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EFFECT OF DIFFERENT FORMULATIONS OF *METARRHIZIUM ANISOPLIAE*-BASED INSECTICIDE ON ITS EFFECTIVENESS AGAINST *SPODOPTERA FRUGIPERDA* ON CORN

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A B S T R A C T

The fall armyworm (FAW) (*Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is one of the most catastrophic pests of corn, the third most important food crop worldwide. In 2019, the pest was first recorded in Indonesia. Currently, the pest has been reported causing damaged to corn crops in most provinces of the country. Farmers depend primarily on insecticide use to manage the new invasive pest. To avoid the potential negative impacts of insecticide use, alternative control measures should be developed, including biological control. Fungal entomopathogen, *Metarrhozium anisopliae,* has been used as an effective mycoinsecticide against the pest in other countries. Formulation is very important to be considered in developing a new bioinsecticide because it affects the effectiveness, sporulation rate, and conidial viability of the entomopathogen. Thus, the study objectives were to determine larval mortality of *S. frugiperda* applied with different formulations of *M. anisopliae.* In addition, conidial density and viability of *M. anisopliae* in different formulations and incubated at different temperatures were also evaluated. The results showed that *Metarrhizium anisopliae,* prepared in powder formulation had better qualities in comparison to those cultured in pellet and pasta formulations. The fungus cultured on rice powder had higher pathogenicity against *S. frugiperda* larvae. Ten days after powder formulation was applied, 76% of the treated insects died, which was significantly higher than the percentages of dead insects in pellet and pasta formulations (56 and 52%, respectively). Similarly, conidial density was significantly higher for powder (5.21 x 10^6 conidia/ml) than pellet (2.79 x 10^6 conidia/ml) and pasta (0.85 x 10^6 conidia/ml) formulations. The percentages of the treated larvae becoming pupa were 11, 21, and 22% for powder, pellet, and pasta, respectively. Therefore, the powder formulation should be used in proliferation of the fungus in mycoinsecticide production.

Keywords: Corn, *S. frugiperda*, *M. anisopliae*, Formulation, Viability, Virulence.

INTRODUCTION

The fall armyworm (FAW) (*Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is one of the most damaging pests of corn (*Zea mays* L.) (Goergen *et al*., 2016). The larvae feed on young curl of leaves, ears, and tassels, inflicting significant loss to corn crop (Cruz *et al*., 1983). The insect can cause yield losses up to 58% on corn (Kumar *et al*., 2022; Kenis *et al*., 2022). This pest has been reported attacking more than 350 plant species, including several important food commodities, such as rice and sorghum (Montezano *et al*. 2018). *Spodoptera frugiperda* is originally from tropical and subtropical areas of America and currently has been found in 109 countries all over the world (Kenis *et al*., 2022).

In Indonesia, FAW first reported in the provinces of West Sumatera (Sartiami *et al*., 2019) and South Sulawesi (Nonci *et al*., 2019). Currently, the pest has been reported in most provinces of the country and has caused substantial losses to farmers (Thamrin *et al*., 2022). Because the insect is a new invasive pest in the country, the farmers primarily rely on the use of synthetic insecticides to manage the pest. However, excessive use of insecticide can raise more problems, including insecticide resistance, natural enemy's death, and health and environmental issues. Fall armyworm has been reported resistant to numerous insecticides in Puerto

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Rico, including flubendiamide, chlorantraniliprole, and methomyl (Gutiérrez-Moreno *et al*., 2019). Therefore, alternative control measures that are effective but safer to non-target organisms and the environment, such as biological control agents should be developed. Entomopathogenic fungi are potential substitutes to insecticides because they are effective, efficient, target-specific, safe to non-target insects, easy to develop, and environmentally friendly (Uge *et al*., 2021; Susanti *et al*., 2023). One of the most commonly used entomopathogenic fungi in pest control is *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ascomycota: Hypocreales) (Chaneiko *et al*. 2019). The fungus accounted for 34% of all mycoinsectides available commercially worldwide (De Faria and Wright, 2007; Yin *et al*., 2021). *Metarhizium anisopliae* has several properties that make it ideal to be incorporated into an integrated pest control (IPM) strategy, namely it is virulent to all life stages of its insect hosts, does not leave toxic residues on plants like other chemical pesticides do (Manson and O'Sullivan 2006), and does not harm human health (Humairoh *et al*., 2013). In addition, *M. anisopliae* can live endophytically in plant tissues so it enhances pest control effectiveness (Aynalem *et al*., 2022). This fungus weakens and eventually kills its insect hosts by infecting them. The infection process begins with penetration through the insect cuticle, either mechanically or chemically by utilizing toxin actions (Syahrawati and Mardiah, 2011). *M. anisopliae* produces cyclopeptide toxins, destruxin A, B, C, D, E, and desmethyldestruxin (Fauzana *et al*., 2020). The fungal entomopathogen is highly pathogenic against *Spodoptera* spp., including *S. frugiperda* (Permana *et al*., 2022; Bharathi *et al*., 2022; Gomes *et al*., 2023; Wang *et al*., 2023).

