Genetic diversity of Trichoderma spp. from tidal swamp lands of South Kalimantan

by Noor Aidawati

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INVESTIGATION OF *B. BASSIANA* PERSISTENCE AND VIRULENCE FACTOR AGAINST *P. XYLOSTELLA* OF PEATLAND IN CENTRAL KALIMANTAN

ICI PITER KULU[1]*, ABDUL LATIEF ABADI[2], AMINUDIN AFANDHI[2] AND NOORAIDAWATI[3]

[1]Faculty of Agriculture, Palangka Raya University, Indonesia
[2]Faculty of Agriculture, Brawijaya University, Indonesia
[3]Faculty of Agriculture, Lambung Mangkurat Univeristy, Indonesia

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Abstract - Biological control using Beauveria bassiana has very low conidial virulence and persistence. This research aims to study the virulence and persistence factors of *B. bassiana* against *Plutella xylostella*, which was isolated from peatland in Central Kalimantan, Indonesia. There were four isolates of *B. bassiana* (B1M3T3S2, B1P1T1S1, B1M3T3S1, and B2P3T3S1), which were arranged in a Complete Randomized Design. Virulence was tested on *P. xylostella* larvae. Mortality of larvae was observed. Persistence was determined by the calculation of conidia numbers after UV irradiation. Conidia numbers were calculated after inoculation. The virulence test results showed that B1M3T3S2, the isolate with a conidia concentration of 10^8 conidia/mL, caused the highest mortality in *P. xylostella* larvae. The persistence test showed that B1M3T3S2 had the densest spore and highest growth rate. B1M3T3S2, an indigenous isolate, was the best isolate as a biological control agent of *P. xylostella*.

INTRODUCTION

Plutella xylostella Linn. (Lepidoptera; Plutellidae) is the main pest that attacks Brassicaceae farming in Central Kalimantan Province, especially in Palangkaraya, which has the largest production of vegetables. This pest will cause 70-80% damage to farming and without synthetic pesticide application it will cause 100% damage. In the dry season, the harvest will fail if this pest is not controlled.

Commonly, farmers on the mustard farms of Kalampangan District, Central Kalimantan, use synthetic insecticides to control *P. xylostella*. Using synthetic insecticides will have negative effects on the environment, such as the mortality of non-target insects as natural enemies (predators and parasitoids) and cause insecticide residue. In addition, long-term pesticide use can cause pest-resistance to pesticides. In order to minimize the utilization of synthetic pesticides against *P. xylostella* a biological control is required as an ecofriendly alternative solution. Biological control is chosen as an alternative method because it has no negative effects on the environment. One biological control of *P. xylostella* is the application of the entomopathogenic mold Beauveria bassiana.

B. bassiana mold can be found easily worldwide due to its multitude of host variations. Hosts of *B. bassiana* are mostly from Lepidoptera, Coleoptera, Hemiptera, Diptera, and Hymenoptera orders (Tanada and Kaya, 1993). Species diversity of B. bassiana is shown in its differentiation of pathogenicity level (Hajek and Leger, 1994). According to previous studies, the differentiation of *B. bassiana*'s pathogenicity level will determine its physiological activity in the biological control of the coffee pest *Heliothis hampei* (Varela and Morales, 1996). One problem of using *B. bassiana* as a biological control is its low conidial persistence, which is caused by environmental factors. Beside of that, another problem is the limitation of its

***Corresponding author's email-** ici_kulu17@yahoo.com

virulent isolate availability. Therefore, this research was done to study the persistence and virulence level of *B. bassiana* against *P. Xylostella*, which was isolated from peatland in Central Kalimantan, Indonesia.

MATERIALS AND METHODS

Materials used in this study were: potato dextrose agar yeast medium, peptone dextrose agar yeast medium, peatland sample, four *B. bassiana* isolates (B1M3T3S2, B1P1T1S1, B1M3T3S1, and B2P3T3S1), sterile aqua dest, and 2nd instar of *P. xylostella* larvae (Kulu *et al.*, 2015). Research was carried out on a mustard farm. The equipments wee used included: scale, hand tally counter, BX41 microscope with DP 26 camera, micropipettes, shaker, incubator, medium bottle (250 mL), oose needle, bunsen, object glass, cover glass, laminar airflow, autoclave, and plastic jar.

