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RESEARCH PAPER

Metarhizium anisopliae Metsch. (Moniliales: Moniliaceae) and *Aglaia odorata* Lour. (Sapindales: Maliaceae) leaf extract: a potent biopesticide cocktail for cabbage leaf caterpillar management

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ABSTRACT

Plutella xylostella Linnaeus (Lepidoptera: Plutellidaae) is an important pest on Brassicaceae plants, especially cabbage. The combination of entomopathogenic fungi and botanical pesticides has been widely developed to increase effectiveness effectiveness against *P. xylostella*. This research aimed to determine the potential of *Metarhizium anisopliae* and *Aglaia odorata* Leaf Extract (*Ao*-LE) as a combined control strategy for *P. xylostella.* The parameters observed in this research were colony growth, viability of conidia of *M. anisopliae*, antifeedant activity, mortality, and lethal time 50 (LT_{50}) of *P. xylostella*. The findings revealed that *Ao*-LE addition maintained colony growth comparable to that of the control treatment, while significantly enhancing the conidial viability of *M. anisopliae*. Notably, 0.80% and 1.00% *Ao*-LE in the *M. anisopliae* suspension resulted in maximum antifeeding activity. Moreover, incorporating 0.20-1.00% *Ao*-LE demonstrably improved *P. xylostella* mortality compared with *M. anisopliae* alone. The combination of *M. anisopliae* 10^7 conidia/mL and 1.00% *Ao*-LE exhibited the lowest LT_{50} value of 2.16 days, highlighting the synergistic effect of the combined treatment. In conclusion, this study provides compelling evidence for the compatibility and synergistic potential of *M. anisopliae* and *Ao*-LE, presenting a promising strategy for the sustainable management of *P. xylostella* infestations.

Key words: chinese perfume plant, compatibility, entomopathogenic fungi, *Plutella xylostella*, synergistic

INTRODUCTION

Plutella xylostella Linnaeus (Lepidoptera: Plutellidae) wreaks havoc on Brassicaceae crops, particularly cabbage. This pest can cause heavy losses in cabbage fields, leading to lower market prices (Parajuli & Paudel, 2019). This necessitates the development of novel and sustainable control strategies to minimize the environmental impacts. Control can be carried out using a biocontrol agent in the form of entomopathogenic fungi or botanical pesticides. Both of these control methods are environmentally friendly (Maina et al., 2018). A combination of entomopathogenic fungi and botanical pesticides can increase their potency.

Metarhizium anisopliae (Moniliales: Moniliaceae) is an entomopathogenic fungus that can infect more than 200 pest species (Kulkarni, 2015). *M. anisopliae* produces a toxin called destruxin, which

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can disrupt the insect's midgut, hemocytes, Malpighian tubules, and muscle tissue, leading to mortality. The use of *M. anisopliae* suspension at a concentration of 10^8 conidia/mL can cause mortality in *P. xylostella* larvae of the fifth instar of over 50%. Tunaz et al. (2019) reported that a suspension of 10^7 conidia/mL causes mortality in *Spodoptera littoralis* fifth instar larvae of 63%.

Aglaia odorata Lour. (Sapindales: Meliaceae) is a plant native to Southeast Asia that contains active ingredients such as alkaloids, saponins, flavonoids, and essential oils, which have potential as botanical pesticides (Efri et al., 2017; Syamsul et al., 2016). The leaves of this plant posses insecticidal compounds of the benzofuran group derived from rocaglamide (Roc), including rocaglamide (A; I; W: AB; J; S; AY), which have an antifeedant effect (Duong et al., 2014). These insecticidal compounds have activity against chewing pests, disrupting larval development (Hall et al., 2017), and include odorin and odorinol pyrrolidine alkaloids that are cytotoxic (Ariani et al., 2019). A concentration of 0.75–1.00% of the *A. odorata* compound extract can cause over 50.0% mortality in *Riptortus linearis* nymphs (Koswanudin, 2011). Iqbal et al. (2015) reinforced this statement by stating that *A. odorata* extract has high effectiveness against *P. xylostella* larvae, and the higher the concentration of the extract, the higher its toxicity.

The combined use of entomopathogenic fungi and botanical insecticides has been widely developed in recent years. These combinations have been proposed as promising strategies for pest control because of their potential synergistic effects (Usha et al., 2014). However, compatibility between the two agents is crucial for achieving synergistic outcomes (Nawaz et al., 2022). Many studies have been conducted on compatibility (Ali et al., 2018; Fernández-Grandon et al., 2020; Jarlina et al., 2015; Nana et al., 2016; Wisuda et al., 2019). Although the individual efficacy of *M. anisopliae* and *Ao*-LE against *P. xylostella* has been reported, their combined effect and compatibility remain unexplored.

