



RESEARCH ARTICLE

Use of aqueous plant extracts to reduce profenofos residues in the leaf of mustard (*Brassica juncea* L.) and suppression of the grasshopper population

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ABSTRACT

Profenofos is an active ingredient from the organophosphate group which residue is often found in various fresh and processed vegetable products. This study aimed to assess the use of aqueous plant extracts of *Sapindus rarak* seeds, *Luffa acutangula* peel and *Centella asiatica* leaves to reduce profenofos residues in leaf of mustard and to evaluate their performance in suppressing the grasshopper populations. *S. rarak* seeds, *L. acutangula* peels and *C. asiatica* leaves were dried and filtered using a 100 mesh sieve. A total of 30 gm samples of each ingredient were mixed with 1000 ml of water and blended at 800 rpm, then filtered using 100 mesh sieve before use. The field experiment was performed by spraying profenofos pesticide with a concentration of 3 ml l⁻¹ at a dose of 30 ml per plant. Two days after the profenofos application, the plants were sprayed with the aqueous plant extracts. Twenty-four hrs aqueous plant extracts application, pesticide residues were detected by the UPLC-MS/MS machine. The phytotoxicity test results showed that the use of aqueous plant extracts at a dose of 30 ml per plant did not cause any phytotoxic symptoms. Furthermore, in the field experiment, the control plants showed a residual value of 2407.62 ng g⁻¹. Results of UPLC-MS/MS showed that the residual value of profenofos in PL treatment (aqueous extract of *S. rarak* seeds) was 1502.05 ng g⁻¹, the recorded residual value in the PP treatment (aqueous extract of *C. asiatica* leaves) was 1316.27 ng g⁻¹ and the residual value in the PG treatment (aqueous extract of *L. acutangula* peels) was 660.71 ng g⁻¹. In the treated plants, the residual value decreased from 37.48% to 72.55%. Furthermore, the number of grasshoppers after the PL treatment decreased and was significantly different from the control. This study provides new information that aqueous plant extracts can reduce the residue of profenofos and suppress the population of grasshoppers in the mustard leaf.

Introduction

The application of synthetic chemical pesticides in Indonesia has now reached an alarming level. The use of chemical pesticides is a means of controlling Plant Pest Organisms (PPO), which farmers in Indonesia most widely use (95.29%) because it is considered effective, easy to use and economically profitable (1). The use of pesticides on agricultural land and plantations occurs from the beginning to the end of the cropping cycle, starting from soil processing, land preparation, plant maintenance, harvesting and even post-harvest (2, 3). Although pesticides benefit in

controlling pests and plant diseases, it needs to be considered its disadvantage: pesticides are bioactive chemicals and are toxic (4). Every poisonous ingredient possesses dangers and harmful in its use, both to the environment and humans (5).

The research results on 315 samples of agricultural products reported that pesticide residues were found in 47% of fresh product samples and 7% of processed food samples (6, 7). Meanwhile, it was reported that pesticide residues were found in 65% of fresh product samples and 10% of processed vegetable samples (8). Those researches indicate that there are still many pesticide residues left in the plants' given

pesticides. The residue is a substance or residual compound from pesticides that is left on the human, animal, plant, air, water and soil tissues. Some of the research results of horticultural crops in the vegetable category ranging from mustard, tomatoes, chilies, cabbage and shallots, detected chlorpyrifos insecticide residues with a value of 0.45 mg kg⁻¹ (9-11).

The residue contained in plants can come from pesticide spraying on the plants. Insecticide residues are present in all plant parts, such as stems, leaves, fruits and roots. Especially for fruit, this residue is present on the surface and flesh of the fruit (12, 13). Even though it has been washed or cooked, this pesticide residue is still present in food ingredients (14). The organophosphate group is the largest amount of pesticides on the market and is widely used in agriculture. It gives a satisfying effect with a low dose; besides, it works quickly and is easily broken down. Organophosphate poisoning can occur by mouth, inhalation and skin. In the body, the organophosphate binds to the acetylcholinesterase (AChE) enzyme, which results in the build-up of acetylcholine in nerves (15). Profenofos is a type of organophosphate insecticide with a maximum residue limit of 5 mg kg⁻¹ in horticultural products as required by the Indonesian National Standard (16).