P. xylostella larvae breeding

P. xylostella larvae were collected from the mustard farm. Larvae were maintained in a plastic jar and fed daily with fresh mustard leaves. The imago which emerged from the pupa were then transferred into a bigger jar and fed with a diet containing 10% honey. Eggs were bred from the imago, then collected into reaction tubes until they became 1st intar larvae. Instar I larvae were taken care of until they became 2nd instar larvae for use in the virulence test.

Virulence test of *B. Bassiana* against *P. xylostella* larvae

This research used 1-day-old 2nd instar of *P. xylostella* larvae. Conidia isolates were 10 mL in volume with concentrations of 10^2 conidia /mL, 10^4 conidia /mL, 10^6 conidia/mL, and 108 conidia /mL. *B. Bassiana* mold suspensions were sprayed on mustard leaves with 20 *P. xylostella* larvae. Treatment was repeated three times. Larvae mortality was evaluated every day for 7 days after inoculation of *B. Bassiana* mold. The experiments were arranged in a completely randomized design (CRD) analysis with four isolate variations. The obtained data were analyzed by variance and Duncan's test at 5% of real level.

Ultraviolet (uv) irradiation on B. bassiana

B. bassiana persistence level was based on the number of conidia surviving after UV irradiation of varying time lenghts (0, 30, 60, 120, and 240 minutes). The experiments were arranged in CRD analysis by using four isolate variations and triplication. The obtained data were analyzed with variance and Duncan's test at 5% of real level.

B. bassiana persistence test in peatland

Peatland sample was sifted and measured for 1 kg, then sterilized using an autoclave on 121 °C, 1.5 atm for 20 minutes. Sterilized peatland was put into a plastic box measuring 25 × 12.5 × 8 cm. *B. bassiana* suspension with a concentration of 10^8 conidia/mL was sprayed onto the sterilized peatland and mixed thoroughly. Observations were made at 0, 24, 48, 72, 96, and 120 hours after inoculation. The experiments were arranged in CRD analysis by using four isolate variations and triplication. The obtained data were analyzed with variance and Duncan's test at 5% of real level.

RESULTS AND DISCUSSION

Virulence of *B. bassiana* isolate against *P. xylostella* pest from peatland in Central Kalimantan

Virulence tests were performed to evaluate the effectiveness of four *B. bassiana* isolates against *P. xylostella* larvae. The mortality percentage of *P. xylostella* larvae after *B. bassiana* application is presented in Figure 1. The results show that all treatments by *B. bassiana* isolate at a concentration of 10^8 conidia/mL show the highest mortality percentage of *P. xylostella* larvae among other concentrations. Isolate B1M3T3S2 with a concentration of 10^8 conidia/mL shows the highest percentage of *P. xylostella* mortality from the fourth until the eight observations. Otherwise, the mortality rate of *P. xylostella* in all of the other treatments is significantly higher than that of the control (Figure 1).

The results of this study show that all of the isolates in a concentration of 10^8 conidia/mL can induce the highest percentage of mortality in *P. xylostella*. According to a previous study, *B. bassiana* is a potential biological control agent for *P. Xylostella* (Valda *et al.*, 2003). This study shows that *P. xylostella* larvae mortality percentage is about 48.75-87.5%. Another study also showed a similar result, with *B. bassiana* causing *P. xylostella* larvae mortality of about 20-94% (Godonou *et al.*, 2009).

Persistence level of *B. bassiana* isolate from peatland in Central Kalimantan against UV irradiation

The persistence of *B. bassiana* isolates were measured from the calculating their conidia, which were irradiated with UV light for varying times of exposure. Those isolates with the highest conidial density have the potential to be used as biopesticides. The average number of *B. bassiana*

