Predaceous Activity and Efficacy of Nematophagous Fungi (*Arthrobotrys oligospora*) on the Larva of Common Housefly (*Musca domestica*) In-Vitro

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ABSTRACT

The study determined the predaceous activity of different spore concentration of *Arthrobotrys oligospora* against larva of common housefly (*Musca domestica*), and the mode of action through microscopic examination at three (3) hours interval for 48 hours.

Synchronized reproduction of adult common housefly to produce similar stage of first larva instar was done. A total of 250 larvae were used in the study where 10 larvae were used for each treatment. There were five treatments; each treatment was replicated five times as follows: T_1- Malathion positive control), T_2- distilled water (negative control), T_3- 2000 spores of Nematophagous Fungi (NF), T_4- 4000 spores of NF, T_5- 6000 spores NF.

The study showed that nematophagous fungi at 4,000 and 6,000 spore levels were comparable to the commercially prepared larvicide (Malathion) in terms of its effects on percentage mortality starting 36 hours until 48 hours exposure. It was effective as biological control based on its the ability to trap and kill the larvae of the common housefly.

INTRODUCTION

The common housefly (*Musca domestica*) is a well- known cosmopolitan pest of both farm and home. These houseflies commonly develop in large numbers in poultry and livestock manure. The indirect damage produced by these insects are the potential transmission of pathogens like bacteria, viruses, protozoa, fungi and nematodes and these are serious problems requiring control.

Some control measures to manage the increase in population of houseflies include good sanitation, use of traps, integrated fly control and application of insecticides or chemical control. Chemical control is the most commonly used method because of their instant preparations and efficacy in controlling flies. With the increasing and a growing public concern about actual or potential problems associated with insecticides, interest in alternative housefly control strategies has increased, like using of biological control.

Biological control can be considered a promising alternative contributing to an increase in the efficacy of verminous control (Valencia *et al.*, 2008). Among the alternative biological controls which are now studied because of its effect on the control of nematodes are the nematophagous fungi.

Nematophagous fungi (*Arthrobotrys oligospora*) are micro fungi that can capture, kill

and digest nematodes (Faedo *et al.*, 1997) They are natural enemies of nematodes and comprise three main groups: the nematode trapping; the parasitic fungi that attack vermiform living nematodes by using specialized structure; and the egg and cyst parasitic fungi that attack these stages with their hyphal tips (Gray, 1985). Although the studies have concentrated on nematode control, this study hoped to find the same effects against the larval stages of the common housefly.

MATERIALS AND METHODS

Culture of Common Housefly Larva

To ensure fair and accurate result of the study particularly on the use of similar stage of *Musca domestica* larva, synchronized reproduction was done. Ten pairs (male and female) of *M. domestica* were collected and cultured in a container box covered with gauze pads and fed with meat and one (1) ml milk (soaked in cotton) to facilitate laying of eggs. The conduct of this study started when enough larvae were already produced.

Preparation of Culture Media

Potato Dextrose Agar (PDA) was used as culture media. Potato infusion was made by boiling 200 grams of sliced (washed but unpeeled) potatoes in one (1) liter distilled water for 30 minutes and then decanting or straining the broth through cheese cloth. Distilled water was added such that the total volume of the suspension was one (1) liter. Twenty grams dextrose and 20 grams agar powder were added. The medium was sterilized by autoclaving at 15 pounds per square inch (100 kPa) for 15 minutes.

Preparation of Culture Plates

Twenty five petri dishes were sterilized and used as culture plates. Each plate was filled with approximately 20 ml of PDA and was allowed to solidify.

Isolation and Culture of Nematophagous Fungi

The fungus *Arthrobothrys oligospora* was derived and cultured from fecal samples from sheep. The pure cultures were mass produced as nematophagous fungi for citrus decline. Mass production was done at the Nueva Vizcaya State University, Bayombong, Nueva Vizcaya. The pure culture was coded as NVSU 212-20012.

Two days before the start of the study, treatments with different concentrations of nematophagous fungi isolated from sheep feces were cultured first. This ensured that the nematophagous fungi would produce their mycelia before placing the larvae.

Neubauer was used to evaluate and count spores of nematophagous fungi for the different concentration Using serial dilution method, the approximate number of spores was achieved (a+b+c+d+e multiplied by 10,0000) and after applying the approximate number of spores, the culture plates were stored at room temperature to facilitate the growth of nematophagous fungi.

Application of Treatments

The nematopagous fungi spores for the different treatments were diluted with 2 ml distilled water. This amount was dropped at the center of media using a pipette. Positive and negative controls treatments were also applied during the start of the experiment. Ten (10) 1st instar larvae of the common houseflies were randomly distributed on each plate.