Based on the above problems, this research is expected to provide valuable insights into the potential of *M. anisopliae* and *Ao*-LE as a combined control strategy for *P. xylostella*. These findings will contribute to the development of environmentally sustainable pest management practices for Brassicaceae.

MATERIALS AND METHODS

Research Site. The research was carried out at the Laboratory of Plant Pests, Department of Plant Protection, Faculty of Agriculture, University of Jember. The research lasted from May to September 2022.

Rearing of *P. xylostella*. *P. xylostella* were obtained from the Indonesian Institute of Sugar and Fibre Research (BALITTAS), Malang, Indonesia. The larvae were reared in 8 × 10 cm plastic jars covered with chiffon fabric and fed with organic cabbage that had been cleaned (Sunarti, 2017). The jars were cleaned, and the larvae were fed once daily. The larvae that transformed into pupae were transferred to another plastic jar. Adults were reared on a diet of honey diluted with water (10% concentration) applied to cotton wool, and cabbage was provided for egg-laying. Eggs (F2 generation) were transferred to different jars until they hatched into larvae. Cabbage for the larval diet was arranged in jars in a sandwich-like position, with larvae placed in between. This procedure was carried out daily and repeated until pupae formed.

Preparation of *M. anisopliae* Fungi. *M. anisopliae* fungi were obtained from the collection of the Laboratory of Plant Pest Control Technology, Department of Plant Protection, University Jember. The renewal of *M. anisopliae* isolates was carried out by inoculating *Tenebrio molitor* larvae. The infected *T. molitor* was cautiously taken and inoculated on PDA (Potato Dextrose Agar) media and incubated for 14 days at 23–25 ºC. The *M. anisopliae* fungi that grew were mass-propagated on corn rice media. The medium was steamed until it was half-cooked (±15 min). The corn rice was then placed in a heat-resistant plastic bag (250 g) and sterilized using an autoclave. *M. anisopliae* cultures were inoculated into the corn rice medium and sealed with a stapler. The cultures were incubated at room temperature (23–25 ºC) for 14 days. The spore density of *M. anisopliae* was counted using the serial dilution method in a test tube containing sterile dH_2O combined with 0.01% Tween-80. A Neubauer improved hemocytometer was used to count under a microscope until a concentration of 10^7 conidia/mL was achieved.

Preparation of *A. odorata* Leaf Extract (*Ao*-LE). *A. odorata* leaves were collected from Sumbersari, Jember, East Java (-8º9'48''S 113º42'58'E), weighing 2 kg. The leaves were then air-dried for approximately 3–4 days to reduce their water content. Dried leaf samples were ground using a blender and filtered through a sieve. The extraction process was carried

out by soaking the *A. odorata* leaf powder in 96% ethanol at a ratio of 1:5. A solvent of 0.01% Tween 80 was added as a solvent and mixed until the solution became smooth. Maceration was carried out for 3 days, with stirring performed six times for 5 min each time. After 3 days, the mixture was filtered using filter paper to obtain the maceration. The maceration was concentrated using a rotary evaporator (HAHNVAPOR HS-2005V-N, HAHSHIN S&T CO., LTD, Republic of Korea) at a temperature of 40 ºC to obtain a thick extract, which was then stored in a refrigerator 4 ºC until it was time to be used for testing.

Effect of Colony Growth and Viability *M. anisopliae* by Adding *Ao*-LE. This test was performed using the media poisoning technique with SDA media combined with concentrations of *Ao*-LE. The treatments used were: A = SDA; B = SDA + 0.2% *Ao*-LE (10 mL SDA + 20 µL *Ao*-LE); C = SDA + 0.40% *Ao*-LE (10 mL SDA + 40 µL *Ao*-LE); D = SDA + 0.60% *Ao*-LE (10 mL SDA + 60 µL *Ao*-LE); E = SDA + 0.80% *Ao*-LE (10 mL SDA + 80 µL *Ao*-LE); F = SDA + 1.00% *Ao*-LE (10 mL of SDA + 100 µL of *Ao*-LE). A Completely Randomized Design (CRD) was employed for this two-stage study. The first stage investigated the effect of *Ao*-LE addition on colony growth and conidia viability of *M. anisopliae*. This stage utilized six treatments with four replicates each (n=24).

