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Fungistatic Effect of *Ipomea Carnea* Extract and *Trichoderma Esperellum* Against Various Fungal Biological Agents

Fungistatic Effect of *Ipomea Carnea* Extract and *Trichoderma Esperellum* Against Various Fungal Biological Agents

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Abstract. The study aimed to measure the fungistatic effect of *Ipomea carnea* and *Trichoderma esperellum* Tc-Jjr-02 biomass extracts against several fungal isolates of biological agents in vitro. Nine selected biological agent fungi were grown in PDA-chloramphenicol media containing 1% fresh extract of *I. carnea* biomass, four of them were grown in dual culture with isolate Tc-Jjr-02 with an incubation period of 96 hours. The results showed that the *I. carnea* biomass extract had a fungistatic effect by inhibiting the growth of the entomopathogenic agents *Metarrhizium anipsoliae* Me-Sdj-16 (27.8±3.2%) and *Beuveria bassiana* Be-Sj-13 (38.1±2.6%) and Be-Sdj-15 (18.6±1.0%), and the biofertilizer agents *Aspergillus* sp. As-Sdj-11 (46.7±1.9%), *Penicillium* sp. Pc-Sdj-14 42.7±3.5%, and *Trichoderma* sp. Tc-Sdj-18 38.5±1.0% at 96 hours incubation period (HAI). The biocontrol agent *T. esperellum* gave a weak fungistatic effect against the biofertilizer agents As-Sdj-11 and Pc-Sdj-07 were 14.4±1.3% and 7.0±1.9% at 96 HAI and did not have a fungistatic effect on the biofertilizer agents *Trichoderma* Tc-Ba-05 and Tc-Sdj-09. Application of *I. carnea* biomass as green manure and some biofertilizer agents can be carried out simultaneously; while the application of *T. esperellum* can be carried out using biofertilizer agents As-Sdj-11 and Pc-Sdj-07 and Tc-Ba-05 and Tc-Sdj-09.

1. Introduction

The implementation of agricultural cultivation technology in the context of a green economy always prioritizes the use of environmentally friendly production resources referring to the 2015 Paris Agreement [1]. The use of biopesticides and biofertilizers is an alternative that gives hope for guaranteeing sustainable agricultural cultivation. This answers the challenge of the fact that in the last decade the application of toxic synthetic chemical pesticides in addition to failing and causing negative impacts in the form of pathogen resistance [2], eradicating organisms that are actually beneficial to plants [3], threatening human health [4], and polluting the environment [5].

The use of plant biomass as fertilizer, the use of effective microorganisms as biological agents of biological fertilizers and as an active ingredient of biopesticides have begun to be widely researched and implemented. In addition to waste or crop residues, the use of wild plant biomass also provides good prospects as an alternative to the use of chemical fertilizers.

Ipomea carnea is one of the wild plants that is widely distributed in wetland agricultural cultivation areas including marginal wetlands. This plant is known to contain various pharmaceutical chemical compounds [6], is used as green manure and can act as a biopesticide to control insect pests [7], and contains various fungistatic compounds that can control plant pathogenic fungi [8].

On the other hand, the use of effective microbial biological agents, especially Trichoderma fungi, has been in an encouraging position considering that various success reports have been inventoried.

Several *Trichoderma* isolates in East Java (Indonesia), including *T. asperellum* isolated from pine plantation forest areas (not agricultural land for food), have also shown outstanding performance in controlling various disease-causing pathogens whose research is supported by the Microbiology and Biotechnology Laboratory, University of Muhammadiyah Sidoarjo. On the other hand, *T. asperellum* can also act as a biofertilizer biological agent, so that its use is at the same time implementing biological fertilizers and bio fungicides in one treatment.

In the activity of protecting plants from the threat of plant-disturbing organisms, microorganisms have begun to be used, including from the group of fungi that have the potential as active ingredients for biofertilizers and bio fungicides. However, in the context of the use of biomass as green manure, this organic material also has the potential to be used as an ingredient for the formulation of various biological agents in the manufacture of biofertilizers and biopesticides that have the potential to be applied in plant protection.

This research is expected to know the extent to which *I. carnea* biomass can inhibit biological agent fungi. On the other hand, it can also be seen how the fungistatic effect of the Trichoderma fungi whose effectiveness as a bio fungicide is known for several fungi that have the potential and can be candidates for biofertilizer biological agents. This information is important in order to assemble treatments for protecting plant health and productivity that are environmentally friendly and sustainable in the future.

