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Endophytic seed with *Beauveria bassiana* and liquid compost: control of pest stem borer of corn, *Ostrinia furnacalis* and increase yield resilient in marginal land?

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Abstract. Corn is an important crop after rice. Tompobulu sub-district, Maros, South Sulawesi has a wide marginal land expected to contribute to corn development. One of main pests is *Ostrinia furnacalis*. *Beauveria bassiana* Vuill is an effective biological control agent as it can be associated with part of plants as endophyte. The study focused on the effect of seed submersion mixed with liquid compost into marginal land. Corn seeds were planted in each plot with a depth of 5 cm and each hole was planted with 1 seed with a spacing of 75 x 20 cm. The fungal isolation was obtained from inoculum cadaver larvae of *O. furnacalis* purified on initial agar medium and propagated on corn medium. The trials were designed with a randomized block design (RBD) consisting of 6 trials (Bisi-2 seeds soaked in aquades (A); Srikandi seeds soaked in aquades (B); Bisi-2 seeds soaked in *B. bassiana* 10⁶ spore/ml suspension for 24 hours (C); Srikandi seeds soaked in *B. bassiana* suspension 10⁶ spores/ml for 24 hours (D); Seeds of Bisi-2 soaked in suspension *B. bassiana* 10⁶ spores/ml for 24 hours + liquid fertilizer (E); and Srikandi seeds soaked in *B. bassiana* 10⁶ spore/ml suspension for 24 hours + liquid fertilizer (F). The highest population of larvae and pupae of *O. furnacalis* in treatment B was 8.35 and the lowest population in treatment E was 3.05. There were no larvae infected by *B. bassiana* fungus in the treatment of seeds soaked with distilled water. However, larvae infected with *B. bassiana* fungus was found in the treatment of seeds soaked in *B. bassiana* suspension 10⁶ spores/ml or added with watering liquid fertilizer. The highest weight of seed weight in Bisi-2 seeds soaked with suspension *B. bassiana* 10⁶ spores/ml.

1. Introduction

After rice, corn is strategic commodity in Indonesia since it has economical and political values and directly associates with national food safety and security. In corn, all parts of the plant can be utilized to support livelihoods and livestock [1-3]. In 2017, the Government is targeting corn production in South Sulawesi at 2.9 million tons, although the current production achievement in 2016 is still in the range of 2,065,125 tons to meet the needs of the domestic corn market [4]. The target has not been achieved due to several factors namely the existence of plant-disturbing organisms and climate phenomena (drought and flood). Plant-disturbing organism is one of the factors that often threaten the efforts to achieve a surplus of corn and horribly wider to national food security.



One of the areas determined by the South Sulawesi Government in supporting a 1.5 million tons surplus program is Maros District. This District has an area of 1,619.12 km² which filled by 14 sub-districts and 103 villages. From the 14 existing sub-districts, Tompobulu sub-district is a target area for corn development that has a large enough potency. Therefore, this sub-district was chosen as a research location. Tompobulu sub-district has mostly marginal land with mountainous topography. Most of the population are farmer community.

In corn ecosystem, pest arthropods such as *Atherigona* sp, *Ostrinia furnacalis* and *Helicoverpa armigera* Hubner are always found and *O. furnacalis* is one of the major pests attacks all phases of corn development. Once it attacks in vegetative stage causing severe damage to the leaves, photosynthesis pathway becomes disturbed [5]. Due to serious damage of the plant tissue, pesticide usage is often applied by farmer. Pesticide apply seems to be ineffective since the larva lives inner stem tissue and unexpected consequence of this is resistance and resurgence, death of non-target organisms, environmental pollution and residues. Alternatively, the use of biological control agent is potential. *Beauveria bassiana* Vuill. is a typical fungus as biological control agent that is currently being developed since it has effective and environmentally friendly role. *B. bassiana* was formulated like pellet alginate [6,7]. Testing *B. bassiana* in vivo by plant root submersion was undertaken and the finding suggests that the fungus can live all parts of corn tissue i.e. root, leaf, kernels and stem [7]. Combination between *B. bassiana* and compost has a potential solution for helping the farmer in marginal land. This paper will examine the association of endophytic fungus as biological control agent and liquid compost to increase yield resilient of farmer community in Tompobulu, Maros South Sulawesi.

2. Material and methods

This research was carried out in corn cultivation on farmer's land in Tompobulu Village, Tompobulu Sub-District, Maros District, Laboratory of Pest and Plant Disease Department, Faculty of Agriculture, Hasanuddin University.

2.1. Land preparation

The land used as a research site was cleaned. It was dig over and made into plots with a size of 5 x 5 m totaling 4 plots. The distance between one plot and another plot was 1 m. Corn seeds were planted in each plot with a depth of 5 cm and each hole was planted with 1 seed with a spacing of 75 x 20 cm.