The colony growth test was performed by inoculating *M. anisopliae* with a cork borer (diameter 5 mm), placing it in the center of the dish (90 mm), and incubated it at room temperature. Colony diameter data were obtained from the average of two measurements (vertical and horizontal) of the fungal colonies. Growth colonies were observed every three days, starting from day 3 (H+3) to day 15 (H+15) of inoculation (Jarlina et al., 2015). The colony diameter was calculated using the following formula:

$$D = \frac{d_1 + d_2}{2}$$

D = Colony diameter of *M. anisopliae;*
d_1 = Vertical diameter of *M. anisopliae* colony;
d_2 = Horizontal diameter of *M. anisopliae* colony.

The viability was tested by inoculating *M. anisopliae* at a concentration of 10^7 conidia/mL onto slide glass, which was added to SDA media with the same concentration of *Ao*-LE treatment and incubated at room temperature in a closed plastic container. Viability data were obtained under a microscope; if conidia had germinated, hyphae were formed (Jarlina et al., 2015). The viability of conidia and the percentage decrease in conidial viability were observed every 12 h for 48 h according to Trizelia & Rusli (2012) using the following formula:

The viability of conidia

$$V = \frac{v_1}{v_2} \times 100\%$$

V = Viability of conidia;
v_1 = Number of germinated conidia;
v_2 = Total number of conidia observed.

The percentage decrease in conidial viability

$$Mr = \frac{M_1 - M_2}{M_1} \times 100\%$$

Mr = Percentage decrease in conidial viability;
M_1 = Viability of conidia on control media;
M_2 = Viability of conidia on treatment media.

Effect Compatibility of *M. anisopliae* and *Ao*-LE Against *P. xylostella* Larvae. This test was carried out using F2 larvae of *P. xylostella* third instar. Larvae were starved for 2–3 h before use. The test was conducted using the contact method by dipping (once) the larvae into the treatment *Ao*-LE for 5 s, then placing them in a plastic jar filled with cabbage leaves as food. The treatments for this test were: A = sterile dH_2O; B = *M. anisopliae* 10^7 conidia/mL + 0.20% Ao-LE; C = *M. anisopliae* 10^7 conidia/mL + 0.40% Ao-LE; D = *M. anisopliae* 10^7 conidia/mL + 0.60% Ao-LE; E = *M. anisopliae* 10^7 conidia/mL + 0.80%; F = *M. anisopliae* 10^7 conidia/mL + 1.00% *Ao*-LE; G = *M. anisopliae* 10^7 conidia/mL; H = 1.00% *Ao*-LE. Each replicate contained 10 larvae A Completely Randomized Design (CRD) was employed for this two-stage study. The second stage evaluated the compatibility of *M. anisopliae* and Ao-LE against *P. xylostella* larvae, employing eight treatments with three replicates each (n=24). In this test, the variables observed were larval mortality, antifeedant activity, and lethal time 50 (LT_{50}). Mortality observations were conducted every day for 7 days. Antifeedant observation was conducted by measuring the leaf weight before and after application every day for 7 days. The LT_{50} value was calculated to determine the time required for each treatment to kill 50% of test larvae.

Mortality of larvae

$$M = \frac{a}{b} \times 100\%$$

M = Percentage of larval mortality;

a = Number of dead larvae;
b = Total number of larvae.

Antifeedant activity

$$PA = \frac{(Bk - Bp)}{Bk} \times 100\%$$

PA = Percentage of antifeedant activity;
Bk = Weight of control leaf;
Bp = Weight of treated leaf.

Data Analysis. The data obtained were analyzed using Analysis of Variance (ANOVA) with Duncan's multiple range test (DMRT) at a significance level of 5%. The LT_{50} value was calculated using probit analysis (Microsoft Excel and SPSS V25; IBM SPSS Statistics, Chicago, IL, USA).

RESULTS AND DISCUSSION

Growth of *M. anisopliae*. The addition of *Ao*-LE to SDA medium had varying effects on the growth of *M. anisopliae* colonies. At 6 days after application (DAA), the colonies grew more slowly compared to the control treatment. At 15 DAA, colony growth was further inhibited with increasing concentration of *Ao*-LE. Although there were differences in the average growth of *M. anisopliae* colonies observed from 9 to 15 DAA across treatments, no significant differences were found based on statistical calculations (Table 1). These results are consistent with those of Nana et al. (2016), who showed that the addition of 1–10% *Calpurnia aurea* leaf extract to SDA medium negatively effected the diameter of *M. anisopliae* colonies, although the results were not significantly different between treatments.

