



RESEARCH ARTICLE

# The utilization of organic adjuvants to increase the effectiveness of chlorpyrifos insecticides against *Spodoptera litura*

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#### Abstract

Public awareness of consuming healthy food with minimal pesticide residues has been on the rise. By contrast, farmers still rely on synthetic chemical pesticides to control pests. The shift from synthetic chemical insecticides to botanical insecticides or bioinsecticides is quite complex, especially in Indonesia. Farmers must have the appropriate technology to be independent of synthetic chemical insecticide. This study aims to test the effectiveness of organic adjuvants to increase the effectiveness of insecticides coupled with chlorpyrifos as an active ingredient. We tested the organic adjuvants containing sulfur, acetic acid, NaCl and aqueous extract of Azadirachta indica leaves. The test involved mustard (Brassica juncea (L.) Czern.) plant on land with a high record of S. litura attacks. The study followed a randomized block design with 8 treatments and 5 blocks, with each block consisting of 20 test plants. The treatments involved water control, chlorpyrifos insecticide at recommended concentration, and a combination of chlorpyrifos insecticide and organic adjuvant. The results showed that the combination of insecticides and organic adjuvants did not have a negative impact on mustard growth, as evinced by the absence of significant difference in all treatments. Furthermore, the combination of insecticides and organic adjuvants reduced the rate of plant damage, the population of S. litura and the population of other insect pests. On the other hand, the combination of insecticides and organic adjuvants did not significantly affect the number of beneficial insects compared with treatment without insecticides. The study documented that the combination of 40 mL L<sup>-1</sup> organic adjuvant + 1.8 mL L<sup>-1</sup> insecticide chlorpyrifos led to the best results. This combination is found the most effective in reducing plant damage and pest population, without any negative impact on beneficial insects. This study has proven the value of organic adjuvants coupled with chlorpyrifos as the active ingredient to reduce the use of insecticides.

#### Keywords

Spodoptera litura, NaCl, sulfur, acetic acid, Azadirachta indica

#### Introduction

Healthy agriculture is a holistic concept aspiring to protect the environment, farmers and consumers of agricultural products. It was reported that healthy agriculture is important because consumer confidence in conventional agricultural products has been declining (1). This phenomenon is inseparable from the fact that many agricultural products, especially vegetables, contain an excessive amount of residue from active pesticides (2). Concrete efforts are needed to reduce the amount of synthetic chemical pesticides along with their negative impacts.

It was stated that farmers around the world have widely used insecticides (3). More than 95% of farmers use synthetic insecticides during crop cultivation. This is also acknowledged that insect pests are the most serious threat to vegetable cultivation (4). Likewise, reports are on the most widely used synthetic insecticide's active ingredient by farmers worldwide is chlorpyrifos (5). Research involving 315 samples of agricultural products reports that pesticide residues are found in 47% of fresh products and 7% of processed food products (6). In addition, a previous study involving 180 samples of vegetables found that pesticides were found in 89% fresh products and 11% processed products (7). Meanwhile, pesticide residues were found in 35% of fresh product samples and 10% of processed vegetable samples (8). Most of the pesticide residues found are from the active ingredient chlorpyrifos. These studies show that there are still a lot of pesticide residues left in plants that are grown with pesticides.

Residues are residual substances or compounds from pesticides left on the human, animal, plant, air, water and soil tissues. According to the Acceptable Daily Intake (ADI), the tolerable amount of chemical substance in the human body without causing health problems is 0.015 mg/kg/day for all types of pesticide residues. This value is equivalent to a concentration of 1 ppm (9). In the long term, the residue that accumulates in the body will not be decomposed, therefore causing chronic diseases, such as kidney disease, cancer and damage to nerve tissue (10). To avoid these destructive effects, pesticide residual levels in various agricultural products, particularly horticultural products, must be reduced. In general, horticultural products are quite vulnerable to pests, particularly insects. As a result of this phenomenon, the usage of pesticides in horticulture goods is relatively high in many nations, including Indonesia.