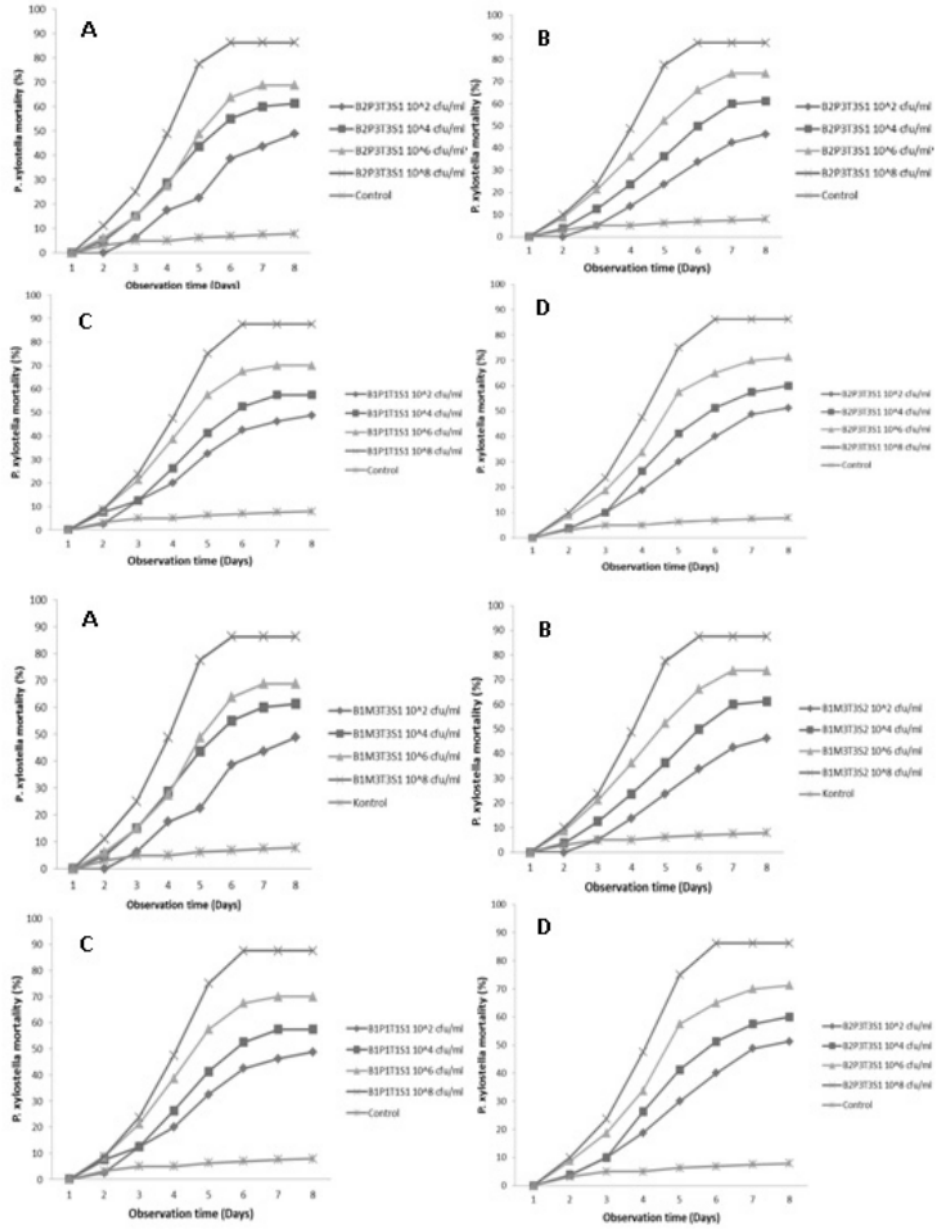

Fig. 1 Percentage of P. xylostella mortality after *B. bassiana* application (A) B1M3T3S1 (B) B1M3T3S2 (C) B1P1T1S1 (D) B2P3T3S1.

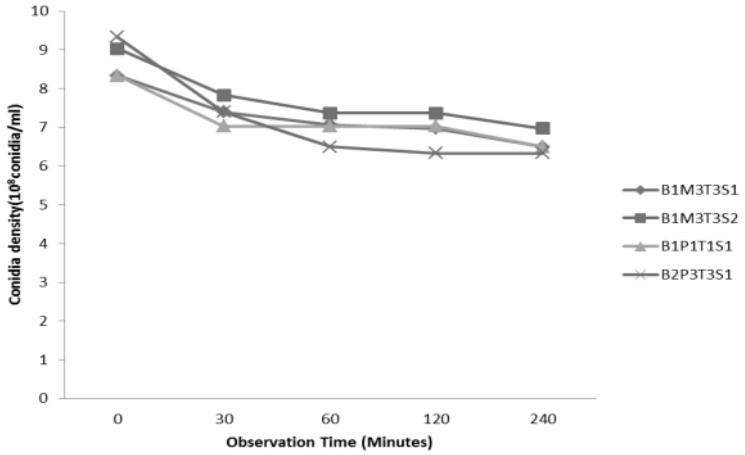

Fig. 2 Mean of the number of *B. bassiana* conidia that survive after UV irradiation under various time of exposure.

