International Journal of Agronomy and Agricultural Research
ISSN: 2223-7054 (Print); 2225-3610 (Online) | IJAAR |
Website: https://www.innspub.net
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RESEARCH PAPER
Vol. 28, Issue: 6, 2026

OPEN ACCESS

Larval development of black soldier fly (*Hermetia illucens* Linnaues, 1758) on organophosphate-treated cabbage

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DOI: https://dx.doi.org/10.12692/ijaar/28.6.9-18

ARTICLE INFORMATION

RESEARCH PAPER

Vol. 28, Issue: 6, p. 9-18, 2026

Int. J. Agron. Agri. Res.

Sucdad

ACCEPTED: 09 June, **2026**

PUBLISHED: 14 June, **2026**

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ABSTRACT

The study conducted in Balili, La Trinidad, Benguet from November 2024 to February 2025 aimed to evaluate the effects of organophosphates (profenofos, malathion, and indoxacarb) on the larval development of Black Soldier Fly (BSF). It assessed growth, development, survival of BSF larvae fed with organophosphate-treated cabbage, organophosphate residue levels, crude protein content of larvae, and the toxicity of organophosphates on BSF larvae. The duration of larvae from 2nd to 6th instar was consistent across treated and untreated groups, ranging from 20 to 23 days with some significant differences in body measurements, excluding 2nd and 3rd instar larvae. Survival rates were significantly different for larvae treated with organophosphates compared to indoxacarb, with malathion-treated larvae showing a survival rate of 30.81%, whereas profenofos and indoxacarb resulted in zero and 0.61% survival rates, respectively, against a control survival rate of 23.61%. Residue levels in cabbage were medium for indoxacarb and low for profenofos and malathion, with no residues found in BSF larvae and frass. Notably, larvae fed malathion treated cabbage had the highest crude protein content at 15.46%, while control larvae had 15.39%. Overall, mortality among BSF larvae varied significantly, with rates from 69.19% to 100%; untreated larvae had a mortality rate of 76.39%, while indoxacarb caused the highest mortality at 100%, followed closely by profenofos at 99.39%.

Key words: Black soldier fly, Cabbage heads, Organophosphate

INTRODUCTION

Insects as food and feed are increasingly seen as a commercially viable mini-livestock alternative to conventional animal production. The Black Soldier Fly (BSF), *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae), had primarily been seen as a pest species, but has recently gained popularity due to its ability to consume and convert a wide variety of organic waste such as vegetable, animal tissues and manure into organic matter. Additionally, the use of its larvae as feed to various livestock further commercialized itself. With the increasing use of BSF as feed, several safety issues arise. One which has received less attention is the effects of contaminant such as insecticides in the feed substrates of the insect.

In livestock, the presence of these contaminants may result to bioaccumulation which may cause residual levels exceeding the legal limits. Moreover, since most persistent synthetic insecticides act against all insects, their residues in the substrate may cause sublethal effects in insects, even at concentrations in the feed substrate that comply with existing legal limits (De Rijk *et al.,* 2021).

Furthermore, according to Aidoo *et al.* (2023), BSF could accumulate undesired substances in their bodies, such as heavy metals, mycotoxins, and pesticides during the feeding process in the larval stage. Cadmium, arsenic, and lead concentration in BSF larvae exceeded the recommended standard for heavy metals occurring in feed and food. Likewise, BSF larvae fed with substrates containing hazardous chemical, such as mycotoxins which exceeds in a current level limit, does not accumulate on larvae. In fact, this may even break down into non-toxic forms, making the larvae potentially suitable as a feed ingredient for farmed animals (Lievens *et al.,* 2021).

Furthermore, Arpilia *et al.,* (2020) showed that BSF larvae could thrive on cabbage waste, tomato, carrot, and mixed waste. Additionally, Alimondo (2019) stated that cabbage was the top one crop produced in Benguet with 25.4 % or 38, 925 metric tons. In terms of the usage of the synthetic chemicals, organophosphates is one of the most commonly used. Similarly, a study conducted by Delima (2012), as cited by Anacin *et al.,* (2017), stated that high concentrations of organophosphate residue was detected in cabbage and cauliflower both in Benguet and Nueva Vizcaya.