In Indonesia, mustard (Brassica juncea (L.) Czern.) is one of the plants reported to contain a substantial amount of chlorpyrifos residue. The high amount of residue of chlorpyrifos in mustard greens results from improper use of insecticides to control mustard pests (11). In various regions in Indonesia, the main problem during mustard cultivation is the attack of the armyworm Spodoptera litura. Yield loss due to S. litura attack is reported to reach 73%, even causing crop failure if the attack occurs when the plants are still young (12). Currently, the control of these pests at the farm level predominantly uses synthetic chemical insecticides. The use of pesticides by farmers is frequently ill-conceived. For instance, farmers frequently spray pesticides despite the absence of pests or the presence of pests in amounts below the economic threshold. In some cases, farmers spray pesticides a few days before

mustards are harvested, which results in a huge amount of residue of insecticides on the plants (13).

Various measures have been made to reduce the dose and the negative impact of pesticide residues. However, the accumulation of pesticide residues in agricultural products remains a complex problem. Various approaches, such as botanical pesticides, have been demonstrated to be effective at controlling S. litura. However, the transition from synthetic to plant-based insecticides cannot be achieved suddenly. Farmers need assurance that plantbased insecticides are equally effective as their synthetic counterparts. To address these challenges, suitable solutions and technology are required. One feasible solution is using organic adjuvants. An adjuvant is an ingredient used as a mixture in the formulation of pesticides (other than solvents or diluents) which serves to modify the properties of active ingredients and increase pesticide effectiveness. Some alternative adjuvants that can be used to suppress pesticide residues are plant extracts, sulfur, lime, and salt (14).

In general, organic adjuvants can be classified into 2 groups. First, a utility adjuvant or spray modifier is an adjuvant used as a mixture in pesticide products to produce active ingredients ready for use. These adjuvants are commonly called carriers. Another type is tank mix adjuvant or surfactant. This is an adjuvant that is used to improve the characteristics of pesticides and increase the effectiveness of pesticides against plant pests or target plants. Also known as SURFace ACTive Agent, this pesticide is applied in the sprayer tank (14, 15). It was reported that adding adjuvant in the form of sulfur to insecticides with chlorpyrifos as the active ingredient can increase the mortality of some fungal pathogens and insect pest (16). A synergistic reaction between organic adjuvants and insecticide's active ingredients has been reported (17).

The abovementioned reports show that the use of organic adjuvants holds the potential to suppress synthetic insecticide residues in the field. However, further testing in a field experiment needs to be done to prove the effectiveness of organic adjuvants. This study aims to find out the effective concentration of organic adjuvants that is more easily accepted by farmers and effective in reducing the use of synthetic chemical insecticides. This is done by examining the effectiveness of organic adjuvants with chlorpyrifos as an active ingredient to reduce the concentration of synthetic insecticides while increasing its effectiveness in controlling important pests and reducing the negative impact of synthetic insecticides on the vegetable.

#### **Materials and Methods**

#### The Time and Site of Research

The research was carried out at the Plant Protection Laboratory of the Plant Protection Study Programme, Faculty of Agriculture, Jember University from August to December 2021. The planting of the test plants was carried out on mustard land managed by a farmer group in Sukorambi village, Sukorambi district, Jember regency, East Java – Indonesia. The land had a prior high record of *Spodoptera litura* attacks.

#### Test Plant

This study used mustard (*B. juncea*) of Tosakan variety as the test plant. Mustard greens were chosen because they had a broad leaf surface to facilitate the analysis of plant damage due to pest attacks. Furthermore, mustard is also the main host of *S. litura*, which is a serious problem for farmers (18). One-week old mustards seedlings were planted in a  $1 \times 1.5$  m seedling bed with basic granule organic fertilizer and NPK fertilizer was applied according to the recommended dose.