The best growth in colony diameter was observed in the control treatment (Figure 1), where the fungus grew in SDA medium mixed with distilled water as usual. The value decreased with increasing *Ao*-LE concentration. However, no significant differences were observed between the treatment and control groups. This is because *Ao*-LE was administered at a low concentration, which did not affect or suppress the growth of *M. anisopliae* colonies. This finding is consistent with Ribeiro et al. (2012), who stated that high concentrations of secondary metabolites

Table 1. Effect of *Ao*-LE addition on *M. anisopliae* colony growth for 15 DAA

Treatment	Colony Growth (cm)				
	3 DAA	6 DAA	9 DAA	12 DAA	15 DAA
A (SDA)	1.79 c	3.08 b	4.16 *	4.86 *	5.23 *
B (SDA + 0.20% *Ao*-LE)	1.76 c	3.05 b	4.10 *	4.66 *	5.20 *
C (SDA + 0.40% *Ao*-LE)	1.19 ab	2.03 ab	3.06 *	4.38 *	5.21 *
D (SDA + 0.60% *Ao*-LE)	1.14 a	1.94 a	2.99 *	4.25 *	4.96 *
E (SDA + 0.80% *Ao*-LE)	1.54 bc	2.56 ab	3.45 *	4.24 *	4.89 *
F (SDA + 1.00% *Ao*-LE)	1.55 bc	2.68 ab	3.71 *	4.19 *	4.54 *

*= not significantly different. Numbers followed by different letters in the same column indicate significantly different results in the 5% DMRT test. DAA = days after application.

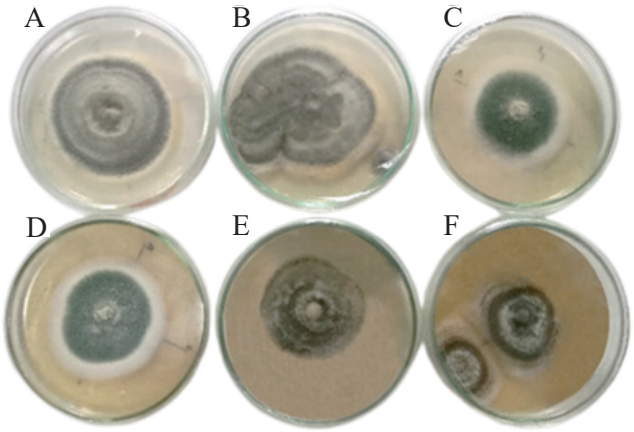

Figure 1. Colony growth of *M. anisopliae* at 15 DAA. A. SDA (control); B. SDA + 0.20% *Ao*-LE; C. SDA + 0.40% *Ao*-LE; D. SDA + 0.60% *Ao*-LE; E. SDA + 0.80% *Ao*-LE; F. SDA + 1.00% *Ao*-LE.

in plant extracts can affect their compatibility with entomopathogenic fungi.

This suggests that *Ao*-LE inhibits *M. anisopliae* growth. The active compounds in *Ao*-LE, such as alkaloids, tannins, and essential oils, can damage cell membranes, cell walls, and enzymes, leading to cell lysis and death. This is consistent with Efri et al. (2017), who state that alkaloids can damage cell membranes through protein denaturation, while tannins damage fungal cell walls through interaction with lipids and amino acids. When tannins penetrate the cell nucleus, the cell will lyse and die. Essential oils affect cell permeability and enzyme response, while saponins and flavonoids can disrupt cell membrane function, causing cells to lyse and die (Li et al., 2022).

Viability of *M. anisopliae* Conidia. The addition of *Ao*-LE to SDA medium had varying effects on the germination or viability of *M. anisopliae* (Figure 2). At 12 hours after application (HAA), the viability percentage of *M. anisopliae* conidia was lower than in the control treatment. The control treatment produced the highest viability percentage, with a value of 62.50%. At 48 HAA, the viability of *M. anisopliae* conidia was higher than 85.00%. Additionally, the germination rate of *M. anisopliae* control reached a maximum value of 100% (Table 2). This indicates that the addition of *Ao*-LE at this concentration did not affect the germination rate of *M. anisopliae* conidia.

Similar results were reported by Hussain & AlJabr (2020), who found that the addition of plant secondary metabolites (1-Chlorooctadecane) in the range of 0.80–4.00 mg/mL did not affect the germination rate of *M. anisopliae*, even producing a very high germination percentage (>96%). According to SNI 8027.2 (2014), conidial viability is considered good if it germinates at 85–100%, medium if it germinates at 70–85%, and poor if it germinates at 55–70%. The higher the viability of entomopathogenic fungal conidia, the higher the pathogenicity of the fungus. The viability of entomopathogenic fungal conidia is the initial stage of the growth phase of the fungus before penetration into the integument of the target insect, and it affects the success of the fungus growing on the body of the target insect (Khairunnisa et al., 2014).