In utilizing this fungus, efforts are needed to maintain its effectiveness and viability for long period of time by using appropriate formulations. Viability is a very important parameter because it determines the growth and the virulence of the fungus (Bamisile *et al*., 2020). The right formulation produces products that have viability and virulence that remain stable when applied in the field and kept in the storage for long period of time (Pasau, 2022). Liquid suspension and dry solid, like powder, pasta, and pellets formulations can be used for mycoinsecticides. Bioinsecticide formulation is used to keep fungal entomopathogens alive, both in dormant and actively growing states (Lapinangga and Bunga, 2023). Therefore, the study objectives were to determine: 1) larval mortality of *S. frugiperda* applied with different formulations of *M. anisopliae*, conidial density and viability of *M. anisopliae* prepared in different formulations and incubated at different temperatures.

MATERIALS AND METHODS

The study was conducted at Biological Control Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Hasanuddin University, Makassar Indonesia.

Conidial Isolate and Formulation Preparation: *Metarrhizium anisopliae* isolate used in this study was obtained from the collection of the Center of Food and Horticultural Crop Protection, Maros District, South Sulawesi Indonesia. The isolate originally isolated from infected *S. frugiperda* in the field. The isolate was grown and purified on potato dextrose agar (PDA) and then mass produced using a rice medium, consisting of 90 g of rice combined with 1% cricket powder. The ingredients were soaked in sterile water for two hours before being placed into heat-resistant plastic ziplock bags and sterilized in an autoclave. After sterilization, it was cooled down before being inoculated with *M. anisopliae* culture grown on PDA. The bags were then incubated in room temperature until the whole rice medium was covered with the fungus. The fungal culture was then used to make mycoinsecticide formulations by following Daud *et al*. (2023).

Pasta formulation: Pasta formulation was made from a mixture of 40 g spore biomass powder with 1.6 g glycerol, 1.7 g N-alginate, 0.2 g urea, and 56.5 ml water. Spores, glycerol, urea, and alginate were put in a container and then mixed evenly. The water was slowly added into the container and the dough was stirred until well mixed to form a pasta formulation.

Powder formulation: Powder formulation was made from 100 g rice media that has been overgrown with *M. anisopliae* fungus from mass propagation. The rice was mashed using a blender to form a smooth powder formulation.

Pellet Formulation: For the pellet formulation, 50 g of spore biomass powder was taken and then mixed with 10 g CMC and 30 ml warm water in one container, then stirred until well mixed and formed a dough. The dough was then molded using a pellet maker to form a pellet formulation.

Greenhouse Experiment: A greenhouse experiment was conducted to determine the mortality rates of FAW larvae applied with *M. anisopliae* prepared in different

formulations: powder, pasta, and pellet.

Insects Culture: To ensure the availability of assay insects, FAW was mass reared in the Laboratory of Plant Pest, Hasanuddin University by following the procedures described by Yusri (2023). The insects used in this study were initially obtained as larvae from corn plantations in Makassar City. *S. frugiperda* larvae were fed on baby corn in plastic containers. After the larvae enter the pupal stage, they were moved into a plastic container containing sterile sand which had been given a little water to keep it humid. After the insect enters the imago stage, the imagoes were moved into a cage using a test tube. The imagoes were fed with 10% honey solution absorbed in a cotton ball hung from the top of the cage using a thread. Fresh baby corn leaves were placed inside of the cage as a substrate for egg laying by the imagoes. The eggs were reared to obtain uniform third instar larvae that were used in the assays.

Corn Plants: Two seeds of corn cv. Ketan Hybrid F1 were planted in each polybag (16 x 20 cm), containing a mixture of top soil and chicken manure compost (2:1). After emergence, thinning was performed and only one plant per polybag was allowed to grow. The plants were maintained by applying NPK fertilizer ten days after planting and watering as needed. Fourteen days after planting, two *S. frugiperda* 3rd instar larvae were infested on the growing point of each test plant using a fine brush.