#### The Production of Organic Adjuvants

Organic adjuvants were produced from a mixture of several organic ingredients chosen for their synergistic effect with the active ingredient, chlorpyrifos. The other materials included sulfur, acetic acid, and NaCl. In addition, aqueous extract of *Azadirachta indica* leaves was used due to their insecticidal properties (19). A total of 30 g of simplicia *A. indica* was dissolved in 1000 mL of water for 24 hrs. The resultant suspension was filtered using a 200mesh filter cloth and then added with 25 g of sulfur, 3 mL of acetic acid, and 3 g of NaCl. The solution was then filtered using a 250-mesh filter cloth to separate the suspension from particles of organic matter. This final solution was then referred to as organic adjuvants.

#### Testing the Effectiveness of Organic Adjuvant in Field Experiment

The mustards were planted on the seedling bed in the experimental field. Mustard was chosen as the test plant because it had a broad leaf surface, therefore making it easier to examine the effectiveness of organic adjuvant. The test was carried out following a randomized complete block design (RCBD) with 8 treatments and 5 replications, with each replication including 20 test plants. A spacing of  $20 \times 20$  cm was applied to ensure optimal growth and accurate observation as required in experimental research. After 5 days after planting (DAP), several treatments as shown in Table 1 were applied to the mustards, with a spray dose of 600 mL per bed. The treatment was repeated every 5 days until the mustard plants were harvested at the 40<sup>th</sup> DAP.

#### Table 1. Research Treatments

Code	Treatment
T1	Water control
T2	3 mL L <sup>-1</sup> profenofos insecticide
Т3	10 mL $L^{\cdot 1}$ organic adjuvant + 2.7 mL $L^{\cdot 1}$ chlorpyrifos insecticide
T4	20 mL $L^{\cdot 1}$ organic adjuvant + 2.4 mL $L^{\cdot 1}$ chlorpyrifos insecticide
T5	30 mL $L^{\cdot 1}$ organic adjuvant + 2.1 mL $L^{\cdot 1}$ chlorpyrifos insecticide
Т6	40 mL L $^{\cdot 1}$ organic adjuvant + 1.8 mL L $^{\cdot 1}$ chlorpyrifos insecticide
Τ7	50 mL $L^{\cdot 1}$ organic adjuvant + 1.5 mL $L^{\cdot 1}$ chlorpyrifos insecticide
Т8	60 mL $L^{\rm \cdot 1}$ organic adjuvant + 1.2 mL $L^{\rm \cdot 1}$ chlorpyrifos insecticide

Agronomic variables were observed during the harvesting. These observation variables were fresh weight per plant, number of leaves, plant height, root length and leaf width.

Furthermore, to observe the effectiveness of organic adjuvants against pests and their safety for beneficial insects, observations were made on the percentage of plant damage caused by pests, the number of *S. litura* per plant, the number of pests other than *S. litura* and the number of beneficial insects. The number of *S. litura*, other insect pests and beneficial insects was counted manually in the morning and evening with the aid of a pitfall trap and yellow pan trap (20).

Observations on the population of *S. litura* and the level of damage to plants were carried out at the 6<sup>th</sup> DAP, 21<sup>st</sup> DAP and 36<sup>th</sup> DAP. Furthermore, observations on the population of pests other than *S. litura* and beneficial insects were done only at 36<sup>th</sup> DAP. Each of the insect pests and beneficial insects was put into a bottle containing 70% ethanol.

#### **Data Analysis**

Data were analyzed using a Two-Way Analysis of Variance (ANOVA). When variance is identified, a follow-up post hoc analysis, Tukey's Honestly Significant Difference (Tukey-HSD) test, will be performed.

#### **Results and Discussion**

#### **Plant Growth**

As seen in Fig. 1, all of the mustards had the same growth performance, regardless of different treatments. This showed that pesticides and organic adjuvants did not inhibit the growth of mustard plants. Based on physical observations, the mustards treated with a combination of insecticides and organic adjuvants did not show any phytotoxic symptoms, such as chlorosis, necrosis, curling, leaf thickening or other symptoms. This showed that the addition of organic adjuvants was proven safe without any negative impact on mustards.