Antifeedant Activity of *P. xylostella*. The weight of food consumed by larvae was calculated as the difference between the weight of food before and after (Table 3). At the 1–4 DAA observation, treatment H produced the lowest food consumption weight, followed by the 5 DAA observation, where treatments C, D, E, and F also produced the lowest weights. The lower the weight of food consumed by larvae,

Table 2. Effect of *Ao*-LE addition on the viability of *M. anisopliae* conidia for 48 HAA

Treatment	Conidia viability (%)		Decline percentage (%)	
	24 HAA	48 HAA	24 HAA	48 HAA
A (SDA)	62.50 b	100.00 *	0.00 b	0.00 *
B (SDA + 0.20% *Ao*-LE)	52.50 ab	98.75 *	16.00 ab	1.25 *
C (SDA + 0.40% *Ao*-LE)	51.25 ab	97.50 *	18.00 ab	2.50 *
D (SDA + 0.60% *Ao*-LE)	52.50 ab	97.50 *	16.00 ab	2.50 *
E (SDA + 0.80% *Ao*-LE)	48.75 a	95.00 *	22.00 a	5.00 *
F (SDA + 1.00% *Ao*-LE)	47.50 a	93.75 *	24.00 a	6.25 *

*= not significantly different. Numbers followed by different letters in the same column indicate significantly different results in the 5% DMRT test. HAA = hours after application.

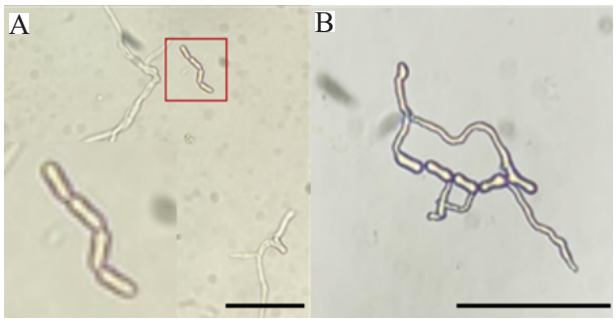

Figure 2. *M. anisopliae* conidia under a microscope (400×; scale bar: 50 µm). A. Conidia not germinate; B. Germinating conidia.

the higher the percentage inhibition of larval feeding activity (Figure 3). The higher the addition of *Ao*-LE to the *M. anisopliae* 10^7 conidia/mL suspension, the greater the antifeedant effect produced. At 5 DAA, treatments E and F showed the highest feeding activity. At the 6–7 DAA observation, the control treatment entered the pupal stage (not counted).

The secondary metabolites of *Ao*-LE act as stomach poisons and enter the insect body along with food. These toxins inhibit electron transport in the mitochondria, thereby inhibiting the formation of energy from food (Ariani et al., 2019). According to Zaynab et al. (2021), saponin compounds are classified into two major groups, steroidal and terpenoid saponins. These compounds contain toxins that decrease the activity of digestive enzymes and food absorption in insects, affecting feeding behavior and leading to death. Additionally, the rocaglamide compound is known to have antifeedant and growth-inhibitory properties for insects (Negi et al., 2016).

The disturbed feeding activity of larvae can lead to a decrease in the supply of necessary nutrients, thus inhibiting metabolism and other vital activities, potentially causing larval death. A higher antifeedant percentage indicates a greater reduction in insect feeding rate. Treatment with *M. anisopliae* at 10^7 conidia/mL can also inhibit larval feeding activity. This is due to the growth of fungal hyphae within the larval body cells, which absorb the larval body fluids for development. This aligns with Gustiana et al. (2019), who also stated that the feeding activity of *P. xylostella* larvae decrease due to infection by *M. anisopliae* as a biological control agent.

Mortality of *P. xylostella*. The mortality of *P. xylostella* larvae was observed over 7 days using a contact method,

Table 3. Effect of compatibility *M. anisopliae* and *Ao*-LE on the weight of food consumed by *P. xylostella* larvae