Conidial Density: Four formulation treatments were evaluated for FAW mortality in the greenhouse, namely P1= Control (distilled water spray), P2 = Pasta formulation, P3 = Pellet formulation, and P4 = Powder formulation. The study was conducted in a completely randomized design with five replicates of five plants each. Before the formulation is applied to corn plants, each type of formulation was separately suspended in distilled water (30 g formulation per 500 ml distilled water). Conidial density of each formulation suspension was determined by using the following formula (Gabriel and Riyanto, 1989):

$$S = \frac{t\ x\ d}{n\ x\ 0.25}$$

S = number of spores/ml, *t* = total number of spores in the observed sample box, *d* = dilution factor, *n* = number of sample boxes observed, 0.25 = a correction factor for the use of small-scale sample boxes in Haemocytometer.

Application of *M. anisopliae* on Corn Plant: *anisopliae* suspension was applied to 14-day old test plants in the afternoon which had previously been infested with 3rd instar larvae at the growing point of the plants. The application was conducted using a hand sprayer with a spray volume of 10 ml per plant. Mortality rate for each treatment was determined by calculating the percent of test insects died due to the entomopathogen. Mortality was calculated using the following formula (Masyitah, *et al.,* 2017):

$$M = \frac{n}{N} \text{ x } 100\%$$

M = mortality, *n* = number of dead insects, *N* = Total number of insects tested.

The first observation was conducted 24 h after the application and every 24 h thereafter for 10 observations.

Pathogen Reisolation: To confirm that the death of the test insects was due to the *M. anisopliae*, the dead insects were surface sterilized using 2% sodium hypochlorite before being placed inside Petri dishes contain PDA. The growing fungus was identified under a dissecting microscope (400x).

Laboratory Experiment: Viability Test: A laboratory experiment was conducted to determine conidial viability of *M. anisopliae* prepared in different types of formulation at different times after application. The treatments were arranged in a completely randomize design in factorial with five replications. The first factor was type of formulation, namely: P1 = Pellet formulation, P2 = Pasta formulation, and P3 = Powder formulation. While, the second factor was storage temperature, namely: 12^0C and 28^0C. To assess the impact of formulation type on the viability of *M. anisopliae* conidia, one g of each formulation was suspended in 40 ml sterile aquadest. One ml of the mixture was diluted in 9 ml of distilled water. One ml of the dilution was put and spread evenly on PDA surface in a Petri dish and then incubated for 24 h in room temperature. The numbers of germinating and non-germinating conidia were counted under a dissecting microscope (400x). Spore viability was calculated using the formula of Gabriel and Riyatno (1989) as follows:

$$\boldsymbol{V = \frac{g}{(g+u)} \times 100\%}$$

V = Conidia viability, g = number of germinating conidia, u = number of ingeminating conidia

The procedures were repeated every seven days for a total of four times.

STATISTICAL ANALYSIS

Data were subjected to arcsin transformation before being analyzed using ANOVA. If significant differences were detected among the treatments, the treatment means were compared using the Least Significant

Difference (LSD) test at 5% significance level.

RESULTS

FAW Larva Mortality: Figure 1 show *S. frugiperda* larva mortality treated with different types of formulation of *M. anisopliae* and observed at different times after the application. In general, the mortality rates for all treatments steadily increased from time to time. For observations 4, 5, 6, 9, and 10, the highest mortality rates were found in powder formulation and they are significantly different from the other formulation treatments. However, for observations 1, 2, 3, 7, and 8, no significant differences among all treatments were detected. Overall, the highest mortality rate was found in the powder formulation, followed by pellet and pasta formulations; and no mortality was found in the control. In the last observation, the powder formulation had the highest mortality (76%), followed by the pellet and pasta formulations with mortality rates of 56 and 52%, respectively. Re-isolation procedures confirmed that all dead larvae were caused by *M. anisopliae* infection.

