide is “residual killing action” that inhibits cholinesterase in acetylcholine hydrolysis into choline and acetate [18]. Contact Copper Oxide (C_uO) fungicide can impair the permeability of the cuticle and change the action site of its enzyme that reduces the affinity of Cu^{2+} [5]. On the other hand, neem extract (*Azadirachta indica*) has diverse effects, because not only that it is consisted of azadirachtin as the major component, but its extract also contains salannin, nimbin, 6-desacetylnimbin, salannidolide, salanninolactone and intermediates [18]. The neem extracts especially azadirachtin acts as a strong antifeedant and “insect growth regulator” and can modify the behavior of target organisms [19-20].

Based on the above-mentioned mode of actions of pesticides, the toxicity of pesticide on predatory mites *A. deleoni* may differ from one pesticide to another. Therefore, the mites may respond differently, and may show different levels of tolerance or resistance, or may also change their feeding behavior. Accordingly, there is a possibility that the selection pressure from generation to generation will change the feeding preference and therefore the predation levels of *A. deleoni* needs to be further studied.

Currently, there is neither information regarding the resistance against pesticides nor the predation capacity and behavior of *A. deleoni* after becoming resistant to various pesticides. Therefore, this study aimed to select pesticide-resistant individuals from a wild-type population and to evaluate whether or not the selected *A. deleoni* population had changed their predation capacity and behavior after being exposed to acaricide (propargite), fungicide (Copper Oxide) and a neem seed extract. These efforts will improve the survival rate of the important biological control agent *Amblyseius deleoni* in pesticide-polluted plantations.

Materials and methods

Parental-mite Population

The parental of *A. deleoni* mites used in this study were taken from a wild type population from Gambung Village, West Java, Indonesia at the coordinate of 107º29′32″ – 107º31′11″ East Longitude and

07º07′18″ – 07º09′11″ South Latitude, with the altitude of 1.350 m above sea level. This area is an isolated tea plantation where no pesticides were used. These mites were tested for its resistance level by selecting the most vulnerable population indicated by the narrowest range of fiducial limit (FL). The selected mites were designated as the parent population with the resistance ratio equals to one.

Resistance Selection

The resistance selections were performed in the Entomology and Parasitology Laboratory of Faculty of Biology, Universitas Jenderal Soedirman, Indonesia. The wild-type predatory mites, *A. deleoni* were exposed to a gradient of concentrations of pesticides to obtain LC_{50} as the basis of selection. The concentrations of 0 ppm, 285 ppm, 570 ppm, 855 and 1140 ppm of propargite acaricide were applied to the wild-type samples, Filial 1 = F1/Generation 1 until F5/Generation 5 of predatory mites *A. deleoni*. Exposures to copper oxide fungicide of 0, 10, 100, 1.000 and 10.000 ppm were performed on the parent samples, F1 to F4, while the concentrations of samples F5 to F12 were increased to 200.000 ppm. The resistance selection of *A. deleoni* against neem seed extracts, with concentrations of 0%, 25%, 50%, 75% and 100%, were applied on the Parent to F8. Ten *A. deleoni* mites were exposed to each concentration of pesticides with 6 (six) replicates.

Preference and Predation-capacity Test

The resistant generations of predatory mites *A. deleoni*, resulted from the experiment were then tested for possible changes in their feeding behavior. The tests included free-choice feeding preference on several alternative prey. The resistant predatory mites were fed on tea pollens, red bean pollens, and *T. urticae* and *Tyrophagus* sp. for preference tests, with 6 replicates. The predation capacity tests of the resistant *A. deleoni* included feeding treatments on the eggs, larvae, nymphs and adults of *T . urticae*. Every treatment was repeated 6 (six) times.

Data Analysis

The data of resistance levels to pesticides were analyzed using a computer application Probit and Logit (POLO). This application was employed to analyze the lethal concentrations 50 (LC_{50}) with 95% Fiducial Limit (FL), the resistance ratio (RR), the slope and the standard errors (SE) and χ^2 at 0.05 error level. The resistant generations of predatory mites *A. deleoni*, resulted from the experiment were indicated by a non-overlapping range of a Fiducial Limit.

