

Biopesticide Formulation Of Bsf Pupa Extract For *Phytophthora* Control In Tomato

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Abstract: *Phytophthora* is a major pathogen that causes serious damage to tomato plants, resulting in reduced yields. The use of biopesticides as an environmentally friendly alternative continues to be developed, but improvements in their effectiveness are still needed. BSF maggot pupae (*Hermetia illucens*) are known to contain bioactive compounds that have the potential as antimicrobial agents. This study aims to characterize the bioactive compounds in a biopesticide formulation enriched with BSF maggot pupae extract and to evaluate their effectiveness and optimal concentration in inhibiting *Phytophthora* growth in vitro and in vivo. The experiment consisted of six treatments: 10% biopesticide without extract and the addition of maggot pupae extract at concentrations of 2.5%, 5%, 7.5%, 10%, and 12.5%. In vitro tests were conducted by measuring the diameter of fungal colonies on PDA media, while in vivo tests were conducted on tomato plants to observe the incidence and severity of the disease. Data were analyzed using homogeneity tests and one-way ANOVA, followed by the BNJ test at a 5% confidence level. The results showed that the addition of BSF maggot pupae extract significantly inhibited *Phytophthora* growth. A concentration of 7.5% provided optimal inhibition of colony diameter and reduced disease severity, while a concentration of 12.5% was most effective in suppressing disease incidence. Therefore, BSF maggot pupae extract has the potential to be developed as an effective and environmentally friendly biopesticide additive for controlling *Phytophthora* in tomato plants.

Keywords: *Phytophthora* spp., Pupa maggot (*Hermetia illucens*.), Biopesticide, In vitro, In vivo, Bioactive compound.

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I. INTRODUCTION

Tomatoes are a horticultural commodity with high economic value and are widely used as a food and industrial ingredient (Ziladi et al., 2021). In Indonesia, tomato production has shown significant growth, with West Java being the largest producing province, reaching 268,073 tons in 2023 (Directorate General of Horticulture, 2024). This data aligns with the 2023 Horticultural Statistics report released by the Central Statistics Agency (BPS), which shows an increase in harvested area and tomato productivity in several strategic areas (BPS Indonesia, 2024).

However, the high potential for tomato production still faces serious challenges in the form of attacks by plant pests (OPT), particularly *Phytophthora* spp., a pathogen that causes leaf blight and fruit rot, which can drastically reduce crop yields (Abad et al., 2023). This pathogen infection has been reported to reduce crop yields by 50–100%, especially in high humidity environments, making it difficult to control conventionally and potentially causing economic losses for farmers (Sihotang et al., 2025). Although synthetic chemical fungicides are effective in suppressing pathogen development, their continued use risks triggering resistance and negatively impacting human health and the environment (Hartono, 2023).

With increasing attention to sustainable agriculture, the use of biopesticides as an environmentally friendly method of controlling plant diseases is increasingly being developed (Yulia et al., 2020). Biopesticides are considered safer for consumers and the environment and can reduce dependence on synthetic chemical pesticides, which have the potential to have negative impacts on health and ecosystems (Sutriadi et al., 2019). Therefore, the search for natural sources of biological control agents continues to be carried out to support sustainable agricultural systems.

One potential source comes from insects, specifically the larvae and pupae of the Black Soldier Fly (*Hermetia illucens*), which are known to contain various bioactive compounds (Surendra et al., 2016; van Huis, 2020). BSF maggot pupae are reported to contain alkaloids, lipids, proteins, and enzymes that have the potential to act as antimicrobial agents and strengthen plant defense systems (Firdaus et al., 2023). These active compounds and enzymes enable BSF maggot pupae to be used as additives in biopesticide formulations to

increase the effectiveness of plant disease control through pathogen inhibition and destruction mechanisms (Wardiman et al., 2024).

Therefore, this study aims to analyze the characteristics of biopesticides with the addition of BSF maggot pupa extract and evaluate its effectiveness in biopesticide formulations against *Phytophthora* spp. in vitro and in vivo, and determine the best concentration that provides optimal protection in tomato plants. The results of this study are expected to provide an alternative for more sustainable plant disease management and support the development of natural biopesticides in modern agriculture..