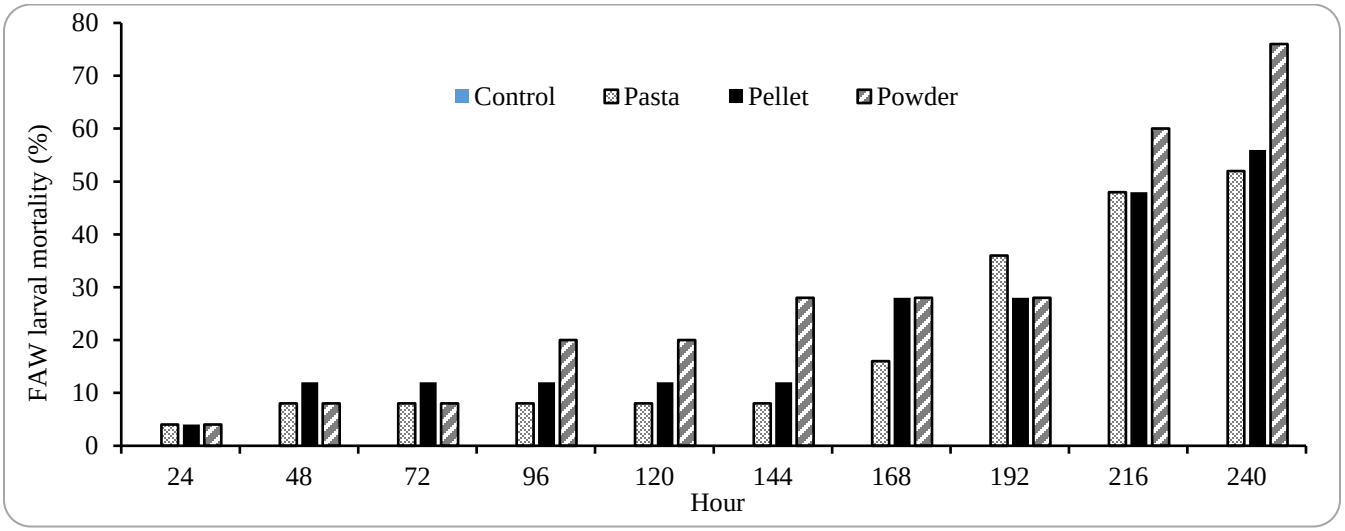

Figure 1. Average larval mortality of *S. frugiperda* applied with different formulations of *M. anisopliae*, powder, pellet, and pasta.

Percentage of Larvae Becoming Pupae: Figure 3 shows percentage of larvae that became pupae after being applied with different formulations of *M. anisopliae*. The lowest percentage of larvae becoming pupae occurred in the powder formulation (11%), which was significantly lower than the other treatments. Twenty-two and 21% of the treated larvae became pupae for pasta and pellet formulations, respectively, which were not significantly different from each other but significantly higher than the powder treatment and significantly lower than the control.

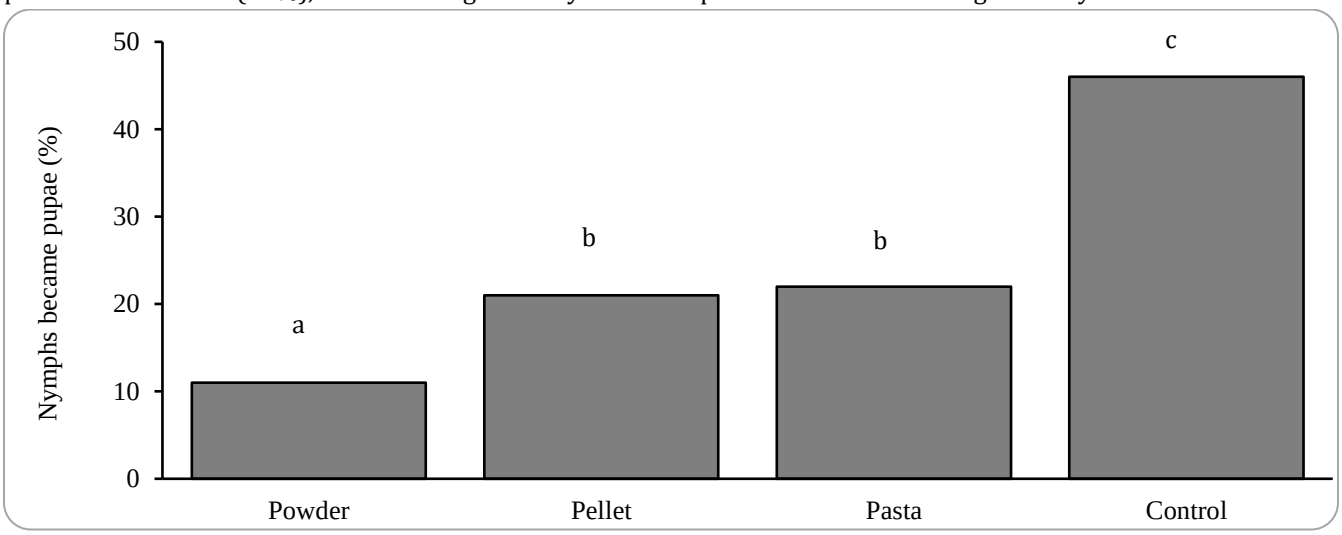

Figure 2. Percentage of *S. frugiperda* larvae developing into pupae on different formulations. Bars with different letters are significantly different ($P < 0.05$).