The study aimed to assess the effects of different organophosphates on the larval development of BSF. Specifically, the study aimed to determine the growth, development survival of BSF larvae fed with cabbage treated with organophosphate; determine the crude protein content of BSF larvae fed with cabbage treated with organophosphate; and determine the lethal toxicity of organophosphates on BSF larvae.

MATERIALS AND METHODS

The materials that were used in the study were 20 liters plastic containers, 1.5-liter plastic bottle, cellophane, and organophosphate insecticides. Also, retrograded rice that was mixed with cabbage served as substrate. A canister, containing 10 ml of 70% ethyl alcohol was used to preserve the BSF larvae. A caliper was utilized to measure the width and length of the larvae, while a digital scale was used to determine the weight. Also, a hygrothermometer was used to measure the humidity and temperature. Additionally, shelf stand for the gallon containers was also utilized.

Preparation of the rearing containers

Sixteen (16) pieces of 20-liter capacity mineral water gallon were modified for the experiment (Fig. 1A). Five holes measuring 12mm were drilled at the bottom of each gallon (Fig. 1C) to serve as exit point of leachate and to facilitate ventilation. Cellophane was placed under the container to trap the leachate. An empty, uncovered, 1.5-L capacity plastic bottle (Fig. 1B) was placed at the rear side of the container to serve as catch basin for the larvae as they self-harvest. One iron stand measuring 113 x 37 inches served as shelf of the 16 containers.

Substrate preparation

Cabbage seedlings with a variety of wonder ball were bought from cabbage seedling growers and were grown in Balili, La Trinidad, and Benguet (Fig. 2). The 50-day cabbages were sprayed with the different organophosphates, which served as treatments, according to the recommended rate, interval and direction for use of the insecticide (Fig. 1B). From these, 57 day cabbages were harvested and were chopped into small pieces.

Fig. 1. Experimental set-up: (A) 20-liters gallon (B) 1.5 -liter plastic bottle (C) Holes for drainage (D) BSF rearing set-up

Fig. 2. Substrate prepation: (A) Cabbage plant; (B) Spraying different organophosphate insecticide

The different organophosphates used were mostly used as insecticide by the farmers based on the study of Bajet *et al.,* (2023) and Lu (2010). The treatments, replicated four times, were laid following the Completely Randomized Design (CRD).

The experiment consisted of four treatments. Treatment 1 served as the untreated control and received no insecticide application. Treatment 2 was sprayed with Profenofos at a recommended rate of 2.0–5.0 tablespoons per 16 liters of water and was identified by a yellow color label. Treatment 3 received Malathion at a rate of 2.0–5.0 tablespoons per 16 liters of water and was marked with a green color label. Treatment 4 was treated with Indoxacarb at a recommended rate of 2.0–5.0 tablespoons per 16 liters of water and was identified by a blue color label.

A kilogram of the substrate was fed to 40 - 90g of 7 - day - old BSF larvae. Additionally, a kilogram of retrograded rice was also added per substrate to minimize the emission of foul odor (Fig. 3). The cleaning and replenishment was done once the food was depleted. Feeding was done twice a week.

Fig. 3. Chopped cabbage with retrograded rice

Determination of growth and development of BSF

Ten (10) BSF larvae samples per treatment replication were harvested daily using random sampling. Each larva was placed in a vial containing 70% alcohol after being measured for length and width (mm).

Determination of organophosphate

Content of cabbage

The Rapid Testing Kit for Organophosphate, a colorimetric method analysis designed by the National Crop Protection Center (NCPC) of the University of the Philippines - Los Baños was used in testing the presence of organophosphate in the sample through its standard protocol.

In addition, trickling extraction method was used to process 25g dry samples of chopped cabbage collected from the grown cabbage treated with organophosphate. The glass was swirled for the extract to evaporate until its volume reduced to 0.5 mL. Then, three (3) treated filter paper were placed in a glass side. Each filter paper was spotted five times and a drop of solution O was added before being covered with glass slide. This was then heated in an alcohol lamp for at least two minutes until the filter paper turned slightly brown.

The resulting color was matched in the color chart. As shown on to the color chart (Table 1), the bluish to bluish- grey ring signifies the presence of organophosphate pesticide.