Treatment	Feed consumption weight (g)						
	1 DAA	2 DAA	3 DAA	4 DAA	5 DAA	6 DAA	7 DAA
A (sterile dH_2O)	1.30 *	1.58 c	1.17 c	0.68 d	0.41 c	0.00 a	0.00 a
B (Metar 10^7 + 0.20%)	1.21 *	1.06 ab	0.72 ab	0.30 bc	0.12 ab	0.00 a	0.00 a
C (Metar 10^7 + 0.40%)	1.22 *	1.04 ab	0.57 a	0.17 ab	0.10 a	0.00 a	0.00 a
D (Metar 10^7 + 0.60%)	1.11 *	0.78 a	0.48 a	0.15 ab	0.04 a	0.00 a	0.00 a
E (Metar 10^7 + 0.80%)	1.09 *	0.73 a	0.46 a	0.14 ab	0.00 a	0.00 a	0.00 a
F (Metar 10^7 + 1.00%)	0.90 *	0.63 a	0.42 a	0.09 ab	0.00 a	0.00 a	0.00 a
G (Metar 10^7)	1.23 *	1.42 bc	1.01 bc	0.46 cd	0.30 b	0.18 b	0.14 b
H (1.00% *Ao*-LE)	0.82 *	0.83 a	0.21 a	0.05 a	0.04 ab	0.00 a	0.00 a

*= not significantly different. Numbers followed by different letters in the same column indicate significantly different results in the 5% DMRT test. DAA = days after application.

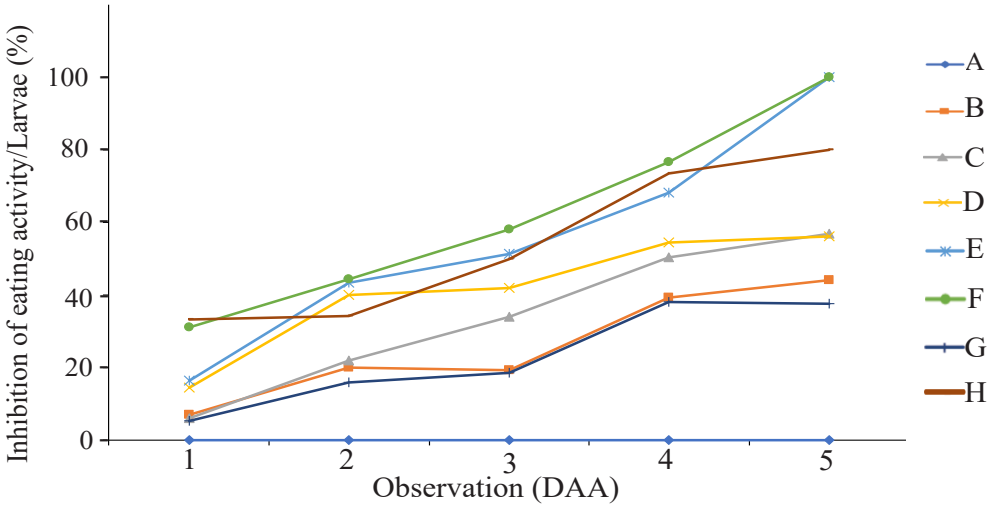

Figure 3. Inhibition of feeding activity of *P. xylostella* larvae for 5 days. A. sterile dH_2O; B. Metar 10^7 + 0.20%; C. Metar 10^7 + 0.40%; D. Metar 10^7 + 0.60%; E. Metar 10^7 + 0.80%; F. Metar 10^7 + 1.00%; G. Metar 10^7; H. 1.00% *Ao*-LE.

where the insects were dipped in the treatments. The treatments involving *M. anisopliae* at 10^7 conidia/mL, *Ao*-LE, and their combinations produced varied data on the mortality of *P. xylostella* third instar larvae (Table 4). By the 7th day of observation, all combination treatments achieved a maximum mortality rate of 100.00%, which did not significantly differ from the control treatment A (0.00%) and H (*M. anisopliae* 10^7 conidia/mL, 73.33%). The mortality rate increased with higher *Ao*-LE concentrations, likely due to a synergistic effect between *M. anisopliae* and *Ao*-LE.

The symptoms of death of *P. xylostella* larvae due to *M. anisopliae* and its combination with *Ao*-LE resemble tthose observed with single *M. anisopliae* treatment (Figure 4). This includes the growth of white fungal mycelium that envelops the larvae's body, which later turns green as the larvae mummify. In contrast, the symptoms of larvae death due to *Ao*-LE include a change in skin color to dark or black, followed by larval shrinkage and stiffening.