II. EXPERIMENTAL PROCEDURE

This research will be conducted from September to November 2025. Isolate rejuvenation and treatment will be conducted at the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung. The equipment used in this research are petri dishes, test tubes, loop needles, 250 mL and 500 mL Erlenmeyer flasks, 1000 mL glass backer measuring cylinders, funnels, dropper pipettes, micropipettes, petri dishes, tweezers, bunsen burners, stirrers, rotary evaporators, filter paper, microscopes, cork borders, analytical balances, vortex mixes, autoclaves, incubators, matches, ovens, polybags, and laminar air flow cabinets. The materials used in this research are pure cultures of *Phytophthora* spp., biopesticides obtained from PT. Great Giant Pineapple Lampung, BSF maggot pupae (*Hermetia illucens*), Potato Dextrose Agar (PDA), 96% ethanol, 70% ethanol, distilled water, spirits, and tomato plant seeds. The parameters observed were colony diameter, percentage of inhibition, disease incidence and disease severity, then the data were analyzed using a homogeneity test, followed by an ANOVA test and then analyzed using an honestly significant difference test (HSD).

2.1 Preparation and Extract Preparation

100 grams of BSF maggot pupae, which had been transformed into a simple drug, were taken and dissolved in 700 mL of distilled water in a beaker. The mixture was macerated for 3 days, stirring occasionally. The macerated mixture was then filtered through filter paper and evaporated using a rotary vacuum evaporator at 60°C to obtain a concentrated extract (Budikania et al., 2021).

2.2 Biopesticide Formulation

Table 1. Biopesticide Formulation with BSF Maggot Pupa

| Treatment | Extract Concentration | Biopesticide Volume 10% (mL) | Maggot Pupa Extract Weight (mL) | Solvent Volume (Water) (mL) |
|-----------|-----------------------|------------------------------|---------------------------------|-----------------------------|
| P0 | 0% | 40 mL | 0 mL | 360 mL |
| P1 | 2,5% | 40 mL | 2,5% x 400 = 10 mL | 350 mL |
| P2 | 5% | 40 mL | 5% x 400 = 20 mL | 340 mL |
| P3 | 7,5% | 40 mL | 7,5% x 400 = 30 mL | 330 mL |
| P4 | 10% | 40 mL | 10% x 400 = 40 mL | 320 mL |
| P5 | 12,5% | 40 mL | 12,5% x 400 = 50 mL | 310 mL |

Information:

P0= Biopesticide Control 10%.

P1= Biopesticide 10% + Maggot pupa 2.5%

P2= Biopesticide 10% + Maggot pupa 5%

P3= Biopesticide 10% + Maggot pupa 7.5%

P4= Biopesticide 10% + Maggot pupa 10%

P5= Biopesticide 10% + Maggot pupa 12.5%

2.3 Bioactive Compound Screening

Bioactive compound screening is conducted to identify compounds contained in biopesticide formulations with the addition of BSF (*Hermetia illucens*) maggot pupae. The bioactive compounds tested include alkaloids, flavonoids, saponins, tannins, phenols, steroids, and terpenoids.

2.4 Rejuvenation of *Phytophthora* spp. Isolates and In Vitro Growth Inhibitory Test of *Phytophthora* spp.

A total of 39 grams of PDA medium was dissolved in 1000 mL of distilled water and heated until homogeneous using a hotplate and magnetic stirrer. Next, the medium was poured into an Erlenmeyer flask and sterilized by autoclaving at 1 atm pressure and 121°C for 15 minutes (Putri et al., 2019). *Phytophthora* spp. isolates were obtained from PT. Great Giant Pineapple Lampung. The isolates were then rejuvenated by reculturing them on PDA medium. The purified isolates were inoculated on PDA medium for 7 days, until fungal colonies filled the petri dish (Yulis et al., 2023).

The *Phytophthora* spp. inhibition test was conducted using the previously prepared PDA medium. This method involves mixing a 10% biopesticide formulation with 1 mL of maggot pupa extract (2.5%, 5%, 7.5%, 10%, and 12.5%) per 25 ml of PDA medium, followed by inoculating *Phytophthora* spp. fungi into the center of the medium. This in vitro procedure involves measuring the diameter of fungal colonies on PDA medium mixed with the biopesticide formulation.