The data of feeding preferences and the predation capacity of the resistant generations of predatory mites *A. deleoni* were conducted using a Completely Randomized Design and analyzed statistically using Analysis of Variance, with error level of 0.05 and 0.01 and followed by Least Significant Difference tests with the same error level.

Results and discussion

Commented [3]: In result and discussion you should express your result, explain why the result and discussing with the relevant references

The results showed that LC_{50} of the wild-type *A. deleoni* against a gradient of concentrations of propargite of 483.46 ppm were ranging from 419.61 to 547.52 ppm (Table 1).

Table 1. Resistance tests of parent *Amblyseius deleoni* against propargite on the wild-type population

Generation	N	Range of concentrations (ppm)	**LC_{50}** (95% FL) (ppm)	Slope ± SE (ppm)
Wild type	300	0.00 – 1140	483.46 (419.61-547.52)	5.12±0.53

Table 1 also shows that the resistance levels of the parent population against propargite were more or less the same. The steep mortality data indicated that this range of concentrations was still tolerable by only a small portion of individual mites in the wild-type population. Therefore, the most vulnerable population was then designated as the parent population that presumably they had never been exposed to any pesticide. Thus, the results of the selection on the wild-type population was then used in the subsequent experiments as a standard parent (resistance ratio =1). [21] found a similar result that the predatory mite *Neoseiulus californicus* was highly tolerant to the acaricide-insecticide than the recommended concentration for the use in citrus. This may be due to the fact both *Amblyseius deleoni and Neoseiulus californicus* are both from the same Family, so that they have similar character.

The subsequent resistance selections of *A. deleoni* against propargite acaricide found that the resistance character was first noticeable in the fourth generation (F4), and then increased to reach the ratio of 7.36 times in the sixteenth generation (F16), compared to that of the parent population. The increasing resistance of *A. deleoni* against propargite from Parent (P) to the 16th generation (Table 2).

Table 2. The increasing resistance of *Amblyseius deleoni* against propargite from Parent generation (P) to 16th generation (F16).

Generation	$\mathbf{LC_{50}}$ (95% FL) (ppm)	Resistance Ratio	Slope±SE (ppm)	χ^2(df=22)
P	483.46 (419.61-547.52)[a]	1	5.12±0.53	35.21
F1	517.57 (452.86-583.31)[a]	1.07	5.39±0.55	35.43
F2	566.96 (513.91-620.86)[a]	1.17	5.007±0.52	20.24
F3	596.14 (532.94-662.14)[a]	1.23	4.025±0.46	20.71
F4	799.17 (677.04-986.02)[b]	1.65	2.328±0.41	10.37
F5	1265.94 (1000.30-2006.56)[c]	2.62	2.121±0.44	5.83
F6	1704.94 (1517.08-2129.70)[c]	3.53	4.015±0.83	3.78
F7	2035.12 (1821.59-2211.90)[c]	4.21	4.689±1.27	6.41
F8	2546.38 (2648.48-2462.90)[d]	5.27	12.075±1.67	3.97
F9	2851.47 (2753.30-2981.23)[e]	5.89	11.223±1.78	5.17
F10	3086.59 (2999.20-3190.77)[f]	6.38	13.749±1.99	9.04
F11	3296.36 (3220.90-3376.48)[g]	6.82	17.706±2.26	10.14
F12	3340.01 (3258.10-3408.52)[g]	6.91	21.214±2.73	8.64
F13	3449.32 (3377.90-3516.20)[g]	7.13	21.586±2.63	11.34
F14	3507.13 (3428.30-3568.12)[g]	7.25	29.400±4.06	9.63
F15	3554.78 (3483.50-3613.61)[g]	7.35	29.257±3.78	11.48
F16	3557.53 (3478.70-3621.14)[g]	7.36	26.506±3.44	14.23

Note:

- The number (N) of individuals each generation was 300.
- FL (Fiducial Limit) is the upper and lower limits of the range of pesticide concentrations with confidence limit of 95%. The values of 95% FL followed by the same small letters, indicated that there is no significant difference, based on statistical tests using Probit and Logit (P>0.05).