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Table 1. Rapid test kit color chart for organophosphate

Residue level	Color description	Color chart
Negative	No color	
Low	Bluish	
Medium	Bluish-grey	
High	Deep blue	

Determination of the residue content of the BSF larvae and frass

Twenty-five grams (25g) of 6^{th} instar BSF larvae were collected per treatment, and were macerated for residual content determination using Rapid Test Kit for Organophosphates (Fig. 4). Similarly, 25g of frass were collected per treatment for residue analysis.

Fig. 4. Twenty five grams of 6th instar BSF larvae for residual analysis

Determination of the crude protein of the bsf larvae

A hundred grams (100 g) of 6^{th} instar BSF larvae were weighed per replication, and were pooled to have 400g of BSF larvae per treatment. From this, 250g were obtained and brought to the Department of Science and Technology for crude protein analysis.

Temperature and humidity

A hygrothermometer was used to measure the temperature and humidity of the study site. This is to determine the effect of the said factors in the larval development of the BSF.

Data gathered

Larval measurement (mm)

This is the width and length of the larvae grown on the different organophosphate. Ten samples were collected and measured using a digital caliper.

Change in larval weight (g)

This refers to the change in the weight of the larvae before being fed with the substrates and after they start to self-harvest from the substrates.

Larval duration

This refers to the number of days of BSF larval development per instar.

Percent survival

Percent survival was computed using the modified formula of Kumar *et al.* (2015).

Percent survival= {(Total number of alive larvae−Total number of dead larvae)/Total number of alive larvae}×100

Organophosphate residue content of treated cabbage, BSF larvae and frass

This is the organophosphate residue content of the treated cabbage and BSF larvae fed with treated cabbage determined using the Rapid Test Kit color chart of NCPC, UPLB. Levels were classified as low, medium, and high.

Protein content

This is the crude protein content of the BSF larvae fed with the different cabbage treated with organophosphate.

Percent mortality (%)

Percent mortality was computed using the modified formula of Kumar *et al.* (2015).

Percent mortality={(Total number of larvae−Total number of larvae alive)/ Total number of larvae}×100

Statistical analysis

One-way Analysis of Variance (ANOVA) for CRD was used to determine the significant effect of the different treatments, and Tukey's test was used to determine the difference among the treatments at a 5% level of significance.

RESULTS AND DISCUSSION

Larval growth, development and survival of BSF fed with cabbage treated with organophosphate

The BSF undergoes complete metamorphosis, progressing through six instar larval growth stages. As the larvae progress through each instar, they increases in size with each molt until they reaches 6^{th} instar (Fig. 5). The second instar of BSF larvae ranges from 10.14 mm to 11.44 mm in length and 2.79 mm to 3.14 mm in width (Table 2). Among the organophosphates (OP), profenofos resulted in the smallest size in both length and width.

Table 2. Measurement (mm) of the different larval stages of BSF fed with cabbage treated with organophosphates

Treatments	Larval instars									
	2nd instar		3rd instar		4th instar		5th instar		6th instar	
	L	W	L	W	L	W	L	W	L	W
Untreated (Control)	11.31^{a}	2.93^{ab}	15.68^{a}	3.40^{a}	17.73^{a}	3.72^{a}	18.63^{a}	3.88^{a}	19.40^{a}	4.19^{a}
Profenofos	10.14^{c}	2.79^{b}	$13.95^{c.}$	3.27^{a}	15.55^{c}	3.41^{b}	16.89^{c}	3.52^{b}	17.15^{b}	3.58^{b}
Malathion	10.75^{b}	3.14^{a}	13.26^{d}	3.31^{a}	16.25^{b}	3.39^{b}	17.72^{b}	3.82^{a}	19.00^{a}	4.12^{a}
Indoxacarb	11.44^{a}	2.92^{b}	14.58^{b}	3.14^{a}	15.72^{c}	3.38^{b}	16.10^{d}	3.31^{c}	-	-

*Means of the same letter in a row do not differ significantly at 5% (Tukey), L=length, W=width, '-' = complete larval mortality.

Fig. 5. Measurement (mm) of BSF larvae from 2nd instar to the 6th instar (50 mag.)