Colony Diameter, according to Nawawi (2001), the formula used is:

$$d = \frac{(AA) + (BB) + (CC) + (DD)}{4}$$

Description:

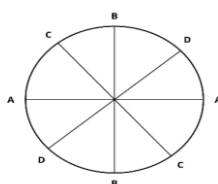
d = Diameter of *Phytophthora* spp. fungal colony (cm)

AA = Horizontal diameter of *Phytophthora* spp. fungal colony (cm)

BB = Vertical diameter of *Phytophthora* spp. fungal colony (cm)

CC and DD = Diagonal diameter of *Phytophthora* spp. fungal colony (cm)

The diagram for measuring the growth rate of *Phytophthora* spp. fungal diameter is shown in Figure 6.



Percentage of Inhibitory Power, the formula for the percentage of inhibitory power against the growth of *Phytophthora* spp. fungi, according to Yulis et al., (2023), is:

$$P = \frac{Dk - Dp}{Dk} \times 100\%$$

Description:

P = Percentage of inhibition (%)

Dk = Diameter of control colony (cm)

Dp = Diameter of treatment colony (cm) Kejadian penyakit

2.5 Preparation of *Phytophthora* spp. Conidial Suspension

Phytophthora spp. fungi that had been cultivated for 7 days on *Potato Dextrose Agar* (PDA) were collected using a sterile loop and placed in a test tube containing 10 mL of sterile distilled water. The suspension was shaken or homogenized using a vortex for several minutes to release spores from the colony surface. The density of the spore suspension was measured using a hemocytometer, then serial dilutions were performed to obtain a spore concentration of 10^5 spores/mL as the inoculation standard for in vivo testing (Latifah et al., 2018).

2.6 In Vivo Test of Maggot Pupa Extract Concentration in Biopesticides

Sterile tomato seeds were planted in seedbeds filled with perforated seedling medium. One to two seeds were placed in each hole, then the holes were closed. The planting medium was placed in the seedbeds, and the seeds were grown in the seedbeds for 18 days (Nazari et al., 2020) until they developed 4-5 leaves (Zebua et al., 2019). They were then transferred to 30 x 30 cm polybags in the morning and acclimatized for 7 days (Nazari et al., 2020).

Pathogen inoculation was carried out on the 25th Day After Planting (DAP) by spraying the upper surface of the plants. Biopesticide was applied after the pathogenic fungus was inoculated on the tomato plants on the same day. Tomato plants were watered twice daily, in the morning and evening. If the growing medium is sufficiently moist, watering is done once a day (Kartika et al., 2015).

The biopesticide formulation was applied to tomato plants once, with applications occurring every seven days for four weeks: on days 7, 14, 21, and 28 Days After Inoculation (DAI) (Mare et al., 2023). Data collected included disease incidence and severity observed over four weeks. The parameters observed are the percentage of disease incidence and disease severity.

The formula for the percentage of disease incidence (KP) according to Nindias et al., (2020) is as follows.

$$KP = \frac{n}{N} \times 100\%$$

Description:

KP = Disease Incidence (%).

N = Total number of plants observed.

n = Number of chili plants infected with *Phytophthora* spp.

Disease severity, based on plant disease resistance criteria according to Efri (2010).

$$KP = \frac{\sum(n \times v)}{Z \times N} \times 100\%$$

Description:

KP = Disease Severity.

n = Number of spots caused.

v = Score value for each spot class.

N = Number of fruits observed.

Z = Score value for the highest spot extent class.

III. RESULTS AND DISCUSSIONS

3.1. Bioactive Compound Test Results

Table 2. Test Results for Bioactive Compound Content of Biopesticide Formula

| Test Name | Result | Description | |
|-----------|-------------------|-------------|------------------------------|
| Alkaloid | <u>Mayer</u> | + | Orange-yellow color forms |
| | <u>Bouchardat</u> | + | Orange/brick red color forms |
| | <u>Dragendorf</u> | + | Brown-orange color forms |
| Flavonoid | | + | Yellow color forms |
| Tanin | | + | Green color forms |
| Saponin | | + | Stable foam forms |
| Fenol | | + | Green color forms |
| Steroid | | + | Green color forms |
| Terpenoid | | + | Red color forms |

Description: (+) = contains test compound

(-) = does not contain test compound

The results showed that the bioactive compound content that had been carried out showed that all secondary metabolite groups tested—alkaloids, flavonoids, saponins, tannins, phenols, steroids, and terpenoids—showed positive reactions. Furthermore, the antioxidant and antimicrobial activity in maggot larvae or pupae was associated with the presence of phenolic compounds and other active metabolites (Almeida et al., 2022). Thus, the test results showing all active compounds strengthen the evidence that maggot pupae have a rich bioactive composition, and variation in results between studies is likely influenced by differences in solvents, extraction techniques, and sample conditions.