The use of alternative pests and plant disease control techniques such as biopesticides and botanical pesticides is currently not very popular among farmers (17). Most farmers prefer to use synthetic chemical pesticides to control pests and plant diseases because they give a fast result and easy to apply (18). Meanwhile, biopesticides and botanical pesticides require a long time and process to be entirely accepted by the farming community (19). Based on this phenomenon, the residual contamination of active pesticide ingredients in conventional agricultural products is complicated to avoid. Concrete solutions are needed to suppress and even remove the residue of active pesticide ingredients that are easy to apply and applied in the field.

Several botanical ingredients such as *Sapindus rarak* seeds, *Luffa acutangula* peels and *Centella asiatica* leaves are reported to contain high saponin content (20-22). Saponins are compounds of the glycoside type. Glycosides are compounds composed of glycons (glucose, fructose) and aglycones (23). Saponins are known as natural detergents, with molecular structure consisting of more than one sugar chain. With its nature as a natural detergent, saponins can dissolve various kinds of substances, including the active pesticide ingredients (24). Generally, one character shown by plants containing saponins is when dissolved or shaken with water will produce foam. The foam that appears is amphipathic glycosides. Foam from this group is stable and does not easily disappear (25, 26).

Saponins' ability to dissolve various substances cannot be separated from the natural glycoside components they contain (27). There are two types of natural glycosides in saponins: alcohol triterpenoid glycosides and steroid structural glycosides. Both

types of natural glycosides are soluble in water and alcohol but insoluble in ether (28, 29). Its water-soluble nature will facilitate application in the field because it does not require special solvents that are potentially difficult to get by the farmers. The use of saponins as on-farm pesticide washing-treatment has not been potentially harmful to plants. This is supported by the statement, saponins not only has dissolving effect, but also is biodegradable and easy to clean using water (30).

In previous studies, saponins from various plant extracts were reported to reduce surface tension (31). The lower the surface tension, the easier the pesticide active ingredients will be washed from agricultural products (24). The use of saponins for on-farm pesticide washing-treatment is a solution that is effective, environmentally friendly, inexpensive and easy for farmers to apply. However, until now, there have been no reports on the use of *S. rarak*, *L. acutangula* and *C. asiatica* as pesticide washing agents directly in the field. The discovery of a pesticide residues washing method in the field will provide a new information and become new recommendations in the context of implementing healthy agriculture.

Apart from being potential as a washing agent for pesticides in the field, the use of aqueous plant extracts also has the potential to reduce pest populations. It was reported that the aqueous extract of *S. rarak* can act as a repellent for some insects (32). In addition, *S. rarak* extract was also reported to be insecticidal against the larvae of *Crocidolomia pavonana* (33). It was also reported that *L. acutangula* extract also has the potency as a potential source of botanical pesticides to suppress the population of several types of pests (34). This fact shows that the extracts from the selected plant have a dual potential, namely as a washing agent for pesticides as well as for botanical pesticides. This study is aimed to evaluate the effectiveness of aqueous plant extracts from *S. rarak* seeds, *L. acutangula* peels and *C. asiatica* leaves as a pesticide washing agent as well as its effectiveness in suppressing grasshopper population in leaf of mustard.

Materials and Methods

Time and Place of Research

The research was conducted from October 2020 to January 2021. The research was carried out in three places, namely the Laboratory of Plant Pest and Disease Control Technology, in greenhouses, and on the experimental field belonging to the Plant Protection Study Programme, Faculty of Agriculture, University of Jember, Indonesia.

Sources of Plant Materials

Sources of plant matter, namely *S. rarak* seeds, *L. acutangula* peels and *C. asiatica* leaves were obtained from traditional markets in Jember, East Java, Indonesia. All the materials obtained are clean, so washing is not carried out prior to the drying process. The plant matter obtained was dried in the greenhouse until the water content was \pm 13-15%.

The sample drying process was performed carefully to avoid direct sunlight exposure. The dry sample was then chopped and filtered using a 100 mesh sieve.

Producing Aqueous Extracts of Plant Materials

The aqueous plant extracts was made by mixing 30 gm of chopped plant powder with 1000 ml of water. The final concentration of the aqueous extract is 30.034 ppm. Mixing was done using a blender at 800 rpm for 5 min. The mixed material was then filtered using a 100 mesh sieve to filter out any residual plant powder that can interfere with the sprayer. The aqueous plant extracts obtained was used for the next test (35).