During the third instar, larvae measure between 13.26 mm and 15.68 mm in length and 3.14 mm to 3.40 mm in width. Indoxacarb produced the smallest width, while malathion resulted in the shortest length during this stage. In the fourth instar, larval length increases to 15.55 mm to 17.73 mm, with a width range of 3.38 mm to 3.72 mm. In particular, profenofos and indoxacarb shows the smallest length and width, respectively, at this stage. The fifth instar larvae range from 16.10 mm to 18.63 mm in length and 3.31 mm to 3.88 mm in width, with indoxacarb producing the smallest size in both length and width. Finally, sixth instar larvae measure between 17.15 mm and 19.40 mm in length and 3.44 mm to 4.19 mm in width. With this, there was a significant difference in the size of the BSF larvae fed with treated cabbage (Table 2).

Moreover, raw cabbage has a protein and fat content of 1.1g and 0.1g, respectively while rice has 2.36g and 0.19g fat. These amount were assumed as the content of the treatments fed to the BSF larvae that affected its growth and development. According to the study of De Rijk *et al.* (2021), it was found that chlorpyrifos had no impact on the growth or survival of BSF larvae during the particular developmental stage, which was between 7 and 14 days of age, which corroborates with the result of the study.

Table 3. Weight (g) of BSF larvae fed with cabbage treated with organophosphate

Treatment	Initial weight (g)	Final weight (g)
Untreated (Control)	74.49^{a}	146.30^{a}
Profenofos	71.73^{a}	3.48^{b}
Malathion	75.30^{a}	128.83^{a}
Indoxacarb	74.80^{a}	0.00^{b}

*Means of the same letter in a row do not differ significantly at 5% (Tukey)

The initial weight of the BSF before feeding with treated cabbage had no significant differences, which means that the larvae has equal weight before feeding with organophosphate treated cabbage varying between 71.73g and 75.30g (Table 3). However, significant differences were observed in the final weight of BSF larvae, which ranged from zero to 146.30g when fed with the treated cabbage. The larva in the control has the highest final weight of 146.30g, followed by larvae with 128.83g and 3.48g when fed with cabbage treated with malathion and profenofos. Indoxacarb results in complete mortality of the BSF larvae, leading to zero weight.

Larvae reared on the different substrates do not differ significantly, with larval duration up to 23 days (Table 4). The untreated (control) has the longest duration, lasting 23 days, followed by larvae fed with cabbage treated with profenofos and malathion, each with a duration of 21 days, while indoxacarb exhibited the shortest duration, lasting only 16 days due to complete mortality of the larvae in the 6th instar, larvae exhibit increasing mortality rate starting from the 4th instar.

The larval duration of the BSF in the study is shorter than the observed duration in the studies that are not treated with organophosphate (OP). This was supported by the study of Bailey (2016) that observed 18 days and that of Pacleb (2021) that recorded a range of 38.89 to 46.96 days. According to De Rijk *et al.* (2021), OP can disrupt the normal growth cycle of BSF larvae, potentially prolonging or shortening its development depending on the concentration and exposure duration. Another possible reason is the variation of substrates used. Bailey utilized vegetable waste (lettuce and squash), fish (tilapia), and cafeteria scraps (pasta, rice, meat, and salad), resulting in a larval duration of 18 days. In contrast, Pacleb (2021) reared BSF using vegetable and fruit trimmings, chicken dung, and a mix of rice bran and kitchen waste, with larval duration ranging from 38.89 to 46.96 days. In this study, cabbage mixed with retrograded rice served as substrates. Cabbage contains 22.0g of calories, 1.10g of protein, 5.2g of carbohydrates and 0.1g of fat. Cooked rice contains 130 calories, 28.7g of carbohydrates (10% DV), 2.36g of protein (5% DV), and 0.19g of fat.

Table 4. Larval duration (days) of BSF larvae fed with treated cabbage with organophosphates

Treatments	Larval stages					Total number of days
	2nd	3rd	4th	5th	6th	
Untreated (Control)	3.00^b	5.00^b	4.00^b	4.00^b	6.75^a	23^a
Profenofos	3.50^b	3.50^b	3.25^b	3.50^b	5.50^a	21^a
Malathion	3.75^b	2.75^b	4.50^b	3.50^b	4.50^a	21^a
Indoxacarb	4.25^a	3.75^b	4.75^b	2.75^b	*	16^b

*Means of the same letter in a row do not differ significantly at 5% (Tukey)

As stated by Diener *et al.* (2019), larvae reared on the fruit and vegetable substrate, which has low protein content, had longer development time of 28 days, which contradicts with the result of the study.