These findings (Table 2) showed that the resistance character was inherited from their parent population. This result is in line with the study by [3] who studied compatibility of pesticides with the generalist predatory mite *Amblyseius largoensis*. Propargite is a selective acaricide because exposures to predatory mites *Phytoseiulus persimilis* resulted in lower mortality, thus, this pesticide is recommended to control the population of pest mites *T. urticae*, which is the prey of *P. persimilis* [11] [20].

In contrast to the results of exposure to propargite, the exposure of wild-type *A. deleoni* to 56% copper oxide (C_uO) in this study resulted in that the range of the fiducial limit of LC_{50} was narrower than that of the subsequent experiments (Table 3).

Table 3. Wild-type *Amblyseius deleoni* resistance tests against 56% Copper Oxide (**C_uO**)

Generation	N	Range of concentrations (ppm)	**LC_{50}** (95% FL) (ppm)	Slope ± SE (ppm)
Wild type	300	0.00-10.00	60.63 (36.77-395.21)	1.033±0.121

Table 3 shows that the results of analysis of the exposures of the wild-type population against copper oxide range of Fiducial Limit (FL) was overlapping, with more or less similar slopes. [21] suggested that exposure to the active fungicides mandipropamid, mefenoxam and difenoconazole was less toxic to the predatory mites *Phytoseiulus macropilis* (Banks) and *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae). The difference in the results of this study is most likely to be caused by the type of active fungicide compound rather than the species differences, since both predatory mites belong to the same family (Phytoseiidae) as the one used in this study. Based on the narrow range and overlapping values, the sample population from the test was used for subsequent selection tests of *A. deleoni* against copper oxide, and was then designated as a parent population. The results of the selection of *A. deleoni* against 56% copper oxide (C_uO) up to generation 12 are presented in Table 4.

Table 4. The increasing resistance of *Amblyseius deleoni* against 56% copper oxide (**C_uO**) from Parent generation (P) to 12th generation (F12).

Generation	**LC_{50}** (95% Fiducial Limit) (ppm)	Resistance Ratio	Slope±SE (ppm)	χ^2(df=22)
P	60.63 (36.770-395.21)[a]	1	1.03±0.12	12.0394

Generation	**LC_{50}** (95% Fiducial Limit) (ppm)	Resistance Ratio	Slope±SE (ppm)	χ^2(df=22)
F1	77.61 (47.76-121.54) [a]	1.28	1.03±0.12	17.8839
F2	125.97 (63.08-231.38)[a]	2.08	0.67±0.09	5.7035
F3	245.90 (137.40-431.75)[a]	4.06	0.74±0.09	6.4921
F4	907.03 (456.09-2066.97)[b]	14.96	0.58±0.08	7.3463
F5	3328.80 (2074.00-5583.12)[c]	54.91	1.11±0.13	28.756
F6	8043.28 (5869.26-11368.00)[d]	132.67	1.26±0.19	4.7088
F7	13929.21 (11840.20-16846.50)[e]	229.75	2.22±0.26	20.1566
F8	22712.15 (19612.4-26111.5)[f]	374.62	3.48±0.37	20.7412
F9	34762.00 (29952.70-40305.25)[g]	573.37	3.09±0.39	9.1361
F10	46261.10 (41166.80-51308.92)[h]	763.03	4.27±0.49	4.0731
F11	77223.90 (72009.50-82615.67)[i]	1273.73	6.81±0.78	5.8537
F12	84676.59 (77740.60-90690.30)[i]	1396.66	7.32±1.00	6.7008

Note:

- The number of individuals (N) in each generation was 300.
- FL (Fiducial Limit) is the upper and lower limits of the range of pesticide concentrations with confidence limit of 95%. The values of 95% FL followed by the same small letters, indicated that there is no significant difference, based on statistical tests using Probit and Logit (P>0.05)