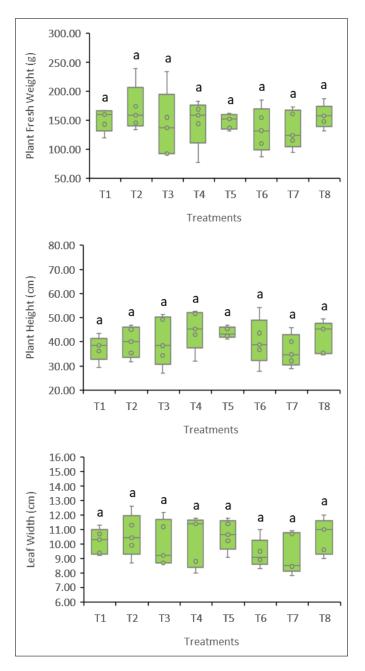
The results of the statistical analysis demonstrated that no significant difference was identified in all observed growth variables across different treatments. In terms of plant fresh weight, T2 (170.40 g) produced the highest average fresh weight, while the lowest average fresh weight was found in T7 (133.40 g). However, this difference was not significant. Furthermore, on the average number of leaves per plant, T2 (13.00) was found superior compared with all treatments, while the lowest average number of leaves was recorded in treatment T3 (10.04). Again, our statistical analysis did not show any significant difference between the treatments.

The results of plant height measurements showed that T4 (44.92 cm) produced the highest average plant height, while the lowest average plant height was identified in T7 (36.30 cm). The measurement results of root length showed that the longest average root length was found in T3 (9.00 cm), with the lowest average root length marked in T6 (8.16 cm). Concerning leaf width, the highest average leaf width was found in T5 (10.63 cm), while the



Fig. 1. Mustards in different research treatments.

lowest average leaf width was found in T7 (9.27 cm). In these 2 observation variables, no significant difference was documented between treatments. The data on plant growth across treatments are presented in Fig. 2.



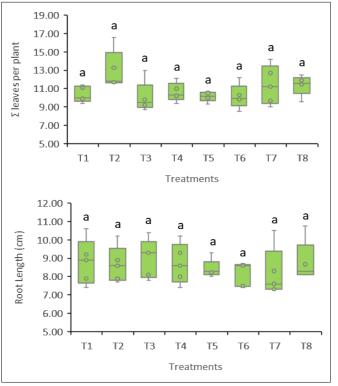


Fig. 2. The mustard growth in different research treatments. Note: different letters on the same graph indicate a significant difference in the Tukey-HSD post hoc analysis with  $\alpha$  5%. (T1) water control, (T2) 3 mL L^1 profenofos insecticide, (T3) 10 mL L^1 organic adjuvant + 2.7 mL L^1 chlorpyrifos insecticide, (T4) 20 mL L^1 organic adjuvant + 2.4 mL L^1 chlorpyrifos insecticide, (T5) 30 mL L^1 organic adjuvant + 2.1 mL L^1 chlorpyrifos insecticide, (T6) 40 mL L^1 organic adjuvant + 1.8 mL L^1 chlorpyrifos insecticide, (T7) 50 mL L^1 organic adjuvant + 1.5 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL L^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL T^1 organic adjuvant + 1.2 mL L^1 chlorpyrifos insecticide, (T8) 60 mL T^1 organic adjuvant + 1.2 mL T chlorpyrifos insecticide, (T8) 60 mL T organic adjuvant + 1.2 mL T chlorpyrifos insecticide, (T8) 60 mL T organic adjuvant + 1

The absence of significant differences between treatments indicated that the application of organic adjuvants did not inhibit mustard growth. All concentrations of organic adjuvants resulted in similar average growth, even compared with control plants that were only treated with insecticides. The safety of organic adjuvants on plant growth was proven by the results of plant growth analysis, which also demonstrated no significant differences between treatments.

