Efficacy of Several Entomopathogenic Microorganism as Microbial Insecticide against Insect Pest on Chili (*Capsicum annum* L.)

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Abstract. The purpose of this study was to apply microbial suspension of which formulated as bio-insecticide to observe its effectiveness in controlling insect pest on chili plants, *Capsicum annum* L. The efficacy of bio-insecticide was evaluated based on potential entomopathogenic microorganisms used in the microbial suspension. The bio-insecticide consisted of entomopathogenic fungal and bacterial suspensions. The experiment was carried out in chili cultivation area in Situ Gede, Bogor. The application used spray method with multiple various doses. The observation showed that the plant treated with spore suspensions of entomopathogenic fungi *Metarhizium* sp. T4B23, B2-2, and cell suspensions of entomopathogenic bacteria Bt + SP4, were able to resist against insect pests, such as whitefly, grasshopper, and armyworm. In addition, the treated plant had more vigorous growth and yield than the control treatment.

1. Introduction
Chili is an important commodity for Indonesian people which must be available as main ingredient for daily consumption. The high demand of chili requires widely cultivation of this horticultural commodity by Indonesian farmers, for example curly chili (*Capsicum annum* L.). The curly chili contains important compounds needed for human health, namely: capsaicin, dihydrocapsaicin, vitamins (A and C), capsantine dyes, carotene, capsorubin, zeasantan, cryptosantin and lutein. In addition, the curly chili also contains minerals such as iron, potassium, calcium, phosphorus and niacin [1].

The needs for curly chilies is increasing every year which indicated by forecasted increasing of total consumption of chilies from 2016 to 2019 (2.90 kg/capita to 3.05 kg/capita) [2]. However, the needs are not met even by an increase in adequate production. Production of chili faces some limitations, one of which is pest and disease incidence in chili cultivations [3]. Many insects are associated with curly chili plants; some important insects are pest, yet there are beneficial insects too such as predators, parasitoids, and pollinating insects. According to the previous research [1], some insects found in chili farming include: Order Lepidoptera (Family Noctuidae), Order Orthoptera (Family Tettigoniidae), Homoptera Order (Famili Cicadellidae), Order Hemiptera (Family Noctuidae), Order Orthoptera (Family Tettigoniidae), Homoptera Order (Famili Cicadellidae), Order Hemiptera (Family Noctuidae), Order Orthoptera (Family Tettigoniidae), Homoptera Order (Famili Cicadellidae), Order Hemiptera (Family Noctuidae), Order Orthoptera (Family Tettigoniidae), Homoptera Order (Famili Cicadellidae), Order Coleoptera (Family Coccinellidae, Scarabaeidae, Curculionidae and Staphylinidae), Order Diptera (Family Dolichopodidae and Syrphidae) and Order Hymenoptera (Family Apidae).
At the level of farmers, farmer groups, and plantation companies, control of insect pest on curly chili plants mostly use chemical insecticides. The chemical insecticide has been causing many environmental problems. Moreover, continued use of pesticides will trigger new problems such as killing non-target insects (parasitoids, predators and other useful insects) or causing insecticide resistance in insect pest. Searching for a more environmentally friendly and effective insecticide is still a much-worked research area. An example of insecticide application alternative is to use microbes as biocontrol agents [4]. Types of microorganisms used as biocontrol agents include: *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces fumosoroseus*, *Lecanicillium lecanii*, *Bacillus thuringiensis* and *Serratia marcescens* [5, 6]. Toxicity of several *Metarhizium* spp against one of important pest of chili plant, *Spodoptera litura*, were attained at conidial density of $10^9$ with 19.79 – 75.70% of *S. litura* eggs were failed to hatch, and up to 58% of the first instar larvae were found dead after 3 days hatched [7].

The microorganism cultures, entomopathogenic fungi and bacteria used in this study were previously isolated, identified and evaluated its toxicity in laboratory scale against *S. litura* [6, 8]. In addition, studies on efficacy of the entomopathogenic microorganism isolates against agricultural insect pests of curly chili plants, *S. litura*, have also been carried out in greenhouse (unpublished work). Therefore, the current research is focused on the effectiveness of these microorganism suspension as biocontrol agents when applied as on chili farming area using the same microorganism cultures.