3.2 In Vitro Test Results of *Phytophthora* spp. Colony Diameter

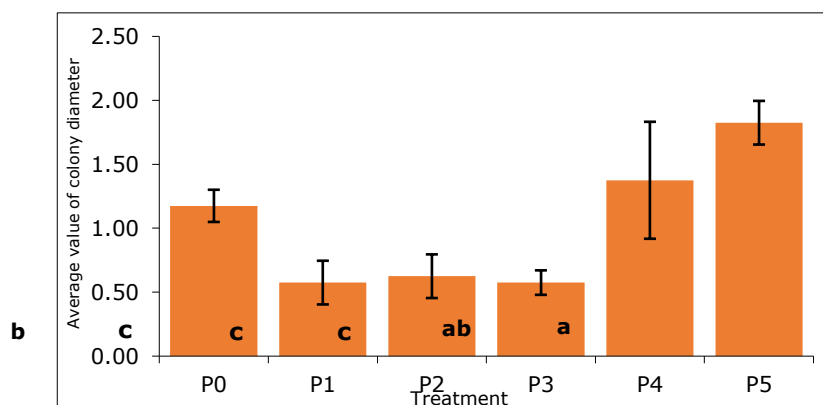


Figure 1. Diameter of *Phytophthora* spp. Under Effect of Biopesticide Formulation.

Figure 1. shows that the largest colony diameter was found at a concentration of 12.5% (P5), followed by a concentration of 10% (P4) and 0% (P0). Then the diameter decreased at a concentration of 5% (P2), decreasing further at a concentration of 2.5% (P1) and 7.5% (P3). The smallest colony diameter diagram was at a concentration of 7.5% (P3) but was not significantly different from 2.5% (P1) and 5% (P2).

The image of the growth of *Phytophthora* spp. colonies on PDA media is presented in the image below.

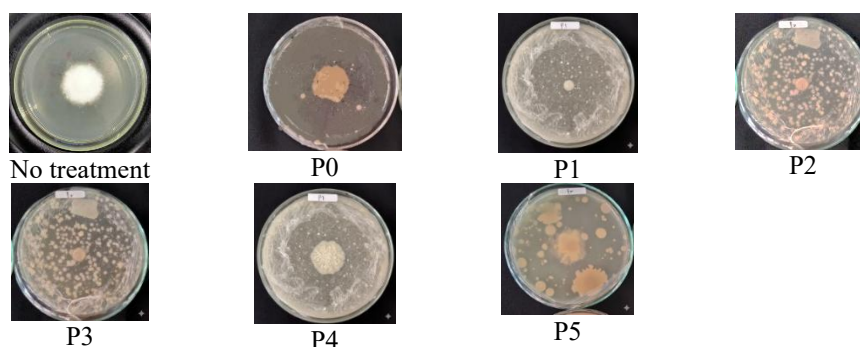


Figure 2. Comparison of *Phytophthora* spp. Colony Diameters.

The results showed that the addition of maggot pupa extract to the biopesticide formula significantly affected the growth of *Phytophthora* spp. colonies. This was evident in the differences in colony diameter between treatments, with the concentration of pupa extract significantly influencing the level of pathogen growth inhibition. Treatment P3 (10% biopesticide + 7.5% pupa extract) produced the smallest colony diameter, thus achieving the highest inhibition against *Phytophthora* spp. This indicates that increasing the concentration of pupa extract to the optimum level can enhance the antifungal activity of the biopesticide formula.

Inhibitory effectiveness is influenced by the secondary metabolite content of maggot pupa extract, which, according to phytochemical tests, contains alkaloids, flavonoids, tannins, saponins, phenols, steroids, and terpenoids. These compounds exhibit antimicrobial activity through various mechanisms, such as membrane and cell wall disruption, inhibition of cell division, and disruption of enzyme activity and pathogen metabolism (Harborne, 2008; Scalbert, 1991; Daglia, 2012; Auza et al., 2025).