Plant growth is influenced by both internal and external factors. The internal factors are generally related to plant physiology and plant genetic activity. In a study conducted tomatoes of different varieties are cultivated with the same technique, but each results in different yields (21). Another study also mentions that different mustard varieties have different growth rates (22). The external factors are generally concerned with weather conditions, soil fertility and nutrient availability (23).

Plants need both macronutrients and micronutrients for a sustainable life cycle. For example, nitrogen (N) plays an important role in the formation of protein and stomata in plants. Furthermore, phosphorus (P) is vital in supporting flowering in plants (24). In this study, the applied organic adjuvant did not pose any impact on plant growth because it did not contain any of the essential nutrients needed by plants.

Insecticides and organic adjuvants play a key role in controlling insect pests, rather than serving as agrochemicals to stimulate plant growth. Furthermore, organic adjuvants are pivotal in increasing the effectiveness of insecticides although applied below the recommended concentration. By contrast, insecticidal active ingredients have no role in promoting plant growth. Furthermore, the contents of adjuvant organic matter, i.e., acetic acid, sulfur, and plant extracts, are also found to exert no major impact on plant growth. Plant growth is more influenced by soil fertility and the availability of nutrients in the soil. This finding accounts for why all treatments in this study produce insignificant results on plant growth.

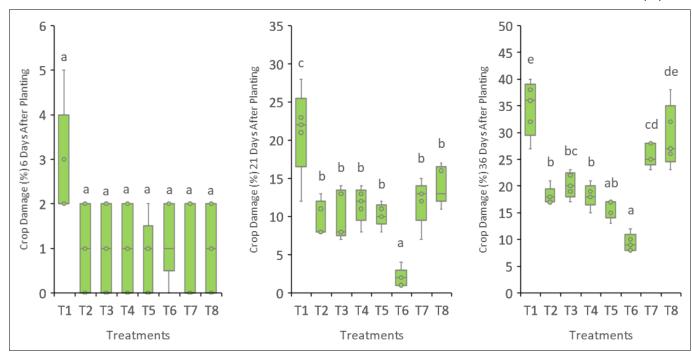
## The Effect of Organic Adjuvant on Pest Attack Rate and the Population of S. litura

The application of organic adjuvants produced different results on 3 observations of plant damage caused by *S. litura* attack. In the first observation at the 6<sup>th</sup> DAP, the % of plant damage caused by *S. litura* was not significantly different between different treatments. Comparing the plants treated with pesticides following the recommended

concentration, plants treated with organic adjuvants, and control plants, the analysis found that the average level of plant damage ranged between 0% and 5%. This occurs because the mustards are still young and the pests do not have the interest to approach them.

Furthermore, in the second observation at the 21<sup>th</sup> DAP, a significant difference was identified in several treatments. Control plants showed the highest level of damage and were significantly different from plants in other treatments. The average plant damage in control plants reached 21.2%. Lower rate of plant damage was marked in all plants treated with pesticides and the combination of pesticides and organic adjuvants. Plants in T2 showed an average damage rate of 9.6%, indicating a lower damage rate of up to 120.83% compared with control plants. T2, however, did not generate any significant difference from T3 (10%), T4 (11.6%), T5 (10.2%), T7 (12%), and T8 (14%). At 21<sup>st</sup> DAP, T6 was found to have 2% damage rate, which was 960% lower than that of control plants (T1). Furthermore, T6 demonstrated 380% lower damage rate than T2.