2.2. Propagation of *B. bassiana*

2.2.1. *Preparation of inoculum in the field.* Inoculum cadaver larvae of *O. furnacalis* died by fungus *B. bassiana* were taken from the cornfield. The fungus was grown on PDA media so that the pure isolates of *B. bassiana* were obtained.

2.2.2. *Preparation of agar media and purification.* The agar media was made from 100 g potatoes, 17 g agar, 20 g sucrose and 1,000 ml distilled water. The media was then sterilized by autoclaving for 2 hours. After it cooled down, the media was poured into petridish as a purification medium for *B. bassiana* fungi.

2.2.3. *Propagation of corn media.* Fungus isolate of *B. bassiana* was obtained from inoculum cadaver larvae of *O. furnacalis* which had been purified on media so that it was then propagated on corn media. Corn media was made from corn seeds that are cooked undercooked for 30 minutes then dried, then put into a heat-resistant plastic bag about 100 g then sterilized with 121°C autoclave for 120 minutes pressure 1 atm. *B. bassiana* in corn media was incubated for 14 days and ready to use.

2.3. Preparation of liquid fertilizer

Preparation of liquid fertilizer was begun by making a series of 3 shelter tab. The first shelter was a tub containing cow dung feces, second shelter containing results of the first disposal and third shelter containing the results of discharge in the form of liquid fertilizer. The results of discharge from third tub produced liquid was used to water the plants so that they did not need to apply chemical fertilizers.

2.4. Treatment, observation and data analysis

The study design was a randomized block design (RBD) consisting of 6 trials and each was repeated 4 times with five replications. The initial observation was conducted in 35 days after plating with 7 days- interval. The total was 120 units of corn. For more detail, trials were structured as following;

- B + aq (A) = Bisi-2 seeds submerged with sterile water
- S + aq (B) = Srikandi seeds submerged with sterile water
- B + Bb (C) = Bisi-2 seeds submerged with *B. bassiana* 10⁶ spore/ml suspension for 24 h
- S + Bb (D) = Srikandi seeds submerged with *B. bassiana* suspension 10⁶ spores/ml for 24 h
- B + Pc (E) = Seeds of Bisi-2 submerged with *B. bassiana* 10⁶ spores/ml for 24 h + liquid fertilizer
- S + Pc (F) = Srikandi seeds submerged with *B. bassiana* 10⁶ spore/ml suspension for 24 h + liquid fertilizer

2.4.1. Observation of *O. furnacalis* population. Observation of larvae and pupae populations was carried out on the leaves through transverse traces on the leaves. Young corncob hairs were also carried out on old corn hairs. Larvae and pupae inside the stem were examined by cutting and dividing the stem segments. *O. furnacalis* taken from plantations was later maintained and fed as needed until death.

2.4.2. *B. bassiana* testing on *O. furnacalis* cadaver. Dead *O. furnacalis* cadaver was grown on PDA media. After the cadaver was covered with *B. bassiana*, the fungus was then observed under a microscope to prove the presence of *B. bassiana*.

2.4.3. Measurement of seed weight. Measurements were made by taking one corncob for each treatment and then weighing it. Data analysis was performed at each observation. If between treatments showed significant differences, Duncan tested would be at a level of 0.05.

3. Results and discussions

3.1. Population density of larvae and Pupa *O. furnacalis*

Table 1 demonstrates to have larva and pupa infestation during observation. The presence of larvae and pupae in early observation can associate with plant damage level. There are 5 stages of insect in finding their host namely habitat discovery, host discovery, host recognition, host acceptance and host suitability [8]. The results of observations of *O. furnacalis* population density for seven observations can be seen in the following table:

Table 1. Density of *O. furnacalis* larva and pupa population in plant development after *B. bassiana* apply

Trial	Plant development (day)							Total
	35	42	49	56	63	70	77	
A	0.85	1.25	1.1	1.3	0.8	0.75	0.55	6.6
B	1.2	1.25	1.35	1.75	1	0.65	0.85	8.35
C	0.4	0.75	0.9	0.9	0.6	0.5	0.35	4.4
D	0.6	0.8	1.05	1.05	0.8	0.4	0.5	5.2
E	0.35	0.5	0.6	0.6	0.4	0.3	0.25	3.05
F	0.45	0.8	0.9	0.9	0.45	0.35	0.3	3.95

In table 1, the highest population of larvae and pupae of *O. furnacalis* was found in trial B (8.35) and the lowest population was in trial E (3.05). Overall, the population of larvae and pupae found during the observation were still low in all trials. The finding suggests that the effect of *B. bassiana* was highly likely to affect the pest population. *B. bassiana* suspension was applied through seed submersion due probably to effect of toxin causing undeveloped population (table 1). Beauvericin is a specific toxin released by *B. bassiana* which was detected in culture and its effect caused to damage Lepidopteran due to the function of hemolysis [7].