The results of statistical analysis using Probit and Logit showed that the LC_{50} between the parent generations, F1, F2 and F3 were not significantly different (P>0.05, overlapping ranges of FL LC_{50}). Based on the comparison of χ2 values against χ2 in the statistical table, it is indicated that the populations of Parent to F3 were dominated by individuals that were susceptible to the pesticide, and proportions of the resistant individuals were very low (slope=1.033 in parent population). However, the exposure to this pesticide in the subsequent experiments resulted in the increasing proportion of resistant individuals, indicated by a significant difference among FL LC_{50} values of *A. deleoni* (P<0.05). Accordingly, the level of resistance (resistance ratio) increased by 14.96 times in generation F4 compared to that of the parent. The increasing proportion of the resistant individuals occurred until 10th generation (F10). Afterwards, the proportion of resistant individuals from F11-F12 only slightly increased.

The increasing ratio of resistance levels to reach 1396.66 times in 12th generation higher than that of the parent indicated that the predatory mite *A. deleoni* was highly tolerant to copper oxide fungicide. Thus, this type of fungicide is not harmful to the survival of these predatory mites. Similar results were also reported by [21], although the active fungicide compounds and the predatory mites species used were different. In addition, [21] found that penetration of copper oxide through the cuticle could cause the chemoreceptors organ to form a permeability barrier in the cuticle to be more selective. Furthermore, the cuticle of predatory mites contains many sterols that reduce its permeability to water and fungicides. However, continuous fungicide selection pressure on the predatory mites from generation to generation can cause the chemoreceptor organs to become thicker and more sensitive [5] [10].

The results of resistance tests on the wild-type population of *A. deleoni* against neem seed extract (Table 5).

Table 5. The results of resistance tests on the wild-type population of *A. deleoni* against neem seed extract

Generation	N	Range of concentration (ppm)	**LC_{50}** (95%FL) (ppm)	Slope ± SE (ppm)
Wild type	300	0.00-100	25.33 (21.68-28.34)	5.833±0.933

Based on the narrow and overlapping range of FL LC_{50} values, the wild-type population was then designated as the parent population, and was used in the subsequent selection tests of *A. deleoni* against neem seed extract (Table 6).

Table 6. Increasing resistance level of *Amblyseius deleoni* against neem extract from generation to generation

Generation	**LC50** (95% FL) (ppm)	Resistance Ratio	Slope±SE (ppm)	χ^2(df=22)
P	24.67 (21.19-27.46)[a]	1.00	6.163±1.063	6.7694
F1	27.62 (24.44-30.52) [a]	1.12	6.168±0.868	10.0704
F2	30.25 (26.46-33.69)[a]	1.23	5.094±0.622	8.4714
F3	32.83 (29.16-36.29)[a]	1.33	5.321±0.607	9.3795
F4	36.93 (33.46-40.35)[a]	1.49	6.107±0.644	10.420
F5	45.86 (41.31-50.37)[b]	1.86	5.787±0.576	25.986
F6	48.73 (44.23-53.21)[b]	1.98	5.114±0.523	19.9165
F7	49.77 (44.49-55.08)[b]	2.02	4.699±0.494	24.941
F8	49.85 (44.87-54.88)[b]	2.02	4.849±0.502	23.296

Note:

- The number of individuals (N) in each generation was 300.
- FL (Fiducial Limit) is the upper and lower limits of the range of pesticide concentrations with confidence limit of 95%. The values of 95% FL followed by the same small letters, indicated that there is no significant difference, based on statistical tests using Probit and Logit ($P>0.05$)

Exposure to the neem seed extracts noticeably increased the proportion of the resistant individuals in F1, F2, F3 and F4 generations, to be more tolerant in the fifth (F5) generation, indicated by the non-overlapping range of FL LC_{50} and the decreasing values of the slopes (Table 6). The increase in tolerant levels of *A. deleoni* towards the neem seed extracts occurred gradually and finally reached 2.02 times in generations F7 and F8 compared to the tolerant level of the parent population. Accordingly, the proportion homogeneity of the tolerant individuals increased, indicated by the decrease in the slope value in the generation F8.