Conidial Density of *M. anisopliae*: Figure 2 shows the effect of formulation types on the viability of *M. anisopliae* conidia. The highest conidial density was found in powder formulation, followed by pellet and pasta formulations which were 5.21 x 10^6, 2.79 x 10^6, and 0.85 x 10^6 per ml, respectively. Mortality rates among the treatments were significantly different from each other.

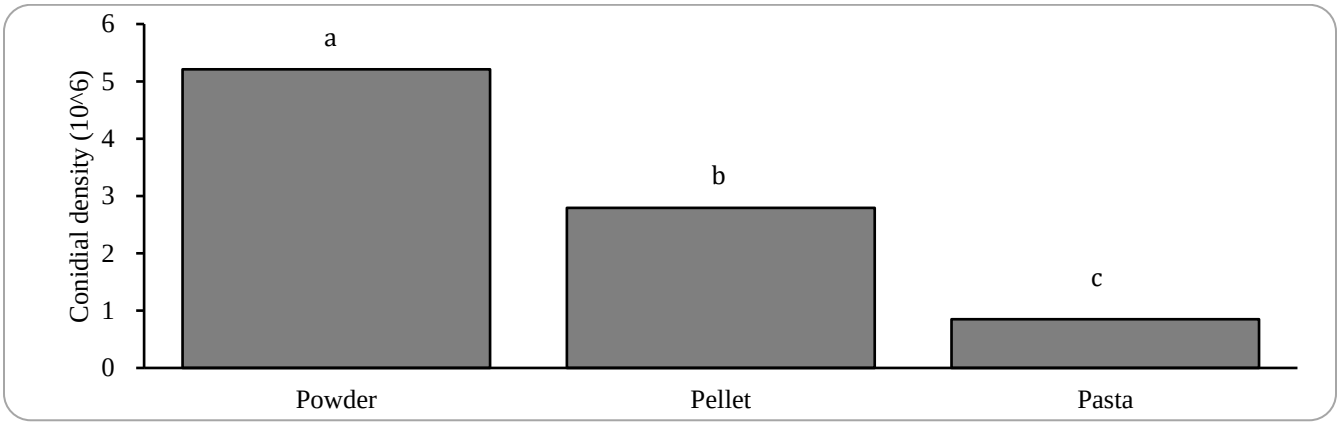

Figure 3. Conidial density (x 10^6) of *M. anisopliae* on different formulations: powder, pellet, and pasta. Bars with different letters are significantly different (P < 0.05).

Conidial Viability: Table 1 shows that for each formulation type, incubation temperature did not significantly affect the spore viability on each observation day, except powder formulation showed significantly higher viability at 12^0C (70%) than 28^0C (38%) on the day 14 observation. On day 7 and 28, significant differences among the treatments were detected on 28^0C treatment. On day 7, powder had significantly lower spore viability than the other formulations, but on day 28, powder had significantly higher spore viability than the other formulations. On day 14 and 21, significant differences among the treatments were detected on 12^0C treatment. On day 14, powder had significantly higher spore viability than the other formulations, but on day 21, powder had significantly lower spore viability than the other formulations.

Table 1. Average percentage of germinating conidia (conidial viability) of *M. anisopliae* on different formulations (powder, pellet, and pasta) and incubation temperatures.

Formulation	7 days		14 days		21 days		28 days	
	12^0C	28^0C	12^0C	28^0C	12^0C	28^0C	12^0C	28^0C
Powder	53 ± 8.8aA	39 ± 6.8bA	70 ± 6.4aA	38 ± 7.8aB	50 ± 1.1bA	53 ± 10.1aA	54 ± 7.9aA	75 ± 7.7aA
Pasta	59 ± 2.2aA	63 ± 4.5aA	47 ± 6.5bA	45 ± 9.9aA	59 ± 3.8aA	48 ± 9.5aA	58 ± 7.4aA	43 ± 8.8bA
Pellet	65 ± 5.1aA	65 ± 7.0aA	50± 11.6bA	35 ± 8.0aA	63 ± 9.5aA	50 ± 8.5aA	56 ± 8.9aA	42 ± 9.3bA

Numbers within a column with the same lowercase letters are not significantly different, while the numbers within a row on the same observation date, with the same uppercase letters are not significantly different (LSD, P < 0.05).