Until now, there is very little information showing the fungistatic effect of extracts of *I. carnea* and Trichoderma fungi on various potential biological agents, so that a strategy that integrates the use of all environmental resources for the control of plant-disturbing organisms and the management of the fertility of the growing media is always filled with concerns that there will be an effect of canceling the role of each other the positive input component of the plant's production. For this reason, research that examines the fungi toxic effects of these two potential resources on several other potential biological agents needs to be carried out.

2. Methods

The research was carried out at the Laboratory of Microbiology and Biotechnology, University of Muhammadiyah Sidoarjo (UMSIDA) from June to September 2021 to test the fungistatic effect of *Ipomea carnea* biomass extracts and *Trichoderma esperrelum* on several biological agent fungi.

2.1. Fungistatic Power of Biomass I. Carnea

The *I. carnea* plant used in this study was taken from around agricultural land in Kedungcangkring village, Jabon district, Sidoarjo regency, East Java province. All fresh *I. carnea* biomass, except roots and flowers, was chopped with a cutter to produce 1-2 cm pieces and crushed using a grinder until smooth. Then 20 g was taken and put into distilled water to a volume of 100 ml and blended for five minutes, then the mixture was filtered to produce a liquid fresh extract. With the addition of sterile distilled water, the concentration of the extract was determined to be 1%. Furthermore, 20 ml of the extract was taken using a measuring pipette and placed into a 9.0 cm diameter petri dish which had been placed in a sterile in case. To sterilize extracts from contaminant microbes, sterilization is carried out using ultraviolet compounds produced from UV lamps in the incase box for 120 minutes. Meanwhile, to make the in vitro test media, 180 ml of PDA-chloramphenicol media was prepared which had been sterilized by means of an autoclave (121 ^{o}C, 1 atm for 15 minutes) which was placed in an Erlenmeyer with a capacity of 500 ml. When the UV sterilization period has passed, the sterile extract is taken with a sterile pipette and mixed into an Erlenmeyer containing PDA-c which is in a liquid state at a temperature of around 50^{o}C; the mixing activity was carried out under a Bunsen fire, and then followed by vigorous shaking so that a homogeneous mixture of the fresh extract of *I. carnea* with PDA-c was carried out as the solvent. Next, the mixture is poured into an empty petri dish to a thickness of 2.0 mm; prior to incubation, the edges of the Petri dishes were sealed with transparent plastic tape to prevent contamination from entering.

After 24 hours of incubation, solid media containing 1% extract of *I. carnea* was ready to be used for in vitro test of the fungitoxicity of extracts against various isolates of biological agents (Table 1),

collection of the Laboratory of Microbiology and Biotechnology UMSIDA, Indonesia. The criteria for potential and candidates have been determined by the Laboratory. Isolates of biological potential fungi are isolates from exploration results which in preliminary tests showed their effectiveness, while isolates of candidates for biological agents are isolates that have passed the effectiveness test phase using indicator plants, especially in testing their ability as bio fertilizers that support plant growth.

Table 1. Fungal isolates of biological agents from the collection of the Laboratory of Microbiology and Biotechnology UMSIDA

1	*Metarrhizium anipsoliae*	Me-Sdj-16	Test isolate; the potential of entomopathogenic agent
2	*Beuveria bassiana*	Be-Sdj-15	Test isolate; the potential of entomopathogenic agent
3	*Beuveria bassiana*	Be-Sdj-13	Test isolate; the potential of entomopathogenic agent
4	*Aspergillus* sp.	As-Sdj-05	Test isolate; candidate for biofertilizer agent
5	*Penicillium* sp.	Pc-Sdj-08	Test isolate; candidate for biofertilizer agent
6	*Penicillium* sp	Pc-Sdj-14	Test isolate; candidate for biofertilizer agent
7	*Trichoderma* sp.	Tc-Ba-09	Test isolate; candidate for biofertilizer agent
8	*Trichoderma* sp.	Tc-Sdj-18	Test isolate; the potential of biofertilizer agent
9	*Trichoderma* sp.	Tc-Sdj-10	Test isolate; candidate for biofertilizer agent
10	*T. esperellum*	Tc-Jjr-02	Isolate tester; bio fungicide agent

Each fungal isolate candidate for biofertilizer biological agents that had been cultured for 10 days in PDA-c media was ready to be used in in vitro assays. From each isolate, culture propagules were sampled with a diameter of 5.0 mm which were taken with an ose needle and placed in the center of a petri dish containing 1% *I. carnea* extract. Simultaneously, propagules of the same size from the same culture were placed in the center of a petri dish with media that did not contain *I. carnea* extract as a control. Observations were made every 24 hours for 4x24 hours, incubation period of 4x24 hours on the radius of the tested fungal colonies. Observations of the fungistatic effect on each biological agent were repeated four times.