The compounds in the neem extract used in this research seemed to contribute similar selection pressures, but caused different impacts, depending on the target individuals and the concentration. This is in accordance with [8]and [5] that the neem seed extract contains various compounds including azadiradione, azadirone, nimbin and salannin, but only azadirachtin acts as a strong antifeedant. In addition, according to [4], different enzymes may catalyze various oxidative reactions of many compounds in the neem extracts. Supposedly, the resistance against neem extracts may continue and develop due to the genetic variations in a population and continuous selection pressures [20].

In this study, we have successfully developed resistant populations of predatory mite *A. deleoni* against propargite acaricide, 56% copper oxide (C_uO) and neem seed extracts. However, we need to test whether or not these pesticide exposures also changed the feeding behavior of the resistant populations of *A. deleoni*. For these purposes, several tests were conducted to study their feeding behaviors.

Regarding the feeding preference, the results showed that the propargite-resistant population of *A. deleoni* preferred *T. urticae* than other alternative prey. This preference was not significantly different from that of the control population (wild type), which had not been exposed to propargite (Table 7). A similar phenomenon was also found in resistant populations to 56% copper oxide (C_uO) (Tables 8) and to neem-seed extracts (Tables 9).

Table 7. The preference of propargite-resistant predatory mites *Amblyseius deleoni* on several alternative preys (data were transformed with √x+0.5)

Types of food	Number of individuals consumed ± SD/24 hours	
	A. deleoni (Control)	*A. deleoni* (propargite resistant)
Tetranychus urticae	2.78 ± 0.35 a A	3.28 ± 0.52 a A
Tea Pollen	2.84 ± 0.38 a A	2.73 ± 0.49 a A
Red bean Pollen	1.51 ± 0.25 b A	1.56 ± 0.69 b A
Tyrophagus sp.	1.86 ± 0.48 b A	1.12 ± 0.55 b A

Note: The numbers in the same column, followed by different letters indicate a significant difference with P = 0.05, while numbers in the same row followed by the same capital letters, indicates that there is no significant difference P = 0,05.

Table 8. Feeding preference of predatory mites *Amblyseius deleoni* resistant to 56% copper oxide (**C_uO**) on alternative foods, (the data was transformed with √x+0.5)

Types of food	Average number of individuals consumed ± SD/24 hours	
	A. deleoni (Control)	56% C_uO resistant *A. deleoni*
Tetranychus urticae	2.78 ± 0.35 a A	2.66 ± 0.52 a A
Tea Pollen	2.84 ± 0.38 a A	3.22 ± 0.37 b A
Red bean pollen	1.51 ± 0.25 b A	1.26 ± 0.32 c A
Tyrophagus sp.	1.86 ± 0.48 b A	1.58 ± 0.46 c A

Note: The numbers in the same column followed by different letters indicate a significant difference with P = 0.05. while numbers in the same row followed by the same capital letters, indicates that there is no significant difference P = 0,05.

Table 9. Feeding preference of predatory mites *Amblyseius deleoni* resistant to neem extract on alternative foods, (data was transformed with √x+0.5)

Types of food	Average number of individuals consumed ± SD/24 hours	
	A. deleoni (Control)	Neem extract resistant *A. deleoni*
Tetranychus urticae	2.78 ± 0.35 a A	2.88 ± 0.68 a A
Tea pollen	2.84 ± 0.38 a A	2.53 ± 0.54 ab A
Red bean pollen	1.51 ± 0.25 b A	2.09 ± 0.59 bc A
Tyrophagus sp.	1.86 ± 0.48 b A	1.40 ± 0.19 c A

Note: The numbers in the same column followed by different letters indicate a significant difference with P = 0.05, while numbers in the same row followed by the same capital letters, indicates that there is no significant difference P = 0,05.