2. Materials and methods

2.1. Isolate preparation and microbial insecticide production

The microbial entomopathogenic isolates used in this study were two fungi: *Metarhizium* sp T4B23 and B22, and two bacteria: *Serratia marcescens* BLSP4 and *Bacillus thuringiensis* (Bt). The Bt isolate was obtained from Institut Pertanian Bogor Culture Collection (IPBCC) while other isolates are collection of Laboratory of Microbiology, Research Center for Biomaterials, LIPI. The fungi were grown on 200 g of sterilized half-steamed rice. The mixture was then incubated for 7-10 days to get a high conidial density of $10^9$ spores/ml (Figure 1B). Meanwhile, the Bt and BLSP4 bacterial isolates were grown on 5 L of nutrient broth media. The bacterial culture was then incubated with shaking condition for 2 days until the cell density reached $10^8$ CFU/ml. Before application, conidia and bacterial culture were diluted in water to achieve the spraying doses.

2.2. Study site, land and experimental plants preparation

The field experiment was conducted in chili farming area in Situgede, Darmaga, Bogor with total area of 1300 m². The trial area was plowed using a tractor and fertilized with the following composition: 200 kg of manure, 100 kg of NPK Mutiara fertilizer, and 50 kg of ZA. The site was plowed according to regular practice of chili farmer and then divided into 40 planting areas (1 x 10 m). Each area was prepared for chili seedlings on 10 x 10 cm of planting space. First, curly chili seeds were sown on nursery beds around the experimental field. The seedlings were transplanted after 14 days after seeding (DAS). Chili seedlings that were unable to survive were replanted with new plant obtained from the commercial vegetable and flower cultivation market in Cipanas, West Java. The total population of survived chili plants on 7 days after planting (DAP) in the trial area was 1180 chili plants. Cultural practices applied were pruning, fertilizing, irrigation, shoot picking and weeding. Fertilizing was done three times using NPK fertilizer, leaf fertilizer and manure while the plants were watered every morning or evening and weeding process was conducted twice a month.

2.3. Evaluation on microbial insecticide formulation in the field

Chili plants were treated with six treatments which consisted of two isolates entomopathogenic fungi *Metarhizium* sp. (isolate B2.2 and T2B23), a consortium of entomopathogenic BLSP4 and Bt, a consortium of T2B23 and BLSP4, Decis® 25 EC and water as positive and negative control. Decis® 25 EC (Bayer Indonesia) contains 25 g/L deltamethrin as active ingredient. Each treatment was conducted in 5 planting areas in which 4 plants were observed and taken as sample in each planting area. Therefore, a total of 20 plant samples for each treatment were used as plant samples.

Spraying application was carried out eight times and divided into 3 stages to the aboveground parts of the plant thoroughly. At the first stage, the spraying was applied to chili plant once a week for 1
month since plant transplanted into the field. The dose used was 200g/100m² for solid inoculum made from *Metarhizium* sp and 1L/100m² for liquid inoculum of BLSP4 + Bt. Then, the second stage spraying was done twice in a month using the same dose as previous stage. The last stage spraying that also carried out twice in a month used 300g/100m² and 2L/100m² for solid and liquid inoculum.

2.4. Observation and data collection
The experimental design used was a randomized block design with 6 treatments and 5 replications. A total of 20 plant samples from each treatment were observed for data collection. The observed parameters were number of plant attacked by insect pest, types of insect pest, plant growth parameters (height and weight of plant at the end of experiment), and weight of harvested chili. Observation of pest attack incidence was conducted 7 times during field trial, every seven days after spraying time, except at the last spraying time. The pest observation data was collected from 4 sampling plants of each treatment by recording the types and number of insect pest found on plants’ surface. For severity of pest attack incidence, scores were made as follows: no incidence; 1 = 1-2% incidence; 3 = 3-5% incidence; 5 = 6-10% incidence; 7 = 11-12% incidence; 9 = >25% incidence.

3. Results and discussion
3.1. Microbial insecticide
Diluted inoculums as microbial insecticide formulation that were ready to applied to chili plants. (Figure 1).