The last observation at the 36<sup>th</sup> DAP found different results. Plants in T7 and T8 showed the highest damage rate, 34.6% and 29.2% respectively. In T2, the damage rate was recorded at 18.2%, which was 90.10% lower than that of T1. In addition, the damage rate in T2 was not significantly different from that in T3 (20.2%), T4 (18.2%) and T5 (15.8%). The treatment associated with the least plant damage was T6 (9.4%), which was 268.08% lower than T1. The damage rate of plants in T6 was also lower and significantly different from those in T2. These data indicate that T6 is the most effective and efficient in suppressing the rate of plant damage. The data on the rate of plant damage is presented in Fig. 3.



The observations marked different S. litura popula-

Fig. 3. Percentage of plant damage after the application of insecticides and combinations of insecticides and organic adjuvants. Note: different letters on the same graph indicate a significant difference in the Tukey-HSD post hoc analysis with  $\alpha$  5%. (T1) water control, (T2) 3 mL L<sup>-1</sup> profenofos insecticide, (T3) 10 mL L<sup>-1</sup> organic adjuvant + 2.7 mL L<sup>-1</sup> chlorpyrifos insecticide, (T4) 20 mL L<sup>-1</sup> organic adjuvant + 2.4 mL L<sup>-1</sup> chlorpyrifos insecticide, (T5) 30 mL L<sup>-1</sup> organic adjuvant + 2.1 mL L<sup>-1</sup> chlorpyrifos insecticide, (T6) 40 mL L<sup>-1</sup> organic adjuvant + 1.8 mL L<sup>-1</sup> chlorpyrifos insecticide, (T7) 50 mL L<sup>-1</sup> organic adjuvant + 1.5 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide.

#### 183 HOESAIN ET AL

tions between treatments. However, at the first observation, at 6<sup>th</sup> DAP, all treatments were not significantly different. The population at the 6<sup>th</sup> DAP ranged from 1 to 3. The increase in population and diversity of treatment effectiveness was first marked since the 21<sup>st</sup> DAP. The observations also found that T1 had the highest number of *S. litura*, with an average of 17.8. A significant decrease was marked in T2 (8.6), which demonstrated a decrease of up to 106.97% compared with T1. The results in T2 were not significantly different from those in T3 (9), T4 (8.8), T5 (8.6) and T8 (10.8). The treatment with the best results at the 21<sup>st</sup> DAP was T6, with an average *S. litura* population of 3.2, which was 456.25% lower than that in T1. When compared with T2, the average of *S. litura* population in T6 was 168.75% lower.

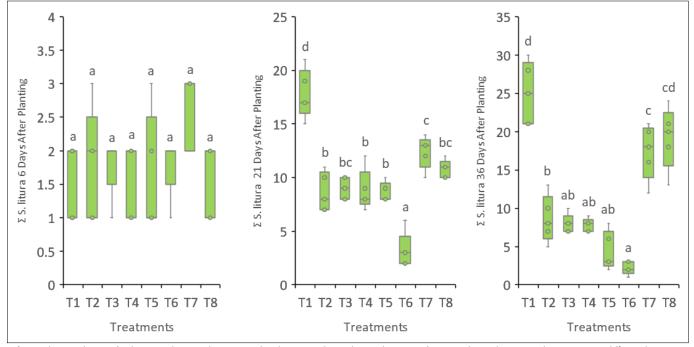
The observations on the average of *S. litura* population at the 36<sup>th</sup> DAP showed that the highest population was found in T1, with an average of population 25. T2 showed a lower population at 8.6. than T1, which was 190.69% lower than that in T1. The analysis results demonstrated that the *S. litura* population in T2 was not significantly different from that in T3 (8), T4 (7.8), T5 (4.4). T6 was proven the most effective in suppressing *S. litura*, with an average population of 2.2. This average was 1,036.36% lower than that in T1. The average of *S. litura* population in the treatments is presented in Fig. 4.

The plant damage due to S. litura attack is generally

organic adjuvants. The combination of insecticide and organic adjuvant was only effective from T2 to T6, where the insecticide concentration was reduced to 40% of the recommended concentration.