P3 treatment at a concentration of 7.5% demonstrated the highest inhibitory effectiveness and is suspected to be the optimal concentration. At lower concentrations, the active compound has not yet reached its minimum effective level, while at higher concentrations, effectiveness decreases, likely due to compound instability or antagonistic interactions between metabolites. Therefore, increasing concentration does not always equate to inhibitory effectiveness (Kurnia et al., 2020).

3.3 In Vitro Inhibitory Percentage Test

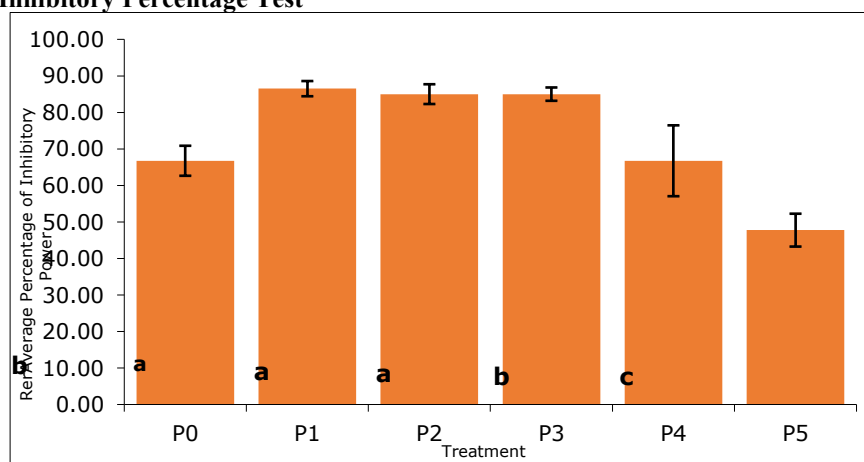


Figure 3. Percentage of Growth Inhibition of *Phytophthora* spp.

The image above shows that the addition of BSF maggot pupa extract to the biopesticide formula on PDA media at varying concentrations affected the percentage of *Phytophthora* spp. growth inhibition. Treatment (P5) showed the lowest percentage of inhibition, then increased at concentrations of 0% (P0) and 10% (P4). The highest percentage was at a concentration of 7.5% (P3), but was not significantly different from concentrations of 2.5% (P1) and 5% (P2).

Based on the data, treatments P1 (2.5%) and P3 (7.5%) showed the highest percentage of inhibition at 86.50%, indicating that the addition of maggot pupa extract at certain concentrations can increase the effectiveness of biopesticides in inhibiting pathogens. The inhibition at these concentrations is thought to be related to the content of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, phenols, steroids, and terpenoids. Alkaloids play a role in inhibiting cell wall formation and fungal protein synthesis, flavonoids interfere with cellular respiration and pathogen cell division, while tannins cause protein precipitation which has an impact on reducing enzyme activity and mycelial viability (Harborne, 2008; Scalbert, 1991; Daglia, 2012; Auza et al., 2025).

3.4 Disease Incidence by *Phytophthora* spp. on Tomato Plants (*Solanum lycopersicum*)

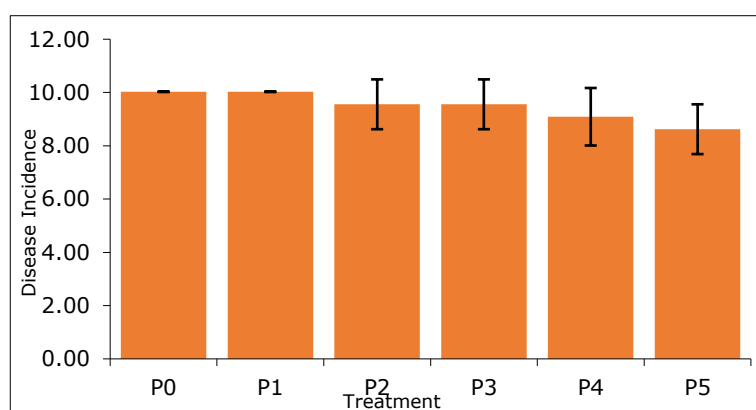


Figure 4. Percentage of Disease Occurrence by *Phytophthora* spp. on Tomato plants.