Phytotoxicity Test

To ensure that the plant extract used was safe for leaf mustard growth, a phytotoxicity test was carried out. The leaf mustard of the *Tosakan* variety was planted in polybags with 15 × 30 cm in a greenhouse. The test was carried out following a completely randomized design pattern with 4 treatments, 5 replications and each replication consisting of 3 test plants. The treatment used was the aqueous plant extracts and water was used as a control. One week after transplanting, the leaf of mustard were sprayed with 100 ml of each treatment's aqueous plant extract. After being given the treatment, the leaf mustard were kept for 3 weeks while observing their development. Phytotoxicity is characterized by the appearance of symptoms such as stunted growth, wilting of the leaves, necrosis or chlorosis on the leaves, shriveled leaves and other abnormal growth symptoms (36).

Field Experiment

The leaf mustards of the *Tosakan* variety were planted on the soil beds in the University of Jember experimental field. The leaf mustard plant was chosen as the test plant because it has a large leaf surface making it easier to be assessed. The test was performed following a randomized block design pattern with 4 treatments, 3 replications and each replication consisting of 20 tested plants. Three weeks after transplanting, the leaf mustard plants were sprayed using synthetic chemical pesticides contain active ingredient profenofos. The pesticide concentration used was 3 ml l⁻¹. The sprayed dose of synthetic chemical pesticides was 600 ml per bed (equivalent to 30 ml per plant).

Two days after the application of synthetic chemical pesticides, the plants were sprayed using aqueous plant extracts. The aqueous plant extracts concentration 3% at a dose of 600 ml per bed (equivalent to 30 ml per plant). Furthermore, the treatments used in the field experiment are presented in Table 1.

Twenty-four hrs after the application of aqueous plant extracts, leaf samples from each treatment were taken and placed in plastic placed in an icebox. The sample was brought to the laboratory for analysis of pesticide residues. In the field experiment, the observed variables observed were pesticide residues attached to mustard leaves and grasshopper

populations after the application of aqueous plant extracts.

Table 1. Treatments used in the study

Code	Treatment
K	Profenofos insecticide
PL	Profenofos insecticide + aqueous extract of <i>S. rarak</i> seeds
PG	Profenofos insecticide + aqueous extract of <i>L. acutangula</i> peels
PP	Profenofos insecticide + aqueous extract of <i>C. asiatica</i> leaves

Pesticide Residue Analysis

Sample extraction was performed on treated leaf mustard samples. Samples in each treatment were blended, then weighed 15 gm in a 50 ml centrifuge tube. The 15 ml of acetonitrile were then added, shaken for 2 min until homogeneous. After that, 6 gm of MgSO₄ and 15 gm of sodium acetate were added. The mixture was shaken for 1 min and centrifuged for 1 min at 6000 rpm. The supernatant formed was taken as much as 8 ml, then transferred to a 10 ml centrifuge tube containing 150 mg MgSO₄, 50 mg PSA, and 50 mg C₁₈, then shaken vigorously for 30 seconds. The mixture was then homogenized with vortex for 1 min and centrifuged for 5 min at a speed of 6000 rpm. A total of 1 ml of the formed supernatant was taken and put into the LC vial to be injected into the UPLC-MS/MS machine (Shimadzu LCMS MS-8060). The pesticide standard used in this research is chlorpyrifos solution analytical standard (99%, Chem Service, USA). The standard series is made in the range 1 - 100 µg as many as 8 points (1; 2.5; 10; 20; 40; 80; 100 µg l⁻¹). The standard solution was stored at -20 °C until analysis (37, 38).

Data Analysis

Phytotoxicity data are presented in descriptive form by explaining whether or not the observed symptoms appear. Furthermore, pesticide residue data is presented in quantitative form by presenting the residual value results from the UPLC-MS/MS method analysis. Grasshopper population data after the application of aqueous plant extracts were analyzed using one way analysis of variance (ANOVA), if there was data diversity followed by further analysis using Duncan's Multiple Range Test (DMRT) with a 95% confidence level.