Organic adjuvants were administered to increase the effectiveness of insecticides at low concentrations, instead of replacing insecticides, because the ingredients could not completely replace the role of insecticides. This finding explains why organic adjuvants are only effective from T2 to T6. In T7 and T8, where the insecticide concentration was declined, organic adjuvants assumedly could not play many roles in increasing insecticide effectiveness.

Insecticides with chlorpyrifos as the active ingredient can control pests, by disrupting the insect neural system, while causing contact poison, stomach poison and respiratory poison. Insects that come into contact with chlorpyrifos insecticide will show abnormal behavior, make irregular movements and experience digestive disorders (26). Organic adjuvants combined with sulfur, NaCl, acetic acid and plant extracts have several mechanisms to increase the effectiveness of insecticides. The first mechanism is increasing the activity of the active ingredients through a synergistic mechanism (27). The second mechanism is resisting the presence of insects through the odor generated by organic adjuvants. In addition, the active ingredient content of *A. indica* extracts is known to have a broad spectrum of insecticidal activity. The main compo-



**Fig. 4.** The population of *S. litura* on the test plants treated with insecticides and a combination of insecticides and organic adjuvants. Note: different letters on the same graph indicate a significant difference in the Tukey-HSD post hoc analysis with α 5%. **(T1)** water control, **(T2)** 3 mL <sup>-1</sup> profenofos insecticide, **(T3)** 10 mL L<sup>-1</sup> organic adjuvant + 2.7 mL L<sup>-1</sup> chlorpyrifos insecticide, **(T4)** 20 mL L<sup>-1</sup> organic adjuvant + 2.4 mL L<sup>-1</sup> chlorpyrifos insecticide, **(T5)** 30 mL L<sup>-1</sup> organic adjuvant + 2.1 mL L<sup>-1</sup> chlorpyrifos insecticide, **(T6)** 40 mL L<sup>-1</sup> organic adjuvant + 1.8 mL L<sup>-1</sup> chlorpyrifos insecticide, **(T7)** 50 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide.

associated with the number of *S. litura*. The higher the population of *S. litura* is found, the higher the plant damage rate will occur (25). In this study, the damage rate to the field and the number of *S. litura* decreased after the treatment involving insecticides following the recommended dose and the combination of insecticides and

nent in neem is azadirachtin, but there are other ingredients, namely meliantriol, salanine, nimbidin, nimbin and other components which are secondary metabolites of this plant. Azadirachtin consists of about 17 components that work by interfering with the growth and juvenile hormones, which disrupts the metamorphosis process and affects the reproduction of adult insects (28, 29).

*A. indica* does not kill pests quickly, but adversely affects feeding power, growth, reproductive power, molting process, mating and sexual communication, egg hatchability and chitin formation. *A. indica* can affect insect behavior by causing stress and starvation. In addition to being an insecticide, *A. indica* also serves as a fungicide, virucide, nematicide, bactericide and acaricide (30, 31).

## The Effect of Organic Adjuvant on Other Pests and Beneficial Insects

The observations on pest populations other than *S. litura* showed that the average population of these pests reached 17 in T1. This figure was not significantly different compared with T2 (14.4) and T3 (13.4). When compared with T1, T4 (11.8), T5 (12.2) and T6 (11) were found to have a significantly lower population of insects, marked by 44.06%, 29.24% and 54.54% lower population respectively. In T7 and T8, a higher average population of other pests was identified, with an average of 13.4 and 18.6 respectively. The data on the number of insect pests other than *S. litura* in each treatment are presented in Fig. 5.