DISCUSSION

Spodoptera frugiperda is a new invasive insect pest of corn that was reported for the first time in 2019 in Indonesia (Sartiami *et al*., 2019; Nonci *et al*., 2019). As an emergency measure, farmers use primarily insecticides to control the new pest. To avoid the negative impacts of excessive use of insecticides, other safer and environmentally friendly control techniques must be developed, such as the use of fungal entomopathogens incorporated into an integrated pest management strategy. One of the entomopathogens that have been reported effective in managing *S. frugiperda* in other places is *M. anisopliae*. In developing a mycoinsecticide, formulation must be carefully chosen because formulation affects virulence, viability, sporulation, shelf life, and application practicality of the product. *Metarrhizium anisopliae* isolate used in this study was originally obtained from Lepidopteran larvae collected from corn fields in South Sulawesi. Local isolate is preferred because it has adapted to local physical and

biological environments. The study was conducted to assess: 1) larval mortality of *S. frugiperda* applied with different formulations of *M. anisopliae* and 2) conidial density and viability of *M. anisopliae* prepared in different formulations and incubated at different temperatures.

The study results revealed that larval mortality rates differed among the formulation treatments (Fig. 1). For powder formulation, larva mortality rate tended to be higher than the other formulations tested. At the last observation (240 h), mortality rates were 76, 56, and 52% for powder, pellet, and pasta formulations, respectively. In contrary, significantly lower percentage of larvae becoming pupae was found in powder formulation; however, percentages of larvae becoming pupae in pellet and pasta formulations were not significantly different each other (Fig. 2). Nutrients in powder formulation are more easily available to the fungus, allowing the fungus to grow quickly and produce more mycotoxin. This is in agreement with Pasau *et al.* (2022) reporting that entomopathogenic fungi that grow on powdery media produce more mycotoxin than those cultured on solid media. The pathogenicity mechanism of entomopathogenic fungi begins when the spores adhere to the outermost cuticle layer of the insect body. Under favorable conditions, after germinating, the spores gradually penetrate the insect cuticle. The fungus secretes toxic chemical compounds into the hemolymph of the insect to overcome its immune system, and it eventually kills the insect. In addition, medium used to grow entomopathogenic fungi also affects fungus sporulation. Our results also showed that powder formulation had the highest conidial density among the treatments (Fig. 3). This is in agreement with Pasau *et al*. (2022) that the carrier material has a significant effect on the density of *M. anisopliae* spores. The success of controlling a pest is highly dependent on the concentration of entomopathogenic fungus spores applied. The more spores attached to the target host, the faster it infects and kills the host (Saputra *et al*., 2013; Daud *et al*., 2019).

Conidial viability (percentage of germinating conidia) is influenced by temperature, humidity, pH, sunlight radiation, and carrier nutrient content. Suitable temperature and humidity for storage reduces the dehydration of the fungus so it can be stored for a long period of time (Hastuti, 2017). However, the temperatures, 12 and 28^{0}C, tested in this study, did not significantly influence the spore germination capacity (Table 1). This is probably attributable to the tested temperatures were within the optimum temperatures for *M. anisopliae* growth. The germination and proliferation of *Metarhizium* are optimum at temperature ranges from 10 to 40°C (Bidochka *et al*., 2001). However, mixed results were obtained from the effect of formulations on the spore viability. For examples, on the 7-day incubation period at 28^{0}C, 39% spore viability on powder formulation, which was lower and significantly different from the other formulation treatments. However, on the 28-day incubation period at 28^{0}C, 75% viability occurred on powder formulation, which was higher and significantly different from the other formulations. Our study on the spore viability was conducted only for a short period of time (28 days) so that differences in shelf lives among the three treatments could not be evaluated. Therefore, it is suggested that such study should be conducted for longer periods, for examples 3, 6, and 12 months.

CONCLUSION

Fungal entomopathogen*, Metarrhizium anisopliae,* prepared in powder formulation had better traits in comparison to those cultured in pellet and pasta formulations. The fungus proliferated on rice powder had higher pathogenecity against *S. frugiperda* larvae, lower percentage of larvae becoming pupa (higher larval mortality), and higher conidial density. Because these are good qualities of mycoinsecticides, the powder formulation should be used in proliferation of the fungus in mycoinsecticide production.

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Melina Melina	:	Conduct research and analyzed the data
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