Results and Discussion

Phytotoxicity of Plant Extracts

Based on the phytotoxicity test, it is known that all aqueous plant extracts used in this study are safe and do not cause phytotoxicity. Compared with control plants, application of aqueous plant extracts with a concentration of 3% did not cause a significant change in the treated plants. All plants showed normal growth were indicated by no symptoms of stunted growth, wilting of the leaves, necrosis or chlorosis appearing on the leaves, shriveled leaves, and other abnormal growth symptoms. Furthermore,

the results of phytotoxicity observations are presented in Table 2.

Table 2. Phytotoxicity observation results

Symptoms	<i>S. rarak</i>	<i>L. acutangula</i>	<i>C. asiatica</i>
Stunted growth	-	-	-
Withered	-	-	-
Necrosis	-	-	-
Chlorosis	-	-	-
Shriveled leaves	-	-	-

Description: (-) no symptoms appeared

Aqueous plant extracts applied in this study did not show any phytotoxicity reactions. Phytotoxic symptoms tend to occur in plants treated with extracts. Extracts usually consist of many combined compounds. Apart from the active compounds, the crude extract also contains other components (39). Methanol extract can consist of a polar component to a nonpolar component. Nonpolar components in oil or concentrated liquids form can damage the wax cuticle of leaves or plant leaf cell membranes (40).

In this study, the absence of phytotoxic effects was suspected due to the ingredients used did not cause cell damage, and the low concentrations used. The active compounds found in *S. rarak* extract include flavonoids, alkaloids, saponins, tannins and polyphenols (41). Meanwhile, *C. asiatica* leaf extract is also reported to contain several active compounds, such as saponins, asiaticocides, thankunicides, isotancunicides, madecassocides, brahmocides, brahminocides, brahmic acids, madasiatic acids, meso-inositol, centellosides, carotenoids, hydrochotillin, vellarine, tannins and mineral salts, such as potassium, sodium, magnesium, calcium and iron (42). The *L. acutangula* extract is reported to contain some active compounds, such as saponins and antioxidants (43). All ingredients in the three extracts are not dangerous or cause phytotoxic at low concentrations.

The Effect of Plant Aqueous Extract on Profenofos Residue

Based on the results of analysis in profenofos residue using the UPLC-MS/MS method, profenofos residue of 2407.62 ng g⁻¹ was found in the control plants (without treatment). The residual value in plants treated with plant extract spraying two days after the application of active ingredient profenofos contained pesticide was shown to be lower than that of the control plants. In the PL treatment, it was found that the detected residue value of profenofos was 1502.05 ng g⁻¹, recorded a decrease of 37.48% compared to the residual value in control plants. Furthermore, in the PP treatment, the detected residue value of profenofos was 1316.27 ng g⁻¹, lower than the PL treatment. When compared with the control, the PP treatment gave 45.32% suppression of the profenofos residue. The highest emphasis was found in plants treated with PG, with a profenofos residual value of 660.71 ng g⁻¹, PG treatment gave an emphasis of 72.55% compared to controls. From these data it can be concluded that the application of plant extract two days after the application of synthetic chemical pesticides can suppress the of profenofos residues in leaf of mustard. The highest suppression was seen in

the aqueous extract of *C. asiatica* leaves treatment. In addition, the data on the profenofos residue on the various treatments tested and the suppression percentage are presented in Table 3.

Table 3. Reduction of profenofos residues in each tested treatment

Treatment	Residual Value (ng g ⁻¹)	Residue Reduction (%)
K	2407.62	0
PL	1502.05	37.48
PP	1316.27	45.32
PG	660.71	72.55

The three aqueous plant extracts tested were effective in suppressing the residues of profenofos pesticides on leaf mustard. The suppression effect occurred due to the washing activity of the profenofos residue on the leaf mustard. One of the compounds playing an important role in washing pesticide residues is saponins (44). The three plant aqueous extract tested were reported to contain saponins. Saponins are glycosides that have aglycones in the form of sapogenins (29). In previous research, it was reported that saponins can reduce water surface tension (31). This phenomenon of decreasing water surface tension causes saponins to form foam on the water surface after being shaken. This property is known for its similarities with surfactant properties. The decrease in surface tension is caused by the presence of soap compounds that break the hydrogen bonds of water (45). In general, the glycone part of saponins consists of sugars such as glucose, fructose and other types of sugar (23). Meanwhile, the aglycone part consists of sapogenins. The amphiphilic nature of saponins is what causes saponins to have the potential as natural ingredients that can function as surfactants (46).