![Figure 1. Mass production of bacterial cells on liquid media (A), conidial production of *Metarhizium* sp. on rice carrier and diluted of inoculums before sprayed to chilli plant (B).](image)

3.2. Efficacy of microbial insecticide in the experimental field
3.2.1. Scoring of plant damage by insect pests. Insect pests that attacked the chili plant in the field were recorded. Most frequently found insect pests were aphids, armyworm, and grasshoppers (Table 1).

| Treatments | Types of insect pest                          |
|------------|-----------------------------------------------|
| Control (-)                        | Ant, Aphids, green grasshopper, brown grasshopper |
| *Metarhizium* sp. T4B23            | Ant, Aphids, green grasshopper, brown grasshopper, army worm |
| *Metarhizium* sp. B2.2             | Ant, Aphids, green grasshopper, brown grasshopper |
| Consortium of Bt +BLSP4             | Ant, Aphids, green grasshopper, brown grasshopper, army worm, planthopper |
| Consortium of microbial entomopathogen | Ant, Aphids, green grasshopper, brown grasshopper |
| Control (+)                         | Ant, Aphids, green grasshopper, brown grasshopper |

The scoring result of insect pest incidence in the experimental area (Table 2) showed that the highest incidence score was 5, detected less than 10% incidence. Among of all treatments, *Metarhizium* sp. T4B23, B2-2, and consortium of microbial entomopathogens treatment did not reach score 5 in 7 times observation.
Table 2. Scoring of pest attack incidence to chili plant in field trial

| Treatments                        | Observation of insect pest incidence |
|-----------------------------------|--------------------------------------|
|                                   | 1st  | 2nd  | 3rd  | 4th  | 5th  | 6th  | 7th  |
| Control (-)                       | 0    | 5    | 5    | 2    | 3    | 2    | 2    |
| T4B23                             | 1    | 0    | 1    | 1    | 2    | 3    | 2    |
| B.2                               | 0    | 2    | 2    | 2    | 1    | 2    | 2    |
| Consortium of Bt+BLSP4             | 0    | 3    | 3    | 5    | 5    | 1    | 1    |
| Consortium of microbial entomopathogen | 0    | 1    | 2    | 2    | 2    | 1    | 2    |
| Control (+)                       | 0    | 3    | 2    | 3    | 5    | 2    | 2    |

Note: 0 = no incidence; 1 = 1-2% incidence; 3 = 3-5% incidence; 5 = 6-10% incidence; 7 = 11-12% incidence; 9 = >25% incidence.

3.2.2. Observation on chili plant growth parameters. The effect of microbial insecticide was also observed on plant growth such as plant height, chilies production per plant, and plant weight at the end of experiment. Measurement of plant growth used 20 plant samples for each treatment. Figure 2 shows the height average of chili at the end of experiment. The figure shows that the highest average of chili plants was found on the plant sprayed with *Metarhizium* sp. T4B23 (70 cm) but insignificantly different to chili plants with negative control treatment (68 cm). Chili plants treated with *Metarhizium* sp. T4B23 also show the highest average of fresh and dry weight of plant mass compared to other treatments, 196 g and 37 g, respectively (Figure 3). Meanwhile, entomopathogenic bacterial consortium treatment resulted in highest yield of harvested chili among all treatment followed by Metarhizium B.2.2 sprayed plants (Figure 4).

Figure 2. Average of chili plants height at the end of experiment.

Figure 3. Average of wet and dry weight of chili plants weight at the end of experiment.
Regarding of very limited water supply at the initial stage of planting, most of chili plants seedling were unable to survive after transplanting process. Therefore, the dead chili seedlings were replanted with new plants from vegetable farming market. The experiment was then continued after frequency of rainfall increased. Chili planting is very dependent on season so that most farmers begins to sow chili seed in dry season due to higher risk of crop failure or yield loss in the rainy season [9]. However, insect pest causes more destruction in dry season, therefore pesticide usage is increased. This study showed the highest incidence of insect pest in control treatment was relatively low, 6-10%. This condition can be caused by experiment carried out during the transition period from the dry season to the rainy season.

The fungal based insecticide used was produced on rice carrier media. Rice was chosen as carrier because the entomopathogenic fungi isolates grew and produced optimal amount of conidia in it, although other alternative carrier media such as corn, wheat, or sorghum can also be used. Rice is suitable for the mass propagation of entomopathogenic fungal conidia because it is common and accessible to find on the Indonesian market and cheaper than wheat, sorghum and PDA. Compared to corn, rice is easier to cook and process, so it requires lower energy.