The prey preference (Table 9) of the resistant population of *A. deleoni* on *T. urticae* might indicate that the selection pressure of propargite favored the individuals with more sensitive chemoreceptor organs. This finding is in accordance to [18] who found similar results on *Phytoseius sp.*. Correspondingly, since propargite has the residual killing action, thus the individuals with more sensitive chemoreceptor organs also possessed less permeable integuments so they were able to minimize the penetration of toxicants to reduce the toxic effects. The selective permeability barrier of the integument relates to the balance between hydrophilic character of the integument and the toxicants that are mostly lipophilic, so the toxicants will stay in or around the membrane of the chemoreceptor organs [3]. [10] assumed that the reduction of the penetration of toxic substances through the integument involves enzymatic oxidative biotransformation. The increased sensitivity of chemoreceptor organs of propargite-resistant predatory mites may have improved the responsiveness of the mites to the existence and the movement of *T. urticae* than other alternative preys such as *Tyrophagus* sp. tea and red bean pollen [20] [22].

Regarding the predation capacity, we also found that *A. deleoni* did not change after being exposed to the selection pressures of propargite, 56% copper oxide (C_uO) and neem extracts (Table 10, 11 and 12).

Table 10. Predation capacity of propargite-resistant *Amblyseius deleoni* on alternative foods (the data were transformed with √x+0.5)

Food (*T. urticae*)	Average number of individuals consumed ± SD/24 hours	
	A. deleoni (control)	*A. deleoni* (propargite resistant)
Egg	2.09 ± 0.21 a A	1.90 ± 0.11 a A
Larva	2.15 ± 0.18 a A	2.41 ± 0.07 b B
Nymph	1.18 ± 0.23 b A	1.51 ± 0.18 c B
Adult	1.02 ± 0.14 b A	0.94 ± 0.13 d A

Note: The numbers in the same column, followed by different letters indicate a significant difference with P = 0.05, while numbers in the same row followed by the same capital letters, indicates that there is no significant difference P = 0,05.

Table 11. Predation capacity of predatory mites *Amblyseius deleoni* resistant to 56% copper oxide (**C_uO**) on alternative foods (the data were transformed with √x+0.5)

Food (various stages of *T. urticae*)	Average number of individuals consumed ± SD/24 hours	
	A. deleoni (control)	*A. deleoni* (**C_uO** 56% resistant)
Egg	2.09 ± 0.21 a A	1.29 ± 0.31 a A
Larva	2.15 ± 0.18 a A	2.18 ± 0.26 a A
Nymph	1.18 ± 0.23 b A	1.29 ± 0.31 b A
Adult	1.02 ± 0.14 b A	1.02 ± 0.14 b A

Note: The numbers in the same column followed by different letters indicate a significant difference with P = 0.05, while numbers in the same row followed by the same capital letters, indicates that there is no significant difference P = 0,05.

Table 12. Predation capacity of predatory mites *Amblyseius deleoni* resistant to neem extracts on alternative foods (the data were transformed with √x+0.5).

Food (various stages of *T. urticae*)	Average number of individuals consumed ± SD/24 hours	
	A. deleoni (control)	*A. deleoni* (neem extract resistant)
Egg	2.09 ± 0.21 a A	2.25 ± 0.17 a A
Larva	2.15 ± 0.18 a A	2.06 ± 0.21 b A
Nymph	1.18 ± 0.23 b A	1.51 ± 0.18 c B
Adult	1.02 ± 0.14 b A	1.05 ± 0.20 d A

Note: The numbers in the same column followed by different letters indicate a significant difference with P = 0.05, while numbers in the same row followed by the same capital letters, indicates that there is no significant difference P = 0,05.

Tables 10, 11 and 12 show that although the selection pressures of propargite, 56% copper oxide (C_uO) and neem seed extracts caused changes in the predation capacity, however, the positive-response stimulating power was not strong enough to significantly change the feeding behavior of predation level of predatory mites *A. deleoni*. These responses were more towards merely adaptive reactions under a selection pressure of the pesticides used in this study. Thus, the selection pressure of propargite, 56% copper oxide (C_uO) and neem seed extracts did not significantly modify the feeding preference of *A. deleoni* on *T. urticae.* Thus, these resistances appeared to be a phenomenon of adaptive reaction [8] as suggested by [23-24] who argued that based on the lethal and sublethal toxicity analysis approach, it was known that the active compound of neem was selective against the predatory mite *Proprioseiopsis neotropicus* (Acari: Phytoseiidae).