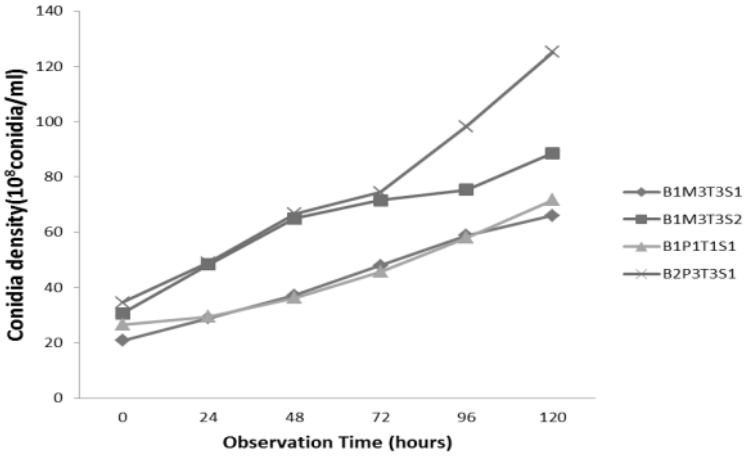

Fig. 3 *B. bassiana* conidial density in peatland after innoculation at varying observation times.

conidia is shown in Figure 2.

UV irradiation time of exposure influences conidia density of *B. bassiana*. The longer the time of exposure, the lower the conidia density. It can be expected that UV light causes damage and inhibits conidial sporulation of *B. bassiana*. This is related to a previous study in which it is stated that UV light has a negative effect on *B. bassiana* and reduces conidial sporulation ability (Trizelia, 2005). UV from direct sunlight can deactivate entomopathogenic mold because it causes cell damage. Cell damage can be expected as a result of DNA mutation after UV irradiation, which causes cell death. Another study states that UV irradiation can cause DNA mutation in microorganisms, which leads to cell death (Inglis *et al.*, 1999). Because of that, UV light can determine the effectiveness of *B. bassiana* as a biological control.

Persistence of B. bassiana in peatland

The persistence of *B. bassiana* provides it with an ability to survive in peatland, which can be observed after the suspension inoculation in peatland with time variations. Results show that the B1M3T3S2 isolate has the highest growth rate among the others (Figure 3). *B. bassiana* is a mold that can survive in saprophyte soil and inside plant tissue (Bruck and Lewis, 2002). Soil is a natural

ecosystem for entomopathogenic mold, acting as a UV shelter with optimum temperature and humidity. In addition, the mold uses decomposed insects and roots as nutrient sources to survive (Leger, 2008). The persistence of *B. bassiana* in soil is determined by certain factors, such as plant residue, rainfall, temperature, humidity, ground water contents, and pH (Bruck and Lewis, 2002; Lingg and Donaldson, 1981). The results of this study also show that *B. bassiana* can grow in sterile peatland which is in accordance with a study by Ling and Donaldson in which it is stated that B. bassiana will grow better in sterile soil than in unsterile soil (Lingg and Donaldson, 1981). Another study indicated that the survival ability of *B. bassiana* conidia is directly determined by physical factors and soil microbial population (Studdert *et al*., 1990). Conidia will survive better in sandy loam soil with low organic matter than in peatland with high organic matter. These results support the notion that *B. bassiana* can potentially be used as micropesticide that has the ability to survive in any soil conditions.

B. bassiana mold as a biological control agent can be achieved using varying methods. It can be applied directly to ecosystem or by inoculation. The successful rate of *B. bassiana* inoculation is influenced by the similarity between the isolate's original habitat and its place of inoculation. A previous study reported that the application of a *B. bassiana* isolate in a cabbage farm ecosystem, which is a similar environmental condition to the isolate's origin is more persistent than isolates from different environmental conditions (Trizelia, 2005).

CONCLUSION

Various factors can influence the successful use of pathogenic *B. bassiana* mold as a biological control for pest. Pest control will be more effective if a high virulent and persistent isolate of *B. bassiana* is available in environment. Results of this study show that each isolate has different levels of virulence and persistence. The highest virulence level of *B. bassiana* against *P. xylostella* (87.5%) is shown by the B1M3T3S2 isolate with a concentration of 108 conidia/mL. This isolate also shows the highest persistence in a mustard farm after UV irradiation, which supports the theory that it can be used as a biological control agent.

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