According to Fernández-Grandon et al. (2020), the bimodal effect refers to the occurrence of multiple infection mechanisms from the combination treatment of entomopathogenic fungi and plant extracts. The metabolism of the larvae's body is disrupted by *Ao*-LE entering the body, making the pest individuals more susceptible to infection by *M. anisopliae*. Aw & Hue (2017) reported that *M. anisopliae* infection begins with the attachment of conidia to the epicuticle. To germinate, the fungus utilizes compounds found in the host integument. The conidia then differentiate into appressoria, which exert mechanical pressure and produce toxins or enzymes that degrade the cuticle, allowing *M. anisopliae* to penetrate and utilize nutrients in the hemocoel of the host larva. The rocaglamide compound contained in *Ao*-LE can cause cytotoxic effects on cells of the digestive system, while the destruxin compound secreted by *M. anisopliae* also disturbs the digestive system (midgut). This can result in severe damage to the larvae's digestive system due to the synergistic effect of both compounds (Reddy & Chowdary, 2021).

This study showed that the larval mortality percentage due to the addition of *Ao*-LE to the *M.*

Table 4. Mortality of *P. xylostella* larvae

Treatment	Mortality (%)						
	1 DAA	2 DAA	3 DAA	4 DAA	5 DAA	6 DAA	7 DAA
A (sterile dH_2O)	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
B (Metar 10^7 + 0.20%)	0.00 a	20.00 b	46.67 c	50.00 c	76.67 cd	93.33 c	100.00 c
C (Metar 10^7 + 0.40%)	0.00 a	20.00 b	43.33 c	63.33 cd	83.33 d	96.67 c	100.00 c
D (Metar 10^7 + 0.60%)	0.00 a	23.33 b	56.67 c	70.00 cd	90.00 d	100.00 c	100.00 c
E (Metar 10^7 + 0.80%)	0.00 a	23.33 b	53.33 c	76.67 d	93.33 d	100.00 c	100.00 c
F (Metar 10^7 + 1.00%)	0.00 a	33.33 b	56.67 c	86.67 e	96.67 d	100.00 c	100.00 c
G (Metar 10^7)	0.00 a	0.00 a	16.67 b	30.00 b	43.33 c	60.00 b	73.33 b
H (1.00% *Ao*-LE)	6.67 b	26.27 b	43.33 c	73.33 cd	93.33 b	100.00 c	100.00 c

Numbers followed by different letters in the same column indicate significantly different results in the 5% DMRT test.

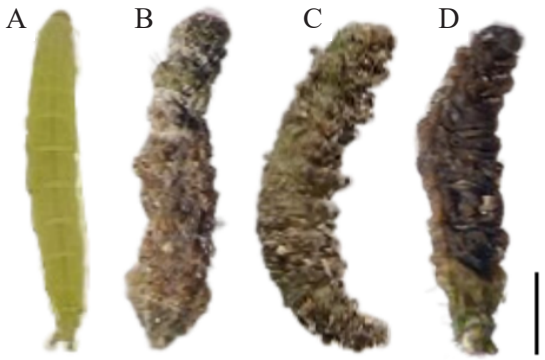

Figure 4. Symptoms of larval death at 7 DAA (Scale bar: 2 mm). (A) Healthy *P. xylostella* larvae; (B) *P. xylostella* larvae die due to the combination; (C) *P. xylostella* larvae die from *M. anisopliae* 10^7 conidia/mL; (d) *P. xylostella* larvae died due to 1% *Ao*-LE.

anisopliae suspension is quite high, despite *Ao*-LE affecting the growth and viability of *M. anisopliae*. Besides the bimodal effect, this high mortality was attributed to the isolate's high virulence against the test insect. According to Trizelia et al. (2018), the origin of the isolate also influences insect mortality due to variations in enzyme production during the infection process. Additionally, the relatively low concentration of *Ao*-LE administered did not significantly impact *M. anisopliae*'s ability to infect the test insect. This aligns with Ribeiro et al. (2012) assertion that the compatibility of entomopathogenic fungi with plant extracts depends on the secondary metabolite composition, which can negatively affect the fungus.

In general, the behavioral changes observed in the test larvae after treatment include slowed movement, remaining still when touched, and decreased appetite until they stop eating and eventually die. Similar observations were reported by Masyitah et al. (2017), who note that larvae attacked by entomopathogenic fungi exhibit reduced appetite, slow movement, silence, and subsequent mortality. Visually, the symptoms of death in *P. xylostella* larvae include the growth of white *M. anisopliae* fungal mycelium covering the body, which later turns green. Typically, dead larvae have hardened bodies and undergo mummification as their body fluids are consumed by the fungus (Khairunnisa et al., 2014).

The symptoms of larvae death due to *Ao*-LE include a darkening or blackening of the skin, with the body becoming increasingly shriveled and stiff. This is consistent with Ariani et al. (2019), who reported that insecticides derived from *A. odorata* can cause larvae to become shriveled and stiff, unresponsive to touch, and with darkened skin color.