Conclusions

The selection pressures resulted in the predatory mites *A. deleoni* to develop resistance against propargite, 56% copper oxide (C_uO), and neem seed extracts, but did not affect the feeding preference and the predation capacity of the predatory mite *A. deleoni* on T. urticae

Acknowledgements

The authors would like to thank the Ministry of Research, Technology and Higher Education of Indonesia for the National Strategic Research Grant.

References

Commented [4]: Pelase using standart of refetence list

[1] BH Budianto and E Basuki. The Predation ability of temperature resistant *Amblyseius* sp. to *Tetranychus urticae. J Trop Plant Pest Dis*. 2013; **13 (01),** 35–41

[2] J Park, K Minsik, J Lee, K Shin, SE Lee, J Kim, K Cho. Sublethal effects of fenpyroximate and pyridaben on two predatory mite species, *Neoseiulus womersleyi* and *Phytoseiulus persimilis* (Acari, Phytoseiidae). *Exp Appl Acarol.* 2011; 54,243–259. http : //doi.org/10.1007/s10493-011-9435-7

[3] R Rocha, D Silva, AV Teodoro, MD Jesus, DS Silva, PR Reis and SS Silva. Compatibility of pesticides with the generalist predatory mite *Amblyseius largoensis* (Acari: Phytoseiidae). *Rev Colomb Entomol.* 2015; 41, 76-80

[4] R. T. Gahukar. Plant-derived products in crop protection: effects of various application methods on pests and diseases. *Phytoparasitica.* 2016; 44, 379–391. http://doi.org/10.1007/s12600-016-0524-3

[5] MT Fountain and N Medd. Integrating pesticides and predatory mites in soft fruit crops. *Phytoparasitica*. 2015; 43, 657–667. http://doi.org/ 10.1007/s12600-015-0485-y

[6] X Zhang, J Lv, Y Hu, B Wang, X Chen, X Xu and E Wang. Prey Preference and Life Table of *Amblyseius orientalis* on *Bemisia tabaci* and *Tetranychus cinnabarinus*. *PLOS one*. 2015; 10, e0138820. http://doi.org/10.1371/journal.pone.0138820

[7] CC Childers and HA Denmark. Phytoseiidae (Acari: Mesostigmata) within citrus orchards in Florida: species distribution, relative and seasonal abundance within trees, associated vines and ground cover plants. *Exp Appl Acarol*. 2011; 54,331–371 http://doi.org/ 10.1007/s10493-011-9449-1

[8] FR Afshar and M Latifi. Functional response and predation rate of *Amblyseius swirskii* (Acari: Phytoseiidae) at three constant temperatures. *Persian J. Acarol.* 2017; 6, 299–314. http://dx.doi.org/10.22073/pja.v6i4.32392

[9] M. Maroufpoor, Y Ghoosta and AA Pourmirza. Toxicity of Diazinon and Acetamipridon on Life Table Parameters of the Predatory Mite, *Neoseiulus californicus* (Acari: Phytoseiidae) when Fed on the European Red Mite *Panonychus ulmi* (Koch). *Egypt J Biol Pest Co.* 2016; 26, 15-19

[10] FJ Calvo, M. Knapp, YM van Houten, H Hoogerbrugge and JE Belda. *Amblyseius swirskii*: What made this predatory mite such a successful biocontrol agent?. *Exp Appl Acarol*. 2015; 65:419–433. http://doi.org/10.1007/s10493-014-9873-0

[11] EMA El-Saiedy, MF Hassan, AF El-Bahrawy, GA El-Kady and MS Kamel. Efficacy of Two Phytoseiid Predators and a Biopesticide Against *Tetranychus cucurbitacearum* (Sayed) (Acari: Tetranychidae) on Eggplant at Ismailia Governorate, Egypt. *Egypt J Biol Pest Co.* 2015, 25, 71-74