The formula used to determine the percentage of the fungistatic effect of the extract on the fungal colony growth of the biological agent tested was as follows (1):

$$IC = (ra - rb).ra - 1.100\% \quad (1)$$

with explanations: IC percentage inhibition of fungal colony growth of biological agents; ra and rb are the radii of colony growth on PDA-c media without and with *I. carnea* extract, respectively.

2.2. T. asperellum Fungitoxic Test

In this test, all of the candidates isolate for biofertilizer biological agents (Table 1) were grown simultaneously on PD-c media. Tc-Sdj-07, Tc-Ba-05, and TSj-13. After the 10-day growth incubation period ended, culture samples of the isolate fungus *T. asperellum* were taken as a tester at a position in the centerline of the petri dish, a quarter of the distance from the edge of the petri dish. Furthermore, it is placed in line with and opposite the propagule test isolate, the culture of one isolate of the biological agent being tested, which is a quarter of the distance from the edge of the opposite cup or half the length of the diameter of the cup from the test isolate propagule. As in the test with *I. carnea* extract, this test also used control, namely the isolates tested were grown in a single medium or without dealing with the propagules of the test isolate. The test was repeated three times. Colony growth observations were carried out every 24 hours, and the inhibition was calculated as in Formula (1), provided that *r*a was the growth radius of the test isolate colonies on dual culture media, while *r*b was the growth radius of the test isolate colonies on monoculture media.

3. Results and Discussion

3.1. Fungistatic Power of Bimass I. Carnea

3.1.1. Inhibition of Fungal Isolates of Potential Bioinsecticide Agents

The results of the inhibitory effect of the fungistatic effect produced by the biomass I carnea extract are shown in Figure 1. The extract inhibited the growth of colonies of the fungus *M. anipsoliae* (Me-Sdj-1), as well as against Be-Sdj-1 and Be-Sdj-15 at 72 an 96 hours after inoculation (HAI). The growth of the two isolates of *B. bassiana*, the entomopathogenic fungus, was supported by *I. carnea* extract for up to 48 HAI.

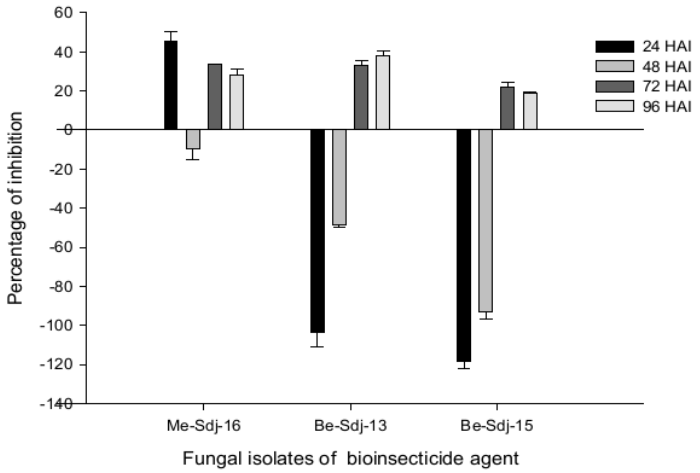

Figure 1. Percentage of inhibition of I. carnea biomass extract against three fungi isolates of entomopathogenic agents

3.1.2. Inhibition Against Trichoderma Isolate Biofertilizer Agents

The fungistatic inhibitory effect of I. carnea biomass extract was seen in *Trichoderma* sp. Tc-Ba-05 and Tc-Sj-1, however, supported the growth of Tc-Sdj-15 isolates, especially at 24 HAI (Figure 2).

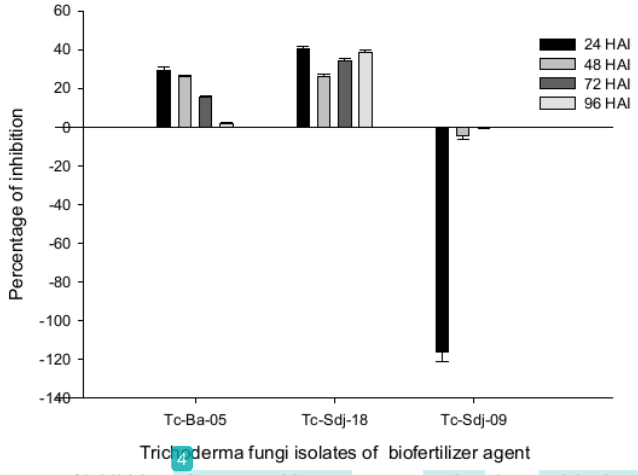

Figure 2. Percentage of inhibition of *I. carnea* biomass extract against three Trichoderma fungi isolates of biofertilizer agents