Experimental Treatment and Design

The experiment was laid out following the Completely Randomized Design (CRD) with five treatments each having five replications. The treatments were as follows:

T_1- positive control (Malathion)
T_2- negative control (distilled water)
T_3- 2,000 spores of nematophagous fungi
T_4- 4,000 spores nematophagous fungi
T_5- 6,000 spores of nematophagous fungi

Observation

Each treatment was observed every three (3) hours interval to check for mortality of larva. The number of dead larva in each treatment applied with NF was recorded and examined under the microscope in order to determine the mode of parasitism. Lethal $Dose_{50}$ was also observed in order to evaluate the efficacy of each treatment. Observation of larvae was done until they became adult houseflies. After seven to nine days, adult houseflies that emerged were sprayed with dichlorofention (Gusanex™) in order to count how many survived from their larval stage and how many died in each treatment. The efficacy of the nematophagous fungi was determined based on the number of houseflies that died.

Computation of Percent Mortality of houseflies was based on the following formula:

$$\text{Percent Mortality} = \frac{\text{Number of dead houseflies}}{\text{Total number of houseflies treatment}} \text{ X } 100$$

Data Gathered

The following data were gathered:

1. Number of dead houseflies
2. Number of live houseflies
3. Mode of parasitism
4. Time of death (3 hours interval)

Statistical Analysis

All data were properly recorded, tabulated and interpreted. The analysis of variance (ANOVA) in a Completely Randomized Design (CRD) was used. Tables with data ranging from zero to 30 and zero to 100 was transformed using *square root* and *Arc sign* transformations, respectively. The Least Significant Difference (LSD) was used to compare treatment means.

RESULTS AND DISCUSSION

Table 1 shows the percentage mortality of common housefly (*M. domestica*) larva as influenced by concentrations of nematophagous fungi (*A. oligospora*) at different times of exposure.

At 3-6 hours of exposure only treatment 1 (positive control - Malathion) showed an effect with 38% at 3 hours and 82% at 6 hours which was significantly different from all other treatments.

Results showed that at 9, 12, and 15 hours of exposure, treatment 1(positive control - Malathion) was significantly different from all the other treatments since all experimental units (larvae) were killed after 9 hours of exposure while in treatments 3, 4, and 5 percentage mortality ranged from 6-12% which were comparable with each other but significantly different from treatment 2 (negative control - distilled water) at 0%.

After 18 hours of exposure, treatment 1 (positive control - Malathion) was still significantly different among all the other treatments. In treatments 4 and 5, percent mortality were 38% and 40%, respectively which were comparable but significantly different from treatments 2 and 3, while treatment 3 is significantly different from treatment 2.

At 21, 24, 27, and 30 hours of exposure, treatment 1 (positive control – Malathion) still differed significantly compared to the other treatments. Treatments 3, 4, and 5 have 44% to 80% which were comparable with each other but significantly different with treatment 2 (negative control – distilled water).

After 33 hours of exposure, treatment 1 (positive control- Malathion) was significantly different among all the treatments. Treatment 5 (6,000 spores NF) with 90% mortality was significantly different from treatments 4, 3, and 2 with 78%, 82% and 0%. respectively.

At 36 hours of exposure, treatments 1 (positive control - Malathion) and treatment 5 (6,000 spores of NF) were comparable with

Table 1. Percentage mortality of common housefly (*M. domestica*) larva using different concentrations of nematophagous fungi (*A. oligospora*) after different times of exposure

Treatments	Mean													
	Time of Exposure (Hours)													
	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48
T_1- Malathion (positive control)	38a	82a	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a
T_2- Distilled water (negative control)	0b	0b	0c	0c	0c	0d	0c	0c	0c	0c	0d	0d	0c	0b	0b	0b
T_3- 2,000 spores of NF	0b	0b	6b	12b	26b	30c	44b	58b	64b	70b	78c	82c	92b	98a	98a	100a
T_4- 4,000 spores of NF	0b	0b	10b	16b	26b	38b	50b	64b	70b	76b	82c	90bc	98ab	100a	100a	100a
T_5- 6, 000 spores of NF	0b	0b	12b	16b	28b	40b	54b	66b	74b	80b	90b	96ab	98ab	100a	100a	100a
Level of Significance	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Coefficient of Variation (%)	29.05	21.45	29.30	10.36	12.50	8.97	10.25	9.80	11.36	11.44	12.10	15.69	11.02	5.17	5.17	0

*Legend: ** - Highly significant* *Note: Means with the same letter are not significantly different*

each other and were significantly different from treatments 4, 3, and 2. Treatments 4 and 5 were comparable with each other but significantly different from treatment 2 - distilled water (negative control).

After 39 hours of exposure, treatment 1(positive control - Malathion) was comparable with treatments 4 (4,000 spores of NF) and treatment 5 (6,000 spores of NF) but they weresignificantly different with treatments 3 and 2. Treatments 4, 5 and 3 were also comparable with each other but they were significantly different from treatment 2.

After 42 and 45 hours of exposure, it shows that treatment 1 (positive control-Malathion), treatment 3 (2,000 spores of NF), treatment 4 (4,000 spores of NF), and treatment 5 (6,000 spores of NF) were not significantly different with each other which means that they have comparable effects on the larvae of housefly. All the treatments were significantly different from treatment 2 (negative control - distilled water).