[12] A Janssen and MW Sabelis. Alternative food and biological control by generalist predatory mites: the case of *Amblyseius swirskii*. *Exp Appl Acarol*. 2015; 65,413–418. http://doi.org/10.1007/s10493-015-9901-8 [13]

[13] I Goleva and CPW Zebitz. Suitability of different pollen as alternative food for the predatory mite *Amblyseius swirskii* (Acari, Phytoseiidae). *Exp Appl Acarol* .2013; 61,259–283. http://doi.org/10.1007/s10493-013-9700-z

[14] A Midthassel, SR Leather and IH Baxter. Life table parameters and capture success ratio studies of *Typhlodromips swirskii* (Acari: Phytoseiidae) to the factitious prey *Suidasia medanensis* (Acari: Suidasidae). *Exp Appl Acarol.* 2013; 61,69–78. http://doi.org/10.1007/s10493-013-9682-x

[15] EK Puchalska and M Kozak. *Typhlodromus pyri* and *Euseius finlandicus* (Acari: Phytoseiidae) as potential biocontrol agents against spider mites (Acari: Tetranychidae) inhabiting willows: laboratory studies on predator development and reproduction on four diets. *Exp Appl Acarol*. 2016; 68, 39–53. http://doi.org/10.1007/s10493-015-9973-5

[16] S Parsa, NA Hazzi, Q Chen, F Lu, BVH Campo, JS Yaninek and AA Vasquez-Ordonez. Potential geographic distribution of two invasive cassava green mites. *Exp Appl Acarol*. 2015; 2-15. http://doi.org/10.1007/s10493-014-9868-x

[17] Dl Mutisya, EM El-banhawy, CPM Khamala and CW Kariuki. Management of cassava green mite Mononychellus progresivus (Acari: Tetranychidae) in different agro-ecological zones of Kenya. *Syst Appl Acarol-Uk*. 2015; 20, 39–50. http://dx.doi.org/10.11158/saa.20.1.5

[18] Ż Fiedler and D Sosnowska. Side effects of fungicides and insecticides on predatory mites, in laboratory conditions. *J Plant Prot Res*. 2014; 54. http://doi.org/10.2478/jppr-2014-0052

[19] LC Hewitt, L Shipp, R Buitenhuis and C Scott-Dupree. Seasonal climatic variations influence the efficacy of predatory mites used for control of western flower thrips in greenhouse ornamental crops. *Exp Appl Acarol*. 2015; 65,435–450. http://doi.org/10.1007/s10493-014-9861-4

[20] FSA Mohamed, HE Hussein, ZEA Darwish, SAA Amer, AB Salama and ME El-Desouky. Influence of some Extracts from Three Lamiaceae Plants on Toxicity, Repellency and some Biological Aspects of *Tetranychus urticae* Koch (Acari: Tetranychidae). *Egypt J Biol Pest Co.* 2015; 25, 255-160

[21] Souza-pimentel G C, Reis P R, Marafeli P D P and Alves J P 2017 Physiological Selectivity of Agrochemicals to Predatory Mites of Tetranychus urticae (Acari : Tetranychidae) on Rosebushes Growing in Greenhouse *Int. J. Environ. Agric. Res.* **3** 14–22

[22] GC Souza-Pimentel, PR Reis, PDP Marafeli and JP Alves. Physiological Selectivity of Agrochemicals to Predatory Mites of *Tetranychus urticae* (Acari: Tetranychidae) on Rosebushes Growing in Greenhouse. *Int J Environ Agric Res*. 2017; 3

[23] K Michalska. The effect of predation risk on spermatophore deposition rate of the eriophyoid mite, *Aculops allotrichus*. *Exp Appl Acarol.* 2016; 68,145–154 http://doi.org/10.1007/s10493-015-9998-9

[24] ACB Silva, AV Teodoro, EE Oliveira and AGS Maciel. Lethal and sublethal effects of neem oil to the predatory mite *Proprioseiopsis neotropicus* (Acari: Phytoseiidae). *Rev Colomb Entomol.* 2013; 39, 221-225