3.1.3. Inhibition of non Trichoderma Fungi Biofertilizer Agents

I. carnea biomass extract inhibited the growth of *Aspergillus* sp. As-Sdj-11 and *Penicillium* sp. Pc-Sdj-14, but supports the growth of *Penicillium* sp. Pc-Sdj-14 with the highest support level at 24 HAI (Figure 3).

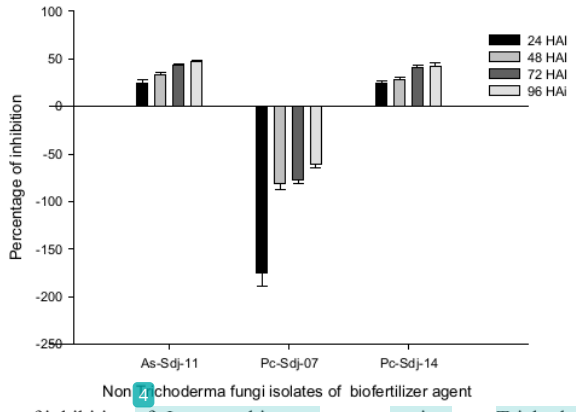

Figure 3. Percentage of inhibition of *I. carnea* biomass extract against non Trichoderma fungi isolates of biofertilizer agent

I. carnea plant biomass appears to contain fungistatic metabolites and has many biological effects on microbes. Some of the reported compounds include caryophyllene, hexadecanoic acid, and terpenes [6,8,9].

Caryophyllene was found to be cytotoxic and antimicrobial [10]. Caryophyllene was found to be cytotoxic and antimicrobial [10]. The effect of caryophyllene in inhibiting the colony growth of several types of fungi tested in this experiment was also reported on several types of fungi including *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Alternaria brassicicola*, *Phoma lingam*, and *Rhizoctonia solani*

[11]. As shown in Figures 1, 2, and 3, the fungistatic effect can be caused by caryophyllene and hexadecanoic acid through inhibition of fungal hyphae cell growth of entomopathogenic agents Me-Sdj-17 and Be-Sdj-13, and Be-Sj-15 at 72 and 96 HAI, *Trichoderma* (Tc-Ba-05 and Tc-Sdj-18), and As-Sdj-11 and Pn-Sdj-14. The terpene oxide form is cytotoxic [12] and has been shown to inhibit various types of microbes [13]. Pyrone derivatives have been shown to have a strong spectrum of fungistatic effects [14,15]. This compound has also been shown to have similar strength to synthetic fungicides [16], not only inhibiting the fungi *Trichomonas vaginalis* and *Candida* spp. [17] also inhibited some of the tested *Trichoderma* fungi.

Especially for *B. bassiana* (Be-Sdj13 and Be-Sdj-15) at 24-48 HAI, *Trichoderma* Tc-Sdj-09 24 HAI, and *Penicillium* sp. Pc-Sdj-07, it seems that there are several compounds contained in the extract that have an effect on supporting the growth of fungal colonies. This shows that the four isolates in this experiment have utilized various compounds contained in the extract. Several Trichoderma isolates with various enzymes and their extracellular metabolites produced were able to convert environmental resources effectively as a source of nutrients [18]. The metabolite oxidation of the extract can be carried out effectively by aerobic organisms [19] including by these tested isolates of *Trichoderma*, *B. bassiana*, and *Penicillium* sp. to produce energy and breakdown of amino acids [20] which are ultimately used as components of fungal biomass.

3.1.4. Inhibition of T. Asperellum Against Candidate Fungi of Biofertilizer Agents

The fungistatic effect of *T. asperellum* activity inhibited the growth of the isolate *Aspergillus* sp. As-Sdj-11 and *Penicillium* sp. Pc-Sdj-07, meanwhile, the inhibition of two Trichoderma isolates was very weak (Figure 4).