At 48 hours of exposure, results show that all treatments with nematophagous fungi were comparable with treatment 1 (positive control - Malathion).

Based on the results of the study, it was proven that nematophagous fungi are comparable with the commercially prepared larvicide (Malathion) after 36, 42, 45 and 48 hours exposure. Malathion however, caused mortality as early as 3 hours after exposure while mortality in nematophagous fungi occurred after nine hours of exposure.

The predaceous activity of this fungi on the larva of common housefly shows that the fungi using its specialized mycelia trapped the larva of common housefly which caused stress and inability of the larva to move and feed resulting to starvation until it died. Valencia *et al.,* (2008) cited that *A. oligospora* penetrates the cuticle of nematode with their thin hyphae which swells to become infection bulbs and this starts to undergo lysis.

The same mode of parasitism was observed in this study on the larva of common

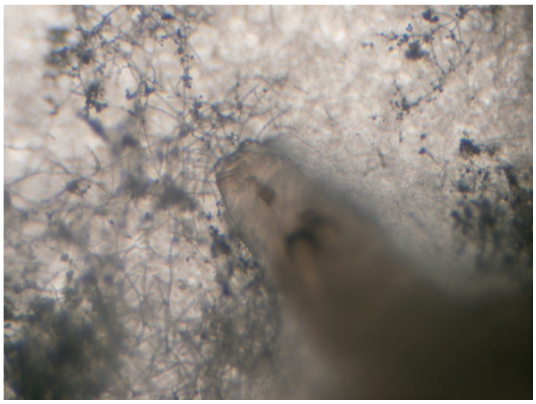

Plate 1. Microscopic examination showing entrapped larva with hyphae on its mouth part

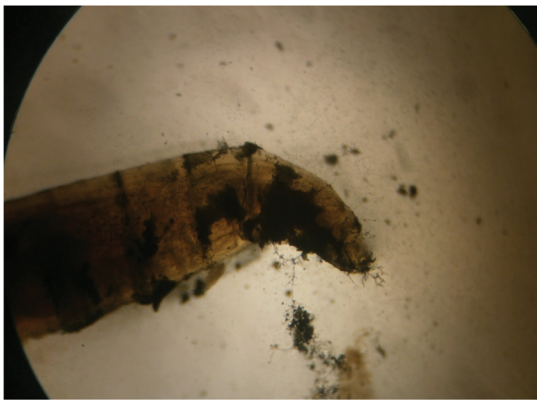

Plate 2. *Arthrobotrys oligospora* penetrates the mouthpart of larva and starts to undergo lysis

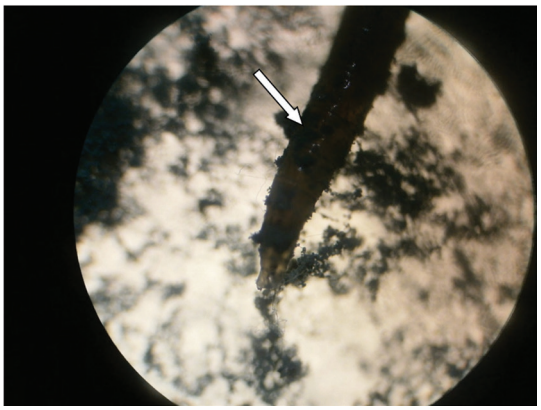

Plate 3. Entrapped larva with thin hyphae of nematophagous fungi. Lysis starts to be seen on its body (black color)

Plate 4. Microscopic examination showing an entrapped experimental sample of eggs of common housefly under 4x magnification

housefly and this was proven using microscopic examination of dead larva on 4x magnification (plates 1, 2 &3). One larva died (2%) in treatment 2 (negative control – distilled water) while 98 % emerged from their pupal stage until it became adult houseflies. Death of the larva in the negative control was attributed to ants that contaminated one petri dish.

Other Observation

Nematophagous fungi can also inhibit further development of common housefly egg. This was proven upon placing of egg on a plate with nematophagous fungi and entrapment using hyphae of NF was observed under the microscope (plate 4).

CONCLUSION AND RECOMMENDATIONS

Conclusion

Based on the result of the study, nematophagous fungi (at 2,000 to 6,000 spores) were comparable with commercially prepared larvicides (Malathion) because of their ability to trap and kill the larvae of the common housefly. The inability of the larva to move and eat allows the nematophagous fungi to penetrate its cuticle. Penetration causes

swelling of larva to become infection bulbs and this would cause the larva to undergo lysis.

Recommendations

Based on the result of the study, 4,000 - 6,000 spores of NF is recommended as alternative to commercially prepared insecticide because of its comparable effect against housefly larvae. It is also recommended that the next study would focus on the following: practical application of nematophagous fungi on chicken feces using alkalinizer to offset the acidity of the feces; conduct histopathological exam in evaluating larvicidal effect of nematophagous fungi; use other alternative culture medium for *A. oligospora* aside from Potato Dextrose Agar; and use other methods of application of treatments.

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