Related to this, BSF larvae are stunted, have curved mouthparts, and have exhibited inactivity, specifically in indoxacarb. It contradicts with the study of Aidoo *et al.* (2023) that showed the accumulation of undesired substances (e.g. heavy metals, mycotoxins and pesticide) in BSF larvae bodies not affecting the biological parameters of BSF larvae.

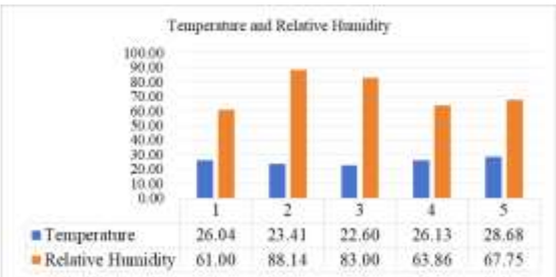

Fig. 6. Correlation of temperature and relative humidity from November 2024 to February 2025

Temperature may have also greatly influenced the shorter larval duration of BSF in the study, with a temperature range from 22.6 to 28.65 ºC and with a relative humidity between 61.0% and 88.14% (Fig. 6). In the study by Bailey (2016), the temperature ranged from 21.6°C to 32.94°C, with room humidity between 58.8% and 99%. Similarly, in the study by Pacleb (2021), the temperature ranged from 15.80°C to 16°C, with room humidity at 93.75%.

Table 5. Percent survival of BSF larvae fed with cabbage treated with organophosphate

Treatment	Survival (%)
Untreated (Control)	23.61^a
Profenofos	00.61^b
Malathion	30.81^a
Indoxacarb	00.00^b

*Means of the same letter in a row do not differ significantly at 5% (Tukey)

Furthermore, BSF larvae percent survival ranges from zero to 30.81(Table 5). The BSF larvae exhibited higher survival when exposed to malathion which is not significantly different to the untreated. Conversely, survival is significantly lower when exposed to profenofos and indoxacarb. According *to* Chatterjee *et al.* (2021), profenofos was toxic specifically for insects compared to mammals, which supports the findings of the study. Furthermore, Alsuhaibani *et al.* (2022) stated that indoxacarb had slight side effects on non-target insects, which contradicts on the result of the study. Based on the labels of toxicity. Whereas, for the various pesticides used, malathion (Malathion) has a green label that indicates low toxicity, indoxacarb (Bombrox) has a blue label that indicates moderate toxicity, and profenofos (Topkill) has a yellow label that indicates severe toxicity.

Table 6. Residue of organophosphate on treated cabbage, BSF larvae and frass

Treatment	Cabbage trimmings	BSF larvae	FRASS
Untreated (Control)	-	-	-
Profenofos	Low	-	-
Malathion	Low	-	-
Indoxacarb	Medium	-	-

Note: - (Negative): No residue detected

Organophosphate residue on cabbage, BSF larvae and frass

Moreover, organophosphate residue on cabbage was detected at a negative to medium level (Table 6). Also, residue was at medium level for indoxacarb and at low levels for profenofos and malathion (Fig. 7). Meanwhile, residue was not detected in both BSF larvae and their frass (Table 5). This implies that BSF larvae have the ability to degrade OP. According to the study of Liu *et al.* (2021) enzymes produced by the gut microorganisms of BSF larvae enabled the hydrolytic breakdown of organophosphate compounds. This process not only neutralizes toxic substances but also improves nutrient availability and enhances the larvae's growth and development. Furthermore, the absence of residue detection may be attributed to the pre-harvest interval (PHI) for all pesticides applied. Demeter Ghana Ltd. (2025) stated that the pesticide underwent natural breakdown processes during the PHI as influenced by sunlight, rainfall, and temperature.

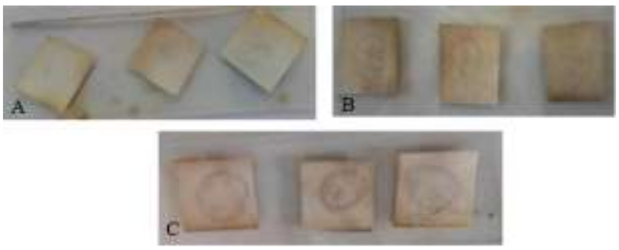

Fig. 7. Residue detected on treated cabbage; (A) Negative; (B) Low; (C) Medium

Protein of BSF larvae

The crude protein content of the larvae fed with cabbage treated with malathion is 15.46%, while the larvae fed with untreated cabbage is 15.39% (Table 7). This is lower compared to the crude protein content of BSF reported by Cheseto *et al.* (2020) which was 30.0 to 46.0% reared on agro-industrial by-products (e.g. dried brewers' spent grains, waste brewer's yeast and cane molasses).