3.2. *The number of infected larvae of B. bassiana in plant development*

Figure 1 shows that no larva was found to be infected in trial A and B, but in other trials F, the number of larvae were found in C (71.4) , D (52.4), E (81.0) and F (47.6) respectively. The dead *O. furnacalis* larvae infected with *B. bassiana* was checked and found white mycelium like powder on the body. The dead larvae were initially blackish brown. Pest infected by *B. bassiana* is shown to have color change of the body’s cuticle before all body covered [9].

3.3. *Weight of seeds on the cob on each test*

The effect of *B. bassiana* to seed weight is seen in figure 2.

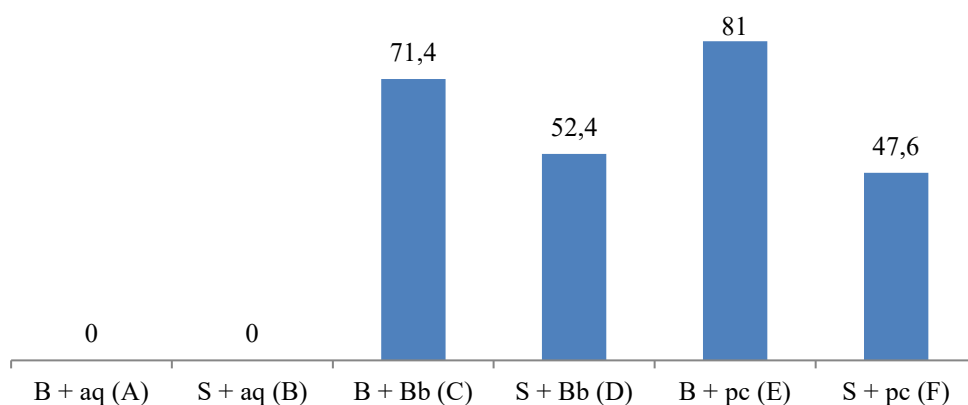


Figure 1. Percentage of *B. bassiana* infected larvae at various plant ages

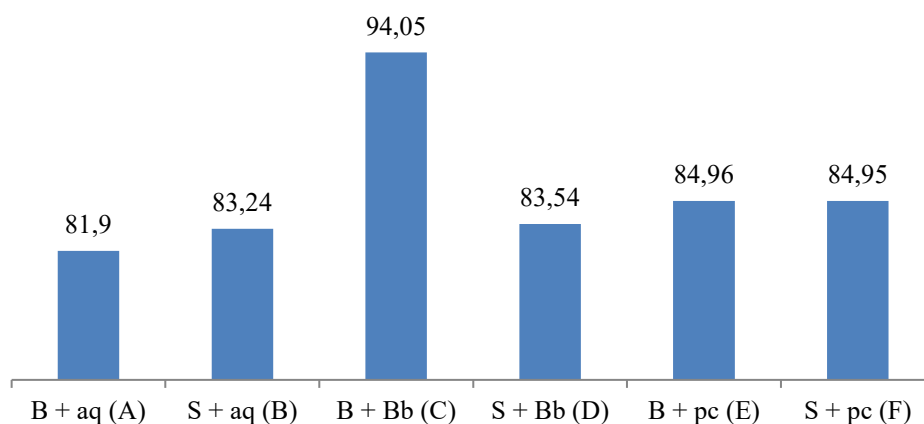


Figure 2. Weight average of seeds per one corncob (g/cob)

The highest average of seed weight was obtained in trial C consisting of combination of Bisi-2 seeds and *B. bassiana* submersion (94.05). the lowest was in control (seed submerged with sterile water). Combination of seed submerged with *B. bassiana* (10⁶) and liquid compost were also much higher than control (84.9) and seems to be insignificant yield with other trials. Organic fertilizer has potential use to support sustainable agriculture particularly soil ameliorants, prevent erosion, soil movement and cracks, improve soil structure, stimulate bacterial growth and propagation. Organic fertilizer contains low levels of N, P, K but contain lots of micro elements. The content of nitrogen in organic fertilizer will be released slowly which will help soil fertility in a long time [10]. Of many benefits of organic fertilizer, the most interesting is that it can be compatibly combined with biological control agent like *B. bassiana*.

4. Conclusion

The average of pest population in field was low and larvae were found to be infected in the plot trials. Combination of seed submersion with 10⁶spores/ml *B. bassiana* suspension and liquid fertilizer were potential to be disseminated to farmer community due to increase of seed weight and control of main pest.

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