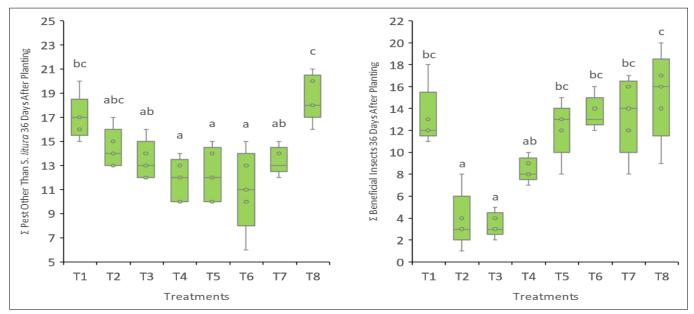
The observations of beneficial insect populations

insects is affected by the application of insecticides and the combination of insecticides and organic adjuvants. The result is almost similar to the outcome of suppressing *S. litura*. The insecticide chlorpyrifos has a fairly broad spectrum. Therefore, other insect pests are also affected by the active ingredients. Chlorpyrifos insecticide residues were reported to affect insect pests above and below ground level (15). This fact points out why the number of insect pests other than *S. litura* was also declined.

This study has found that lower concentration of insecticides increases the population of beneficial insects because the insecticide exerts a distinctive aroma that deters insects. The lower concentration is likely to attract beneficial insects. Beneficial insects are generally found in small numbers in agroecosystems that apply insecticides intensively and/or over a long period. Intensive or longterm application of insecticides results in gradual accumulation of the residues of active ingredients that adversely affect beneficial insects.

#### Conclusion

The combination of chlorpyrifos insecticides and organic



**Fig. 5.** The number of insect pests other than S. *litura* and beneficial insects on plants treated with insecticides and a combination of insecticides and organic adjuvants. Note: different letters on the same graph indicate a significant difference in the Tukey-HSD post hoc analysis with  $\alpha$  5%. (T1) water control, (T2) 3 mL L<sup>-1</sup> profenofos insecticide, (T3) 10 mL L<sup>-1</sup> organic adjuvant + 2.7 mL L<sup>-1</sup> chlorpyrifos insecticide, (T4) 20 mL L<sup>-1</sup> organic adjuvant + 2.4 mL L<sup>-1</sup> chlorpyrifos insecticide, (T5) 30 mL L<sup>-1</sup> organic adjuvant + 2.1 mL L<sup>-1</sup> chlorpyrifos insecticide, (T6) 40 mL L<sup>-1</sup> organic adjuvant + 1.8 mL L<sup>-1</sup> chlorpyrifos insecticide, (T7) 50 mL L<sup>-1</sup> organic adjuvant + 1.5 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant + 1.2 mL L<sup>-1</sup> chlorpyrifos insecticide, (T8) 60 mL L<sup>-1</sup> organic adjuvant +

show a distinctive result. The higher the pesticide concentration is applied, the lower the population of beneficial insects are found. In T1, the average number of beneficial insects was 13.2. However, in contrast, T2 was marked with a population of beneficial insects at an average of 3.8, which was 247.36% lower than that in T1. In T3 to T8, the average number of beneficial insects fluctuated but tended to increase as the pesticide concentration decreased. In terms of an average population of beneficial insects, T4 (8.4), T5 (12.2), T6 (13.6), T7 (13.4) and T8 (15.2) were not significantly different from T1. The data on the number of beneficial insects in each treatment are presented in Fig. 5.

adjuvants has been shown to increase the effectiveness of insecticides with chlorpyrifos as the active ingredient. The study has also demonstrated that the most effective and efficient concentration is 40 mL l<sup>-1</sup> organic adjuvant + 1.8 mL l<sup>-1</sup> insecticide chlorpyrifos. The combination is effective in reducing the rate of plant damage and suppressing the population of *S. litura* as well as other insect pests, without suppressing beneficial insects. Furthermore, this combination does not have a negative impact on mustard growth.

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The population of other insect pests and beneficial

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#### 185 HOESAIN ET AL

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#### **Authors contributions**

MH, APP, and FKA conducted the field experiment and wrote the manuscript; SP and W analyzed the data; and MA co-wrote the manuscript and performed the statistical analysis.

#### **Compliance with ethical standards**

**Conflict of interest**: Authors do not have any conflict of interests to declare.

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