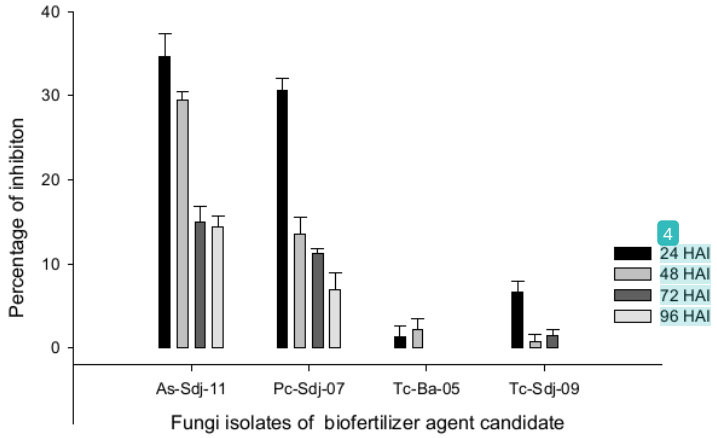

Figure 4. Percentage of inhibition of *T. esperellum* against the fungi isolates of biofertilizer agent candidate

The fungus *T. asperellum* which, prior to species determination based on molecular markers [21], based on morphological characteristics was considered as *T. harzianum*, was effective in controlling the fungus *R. solani* pathogen damping-off on soybean [22], *F. oxysporum* causes root powder of red chili [23], *Phytophthora infestans* pathogen late blight on potatoes [24], and *Colletotrichum* spp. anthracnose pathogen in chili [25], and. This test was conducted to determine whether *T. esperellum*, which was originally intended to be applied as a bio fungicide by soil treatment together with other biological agent fungi that act as active ingredients in biofertilizers, can synergize to provide plant protection and health. The test results as shown in Figure 4 that *T. esperellum* gave a fungistatic effect on *Aspergillus* sp. As-

Sdj-11 and *Penicillium* sp. Pc-Sdj-07. Meanwhile, the fungistatic effect was not seen against the two Trichoderma isolates.

Trichoderma is a fungus that *Trichoderma* is a phytopathogenic fungus with extracellular chitinase activity [26] playing an important role in the process of its anti-fungal behavior [27] in the form of chitin degradation in addition to cellulase activity [28]. The combination of chitin and cellulose as the main constituents of fungal cell walls becomes the target of biological hydrolysis of chitin into N-acetyl-D-glucosamine monomer which will be further used by *Trichoderma* fungi as a source of carbon and energy [29,30]. On the other hand, both the isolate Tc-Jjr-02 as a tester and the two isolates tested (Tc-Ba-05 and Tc-Sdj-09) were from the *Trichoderma* genera which have chitinase enzymes [31,32] which in this test are neutral or neutral. the fungistatic effect of *T. asperellum* Tc-Jjr-02 did not appear to work against Tc-Ba-05 and Tc-Sdj-09.

4. Conclusions

I. carnea biomass extract gave a fungistatic effect by inhibiting the growth of the bioinsecticide agents *Metarrhizium anipsoliae* Me-Sdj-16 (27.8±3.2%) and *Beauveria bassiana* Be-Sj-13 (38.1±2.6%) and Be-Sj-15 (18.6 ±1.0%), and the biofertilizer agents *Aspergillus* sp. As-Sdj-11 46.7±1.9%, *Penicillium* sp. Pc-Sdj-14 42.7±3.5%, and *Trichoderma* Tc-Sdj-18 38.5±1.0% at 96 hours after inoculation (HAI). *I. carnea* biomass extract increased the growth of *B. bassiana* Be-Sj-13 Be-Sj-15 438.3±1.7% and 93.3±3.3%, respectively, at 48 HAI, *Penicillium* sp. Pc-Sdj-07 61.1±3.2% in 96 HAI, and *Trichoderma* sp Tc-Sdj-09 115.7±4.8% in 24 HAI. The bio fungicide agent *T. esperellum* exerted a fungistatic effect at the beginning of the incubation period against the biofertilizer agents *Aspergillus* sp As-Sdj-11 and *Penicillium* sp. Pc-Sdj-07 were 34.7±27% and 30.7±1.3% at 24 HAI, but the fungistatic effect was weakened in 96 HAI to 14.4±1.3% and 7.0±1.9%; meanwhile, the fungistatic effect on the biofertilizer agent *Trichoderma* sp. Tc-Ba-05 and Tc-Sdj-09 were nil at the end of the incubation period.

Implementation of the application of *I. carnea* biomass as green manure and as a biofertilizer agent can be carried out simultaneously; Meanwhile, the application of *T. esperellum* bio fungicide is possible simultaneously with the application of candidate biofertilizer agents *Aspergillus* sp As-Sdj-11 and *Penicillium* sp. Pc-Sdj-07 and *Trichoderma* sp Tc-Ba-05 and Tc-Sdj-09.

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