According to insect pest observation, some insect pests were found in the field, such as aphids, army worm and grasshoppers in large numbers and at certain periods (Figure 5). The grasshopper are short harned species, belongs to the Order Orthoptera, Family Pyrgomorphidae with green or brown in colour and belongs to chewing insect. The insect undergoes partial or incomplete metamorphosis (hemimetabolous) and also a polymorphism. Aphids and army worm are considered as major insect pest of chili farming [10] due to its very rapid population increase and wide host range subsequently causing extreme damage in short time. Aphids belongs to the Order Hemiptera Family Aphididae, a small sized insect (1/32 to 1/8 inch) [11] and including a sap sucking insect. Aphid causes direct and indirect damage to chili plant [12]. Directly, aphids infest their host by sucking fluid epidermal cells of leaf while indirectly, this insect excretes a sugar-rich sticky liquid waste that promotes growth of mold causing disease on host plant resulting in leaf curling and inbition of plant growth. Furthermore several aphids species are vector for plant viral diseases[14]. Army worm included in the Order Lepidoptera Family Noctuidae. The most destructive species is the larval phase of insect pest known as armyworms. Characteristics of this larvae are green with black lines on the abdomen with a body size of \( \pm 2 \) cm. Army worms not only cause damage to the leaves, but also in the generative phase of chili by acting as a fruit borer then causing crop failure.

**Figure 4.** Average of harvested yield per 20 plant samples at the end of experiment.
The most frequently encountered insect pest in the field trial: A) aphids, B) army worm, C) grasshopper

According to scoring of pest attack incidence to chilli plant during field trial, microbial insecticide treated plants showed lower level of insect pest attack at the early stages of plantation compared to negative control treated plant. The highest incidence score (level 5) was just observed at the 4th observation while plants treated as negative control already shown 6-10% pest attack incidence at the 2nd observation. Furthermore, of all treatment treatment of entomopathogenic fungus Metarhizium sp. T4B23 had incidence score that never reaches level of 5 during the observation period. This is suggesting that application of bio-insecticide from the beginning of planting season can be considered as an effective means to prevent the attack of large population of insect pest. Spraying application was carried out in 3 stages, every week or once in two weeks, to ensure the continuity of the prevention of microbial insecticides against pest attacks. The different doses used in the last spraying were intended to anticipate higher possibility of insect pest attacks when plant begin to produce chili.

In addition to observation data on pest attack incidence and their damage, amount of harvested yield at the end of experiment was used as supporting data to see the relationship between bio-insecticides and plant growth. There was a positive relationship between low incidence of insect pest attack and chili production. Chili plants that were sprayed with microbial insecticide had low incidence of insect pest attack and then produced more chillies than that of negative control, and even positive control treated plants. The exception was shown for plant treated with consortium of microbial entomopathogen that produced less chili than that of positive control, but the chili production was still higher than that of negative control treated plant. Chili plants sprayed with consortium of bacteria produced highest amount of chili of all treatment despite having higher level of insect pest attack compared to positive control at 4th observation time and low performance of plant mass suggesting that bacterial based insecticide might potentially increase plants yield. Although not significantly affected harvested yield, plant mass parameters are used to provide prediction about crop yield. Other study has reported similar result using spraying of entomopathogenic fungi as foliar application in combination with different organic amendments such as farm yard manure, neem cake and phosphate solubilizing Pseudomonas fluorescens on chilli in field experiment has shown promising to reduce sucking pest population and increase yield [14]. Oil-based formulation of B. bassiana sprayed with a controlled droplet application has also reported lower incidence of chilli mite compared to other delivery equipments and talc-based formulation [15]. These results indicate that entomopathogenic microbe-based bioinsecticides have the potential to increase plant growth and production rather than merely acting as biological agents to control insect pests in chilli plant.

4. Conclusion
Scoring of pest attack incidence to chilli plant during field trial showed that spraying of bio-insecticide is well applied as soon as seedling transplantation is carried out before insect pests attack. Other than that, important insect pests on chilli plant such as S. litura were relatively low encountered. Plant growth parameters also indicate that the application of bio-insecticides does not interfere with the growth of
chili plants, chilli plants treated with *Metarhizium* sp. T4B23 had the highest plant height and weight. Moreover, the highest yield was obtained from plant treated with bio-insecticides based on Bt + SP4 of all treatment. The entomopathogenic bacteria based bioinsecticide is also very potential to be further developed because the liquid formulation poses its own challenges for shelf life and storage.

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