Table 7. Crude protein of 6th larval instar of BSF fed with cabbage treated with organophosphate

Treatment	Crude protein (%)
Untreated (Control)	15.39
Profenofos	-
Malathion	15.46
Indoxacard	-

Note: all the BSF larvae were dead that no larvae were subjected to crude protein analysis

Due to the insufficient weight of larvae fed with profenofos-treated cabbage and the complete mortality of the BSF larvae fed with indoxacarb, crude protein analysis was not conducted for this treatment.

According to Spranghers *et al.* (2018), the ideal crude protein content of diet for BSF optimal growth is 5.4%. Raw cabbage has a protein content of 1.1g while rice has 2.36g. The substrates did not meet the protein requirement of BSF, probably causing the low protein content of the BSF larvae.

Lethal toxicity of organophosphates on BSF larvae

BSF larvae mortality ranged from 69.19 to 100.% (Table 8). Indoxacarb (100.0%) and profenofos (99.39%) cause higher mortality which are significantly different from the malathion (69.19%). In addition, the dead larvae (Fig. 8) are either white or black in appearance, characterized by an elastic cuticle and a soft body. This result indicates that malathion exhibits lower toxicity which is supported by the study of Newhart (2006).

Table 8. Mortality (%) of BSF larvae fed with treated cabbage with organophosphate

Treatment	Mortality(%)
Untreated (Control)	76.39[b]
Profenofos	99.39[a]
Malathion	69.19[b]
Indoxacarb	100.00[a]

*Means of the same letter in a row do not differ significantly at 5% (Tukey)

The mortality of the BSF larvae can be associated with the toxicity of the organophosphates applied, despite the larvae's low to medium residue levels on cabbage. Both malathion and profenofos showed low residue concentrations however, profenofos exhibited greater toxicity than malathion. According to Tomberlin *et al.* (2002), one potential health effect of pesticide accumulation in BSF larvae was the interference with their life cycle, which may result in increased mortality rates. On the other hand, the excessive moisture of the substrates influenced the mortality rate of the BSF larvae (Anukun *et al.,* 2024).

Fig. 8. Deceased BSF larvae treated fed with treated cabbage with organophosphte

CONCLUSION

The study conducted in Balili, La Trinidad, Benguet from November 2024 to February 2025 analyzed the impact of organophosphates (profenofos, malathion, indoxacarb) on black soldier fly (BSF) larvae. It examined growth, development, survival, and organophosphate residue in treated cabbage, larvae, and frass.

BSF larvae completed six instars in 23 days at temperatures of 22.60°C to 28.68°C and relative humidity of 61.00% to 88.14%. Survival rates varied between 0% and 30.81%, significantly dropping when exposed to profenofos and indoxacarb. Cabbage showed medium residues of indoxacarb and low for profenofos and malathion, while no residue was detected in larvae or frass. BSF larvae fed on malathion-treated cabbage had the highest crude protein content (15.46%), slightly higher than untreated cabbage (15.39%). Mortality rates varied from 69.19% to 100%, with indoxacarb causing the highest mortality.

Overall, malathion and profenofos did not significantly affect larval growth, but toxicity influenced survival. Low to medium residue levels in cabbage can be degraded by BSF larvae, yet protein content in substrates remained inadequate for optimal development.

RECCOMENDATION

Black Soldier Fly larvae fed with cabbage treated with organophosphate with low to medium residue can still complete its life cycle. Although the larvae has low protein content, they are still safe to be fed to other animals, since they have the ability to break down organophosphate residues.

ACKNOWLEDGEMENTS

I express my sincere thanks to Dr. Gemma S. Das-ilen for her valuable guidance. Also, I would like to express my utmost appreciation to the members of the advisory committee–Prof. Lucille M. Faroden, Ms. Nora Hill G. Evasco, Dr. Teresita K. Mangili and Prof. Anna Liza B. Wakat—for their insightful comments, suggestions, and constructive criticism, which greatly enhanced this study.

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