Lethal Time 50 (LT_{50}). The toxicity of the combination of *M. anisopliae* and *Ao*-LE was assessed by calculating the lethal time (LT_{50}), which is the time required to kill 50% of the tested insects. Based on the regression equation of the LT_{50} value, there is a positive correlation between the log of time (days) and the probit value of mortality (Table 5).

The LT_{50} value indicate that higher concentration of *Ao*-LE added to *M. anisopliae at* 10^7 conidia/mL result in lower LT_{50} value, and vice versa. The lowest LT_{50} value was observed in treatment F (*M. anisopliae* 10^7 conidia/mL + 1% *Ao*-LE) at 2.16 days, while the highest LT_{50} value was found in treatment G (*M. anisopliae* 10^7 conidia/mL) at 5.03 days. The higher LT_{50} value in treatment G is attributed to the time required for *M. anisopliae* to infect the test insects, with larval mortality observed starting from 2 DAA. This finding aligns with Masyitah et al.'s (2017) statement that entomopathogenic fungi require time for conidia adhering to the larval cuticle to germinate and form hyphae before penetrating the cuticle and causing mortality.

Table 6 illustrates the relationship between the weight of *P. xylostella* larvae consumption and their mortality. A negative correlation index was obtained, indicating that lower larval food consumption correlates with higher mortality of *P. xylostella* larvae. Similar correlations were reported by Hidayati et al. (2013), who observed that lreduced feeding activity leads to increased mortality in *P. xylostella* larvae. The

Table 5. LT_{50} value of compatibility of *M. anisopliae* and *Ao*-LE against *P. xylostella* larvae

Treatment	LT_{50} (day)	Limit Intervals (day)	Regression Equations	R2	Slope	SE Slope
B (Metar 10^7 + 0.20% *Ao*-LE)	2.68	1.69 – 4.26	y = 8.1493x + 0.6075	0.9230	1.96	1.02
C (Metar 10^7 + 0.40% *Ao*-LE)	2.70	1.95 – 3.72	y = 8.3641x + 0.5786	0.9398	2.78	1.03
D (Metar 10^7 + 0.60% *Ao*-LE)	2.27	1.43 – 3.59	y = 9.0879x + 0.5488	0.9512	2.49	1.22
E (Metar 10^7 + 0.80% *Ao*-LE)	2.38	1.73 – 3.30	y = 9.1939x + 0.5406	0.9614	3.25	1.27
F (Metar 10^7 + 1.00% *Ao*-LE)	2.16	1.67 – 2.79	y = 9.2196x + 0.6851	0.9576	4.55	1.26
G (Metar 10^7)	5.03	4.14 – 6.11	y = 7.5874x - 0.5537	0.8711	2.98	1.30
H (1.00% *Ao*-LE)	2.84	2.45 – 3.29	y = 5.6682x + 2.8697	0.8740	4.05	0.70

Table 6. Correlation between larval feed weight and mortality

Character	Larval feed weight	Mortality
Larval feed weight	1	
Mortality	-0.991**	1

**= very significantly correlated ($p < 0.01$); r table: 0.917.

stomach poison extract compounds enter the digestive system of insect pests through their food, disrupting metabolism, causing feeding disorders, depriving larvae of necessary nutrients, and ultimately leading to death.

CONCLUSION

The results of this study demonstrate that the combination of *M. anisopliae* and *Ao*-LE is both compatible and synergistic. The addition of *Ao*-LE to *M. anisopliae* suspension increased the mortality of *P. xylostella* larvae, with the lowest LT_{50} value observed at 2.16 days after treatment with *M. anisopliae at* 10^7 conidia/mL + 1% *Ao*-LE. Therefore, the combination of *M. anisopliae* and *Ao*-LE shows promise as a biological control agent for *P. xylostella.* The synergistic effects of toxins produced by *M. anisopliae* and *Ao*-LE contribute to the increase mortality of *P. xylostella* larvae.

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AUTHORS' CONTRIBUTIONS

SP and WKW considered as research concept and design. SP and WKW completed the entire fieldwork. APP, and AW perform data collection and/or assembly of data. HSA and ZFNH performed data analysis and interpretation. SP, APP, AW, and HSA performed critical revision of the article. SP, WKW and ZFNH wrote the manuscript. SP, APP, AW, and HSA approved the final manuscript. The authors have provided responses and comments on the research flow, data analysis, and interpretation, as well as the shape of the manuscript. All authors have read and approved the final manuscript.

COMPETING INTEREST

We declare no competing interests, such as financial or non-financial interests, or professional or personal relationships that are directly or indirectly connected to the work submitted for publication.

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