Surfactants are generally used in soap production. Surfactants are molecules with a hydrophilic group and a lipophilic group. The combination of these two groups can unite a mixture of water and oil (47). This property is thought to be the reason why aqueous plant extracts used in this study can wash or dissolve the active ingredients profenofos. Surfactant molecules have a polar part that likes water (hydrophilic) and a non-polar part that likes oil/fat (lipophilic). The polar parts of the surfactant molecule can be positive, negative or neutral (48).

Effect of Aqueous Plant Extracts Applications on Grasshopper Populations

Based on the results of observations on the grasshopper population after application of aqueous plant extracts, the results obtained were quite diverse. In control plants, the average grasshopper population found after application of aqueous plant extracts was 4.67 ± 1.41. The treatment that gave the highest suppression was PL with an average observed grasshopper population of 1.44 ± 1.42. When compared with control plants, PL treatment suppressed grasshopper population by 69.16%. The PL treatment was the only treatment that gave significantly different results compared to control plants. The second highest suppression was given by the PG treatment and PP, with an average of

grasshopper population values of 3.22 ± 2.05 and 3.67 ± 1.66 respectively. Although the population found in the PG and PP treatments was lower than that of the control plants, statistically the PG and PP treatments were not significantly different from the controls. The data regarding the grasshopper population is presented in Fig. 1.

Apart from being effective in suppressing profenofos residues, the application of aqueous *S.*

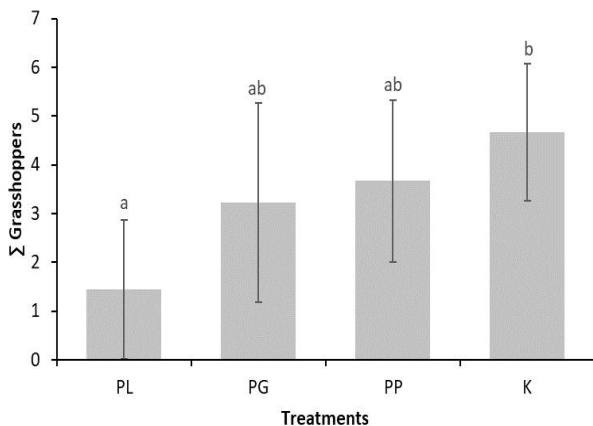


Fig. 1. The observed population of grasshoppers after aqueous plant extracts application.

rarak extract was also known to be effective in suppressing the grasshopper population in this study. The use of *S. rarak* extract to control several pests has been previously reported. For example, it was reported that *S. rarak* extract was effective in controlling pests of golden snails (*Pomacea canaliculata*) in rice (49). In a separate report, treatment with an aqueous extract of 0.80-3.80% resulted in 10-100% mortality of *Crocidolomia pavonana* larvae with the LC_{50} of 1.681% (50). On the other hand, there are reports on the application of lerak extract at the same concentration could result in 1-94% mortality of *C. pavonana* larvae with the LC_{50} of 1.898% (33). Although effective in controlling some pests, reports of *S. rarak* extract for controlling grasshopper populations in the field are still very rare. Other aqueous plant extracts used in this study could not statistically suppress the grasshopper population in the field, presumably because they do not contain compounds that can control grasshoppers, or the concentrations used are not optimal for controlling grasshopper populations.

Conclusion

This study provided new information that the application of aqueous extracts from *S. rarak* seeds, *L. acutangula* peels and *C. asiatica* leaves with a concentration of 3% and a dose of 30 ml per plant can reduce the profenofos residue in leaf mustard by 37.48% to 72.55%. The highest emphasis was obtained on plants treated using aqueous extract of *L. acutangula* bark (72.55%). Apart from being effective in suppressing pesticide residues, the application of *S. rarak* aqueous extract was also

effective in suppressing the grasshopper population in mustard leaf.

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Authors' contributions

All authors have a balanced contribution in designing research concepts, conducting experiments, analyzing data and writing the manuscript.

Conflict of interests

The authors confirmed that this study was conducted without any conflict of interest.

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