The results showed that disease incidence between treatments was not statistically significantly different. The highest disease incidence rates were found at P0 and P1 (0–2.5%), then tended to decrease from P2 to P5, with the lowest value at a concentration of 12.5%. However, the addition of maggot pupa extract to the biopesticide did not significantly reduce *Phytophthora* spp. disease incidence at the 0.05 level. This is likely due to the aggressive nature of *Phytophthora* spp. infections, with high sporulation and infectivity, allowing the pathogen to infect plants before the bioactive compound takes effect, especially if the biopesticide is contact-based and not systemic (Judelson & Blanco, 2005).

Thus, the results of this study indicate that although the maggot pupa extract-based biopesticide formula effectively inhibited pathogen growth in vitro (colony diameter and inhibition), its effectiveness in reducing disease incidence in tomato plants in the field or under living conditions did not show significant differences. This emphasizes the importance of developing biopesticide formulations that are more stable, have better systemic properties, and can maintain the activity of bioactive compounds in complex cultivation environments.

3.2 Disease Severity by *Phytophthora* spp. on Tomato Plants (*Solanum lycopersicum*).

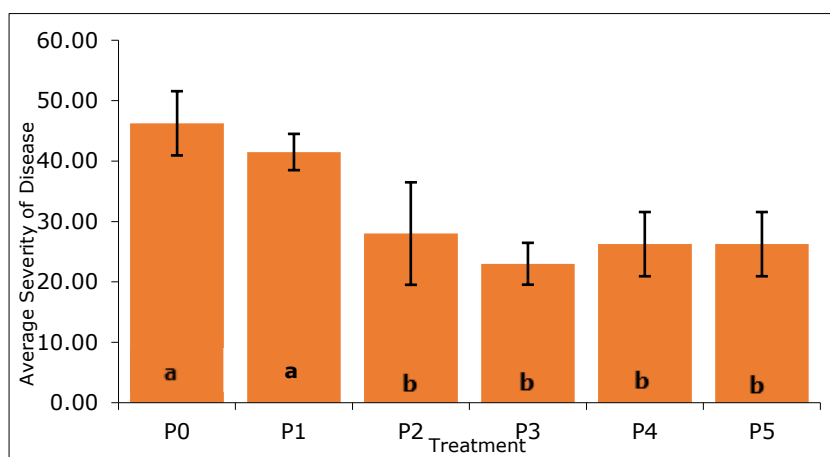


Figure 5. Severity of Disease by *Phytophthora* spp. on Tomato Plants.

Disease severity analysis showed that the addition of BSF maggot pupa extract to the biopesticide formula significantly affected disease severity in tomato plants inoculated with *Phytophthora* spp. The control treatment (P0) resulted in the highest disease severity of 46.25%, while treatment P3 (7.5%) showed the lowest disease severity of 23.00%, indicating a reduction in disease severity of almost half compared to the control. This reduction aligns with previous findings that bioactive compounds can suppress pathogen virulence and symptom development without completely preventing initial infection (Arbai et al., 2020; Faza & Cahyani, 2021). Therefore, BSF maggot pupa extract has potential as a biopesticide to reduce the severity of *Phytophthora* spp. disease in tomatoes, with a concentration of 7.5% being the most optimal, although it does not completely eliminate infection.

The difference in effectiveness between P5 and P3 can be explained by the stability of the active compound. Excessively high concentrations, such as those found in P5, can potentially decrease the stability and biological activity of some metabolites, as well as increase the likelihood of antagonism between compounds (Harte et al., 2024). Consequently, although P5 is able to prevent initial infection (low disease incidence), its ability to suppress pathogen development after infection (severity and colony diameter) is less than optimal. This suggests that the ability to prevent initial infection is not always accompanied by the ability to suppress systemic disease progression.

Thus, the effects of maggot pupa extract treatment differed across parameters, as disease incidence measures the presence or absence of infection, while colony severity and diameter measure pathogen development after infection. P5 was more effective at preventing initial infection, while P3 was more effective at suppressing disease development once the pathogen was already within plant tissue.

IV. CONCLUSION

The biopesticide formula contains bioactive compounds such as alkaloids, flavonoids, tannins, saponins, phenols, steroids, and terpenoids. The addition of BSF maggot pupa extract has been shown to suppress the growth of *Phytophthora* both in vitro (colony diameter and percentage of inhibition) and in vivo (disease incidence and disease severity). At a concentration of 7.5% (P3), the best concentration that affects the growth of *Phytophthora* in vitro and is able to reduce the severity of the disease in vivo, the treatment does not show an effect on the incidence of disease (not significantly different).

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