Eco. Env. & Cons. 28 (September Suppl. Issue) : 2022; pp. (S38-S42)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i05s.007

Adding Several Types of Insect Flour to Increase the Virulence of *Metarhizium anisopliae* Local Isolates against Pests *Cylas formicarius*

Nina Jeni Lapinangga[1], Jemrifs H. H. Sonbai[1] and Jacqualine A. Bunga[1]

[1]State Agricultural Polytechnic Kupang, East Nusa Tenggara Province, Indonesia

(Received 24 February, 2022; Accepted 3 April, 2022)

ABSTRACT

The productivity of sweet potato in Timor Tengah Selatan (TTS) Regency is still far below the national productivity. The limiting factor is the attack of the pest *Cylas formicarius* both in planting and in storage. The aimed of this study was to examine the virulence of local isolates of *Metarhizium anisopliae* fungus cultured on growth media containing chitin. The study was conducted at the Plant Protection Laboratory from May-December 2021. The experiment was arranged using a Completely Randomized Design with five replications. The treatments were the type and concentration of chitin derived from three types of insects and arranged as follows: T0, control without chitin; T1, chitin from *Gryllus assimilis* 0.5%; T2, chitin from *Gryllus assimilis* 1%; T3, chitin from *Locusta migratoria* 0.5%; T4, chitin from *Locusta migratoria* 1%; T5, chitin from *Oryctes rhinoceros* 0.5%; and T6, chitin from *Oryctes rhinoceros* 1%. The results of this study showed that the addition of a chitin source to PDA growing media was able to increase conidia production, viability, and conidia virulence of *M. anisopliae*. Chitin also affects the virulence of *M. anisopliae* in *C. formicarius* which can increase the mortality percentage from 68% to 187%. Chitin in the form of flour derived from the insect O. *rhinoceros* with a concentration of 0.5% was an appropriate concentration to increase the viability and virulence of the *M. anisopliae*, besides that its protein content of 42.43% was high enough to support the production of quality entomopathogenic fungus.

Key words: *Entomopathogenic fungi, Metarrhizium anisopliae, Cylas formicarius, Biological control*

Introduction

One of the limiting factors for sweet potato productivity is *Cylas formicarius pest attack* . Pests damage bulbs not only in planting, but also attack bulbs stored in warehouse (Capinera, 2018). This pest attack can cause 5-97% yield loss (Chen, 2017; Kalshoven, 1981). *C. formicarius* pests can be controlled using entomopathogenic fungi. Lapinangga *et al*., (2018) found the entomopathogenic fungus *Metarhizium anisopliae*, a local isolate of TTS, which is feasible to be developed as a biopesticide to control *C. formicarius* because it has a high mortality rate (80.75%).

The fungus *Metarhizium anisopliae* is easily propagated in vitro, but in the process of propagation usually there is a decrease in quality and virulence. The decrease in the quality of fungal spores was caused by reduced sources of chitin and protein in the propagation medium (Bucarei *et al*., 2016). According to Hanti *et al*., (2012) to increase the virulence of the fungus it is recommended to grow it on media that contains a lot of chitins from insects. The addition of chitin to the growing media stimulates the production of chitinase which functions to increase the ability of entomopathogenic fungal infections (Istiqomah and Fatimah, 2014).

Insect flour contains chitin and high protein so that it can increase the number of infective conidia and virulence against pests (Pramesti *et al*., 2014). According to Herlinda *et al.* (2012) cricket flour (*Gryllus* sp) with a concentration of 0.5% added to potato dextrose extract medium was able to increase the viability of *Beuaveria bassiana conidia* to 80%. The results of research by Nuryanti *et al*. (2013) showed that the fungus *B. bassiana* grown on rice media with the addition of grasshopper flour, caused a mortality of 78% walang sangit. Prayogo *et al*. (2017) stated that the addition of chitin from insects *Gryllus assimilis*, *Perna viridis*, and *Scylla serrata,* although were not able to increase conidia production, did increase the viability and virulence of conidia of the fungus *B. bassiana.* Several studies that have been mentioned have been successful in adding chitin-containing ingredients as a medium for entomopathogenic fungi. The similarity of characteristics as an entomopathogenic fungus is expected to give good results for the growth of local isolates of *M. anisopliae.*

Research examining the use of insect flour containing chitin as a growth medium for *M. anisopliae* is still rarely studied, whereas local isolates of the fungus *Metarhizium anisopliae* have not been reported previously. Based on this, it is necessary to conduct research on the effect of adding several types of insect flour to local isolates of *Metarhizium anisopliae mushroom propagation media on its virulence.*

Materials and Methods

The experiment was arranged using a completely randomized design with five replicates. The treatments were the type and concentration of chitin derived from three types of insects and arranged as follows: T0 = control without chitin; T1 = chitin from *Gryllus assimilis* 0.5%; T2 = chitin from *Gryllus assimilis* 1%; T3 = chitin from *Locusta migratoria* 0.5%; T4 = chitin from *Locusta migratoria* 1%; T5 = chitin from *Oryctes rhinoceros* 0.5%; and T6 = chitin from *Oryctes rhinoceros* 1%. Each treatment was repeated 6 times. Insects used in this study were insects in the imago phase.

- Insect flour manufacture

  Imago *Gryllus assimilis*, *Locusta migratoria,* and *Locusta migratoria* 0.5 kg each were dried in an oven for 24 hours at 70 C. The dried insects were then ground and filtered. The filter results were stored at room temperature. Insect flour was then analyzed for chitin and protein content. The addition of the amount of insect flour in 100 ml of PDA media used the formula by Pramesti (2014): K = Concentration (%) x % chitin content x 100 grams

  Each type of chitin was added to the PDA growth medium. The control treatment only used PDA media without insect flour. The growing medium was then poured into a petri dish.

- Preparation and inoculation of fungi on growing media

Pure culture of *M. anisopliae* local isolate used were derived from previous studies. Mushroom suspension solution made following the Pramesty (2014) procedure by taking and weighing 17 g of rice (starter) that had been prepared previously, then mashed and put into a bottle and added 100 ml of sterile water; then shaken using a shaker for 24 hours. The spore density obtained in a suspension of 100 ml of *M. anisopliae* in this suspension will be used for treatment.

The fungal isolates were then planted and incubated on PDA media enriched with insect flour. The isolates were incubated in dark and humid conditions for 14 days. After 14 days the fungus can be used for mushroom quality measurement and virulence test.

- Mushroom quality measurement was done by:
  a. Calculating conidia viability.
  b. Count the number of conidia.
- Virulence test, with stages:
  a. Preparation of bioinsecticide solution

     The biological insecticide solution was made by taking 10 grams of PDA media made from insect flour that had been overgrown with fungus, crushed and then mixed into 200 ml of distilled water, then stirred until smooth. Furthermore, the mixture was filtered to be applied in the test.

  b. Application of biological insecticides

The application technique used was the contact method. The test insects were put into plastic containers with a diameter of 10 cm and a height of 15 cm, each container containing 20 imago. The application dose of conidia suspension was 200 ml/100 imago which was sprayed all over the body. The test insects were fed with sweet potato tubers. Observations were made every 12 hours after application 8 times. Observations included the mortality of the test insects and the time of death of 50% (LT50). Data obtained from mortality observations were carried out by Analysis of Variance (ANOVA) if there was an effect, further DMRT test was carried out.

Results and Discussion

Conidia Production and Viability *of Metarrhizium anisopliae*

The addition of chitin from three types of insects, both 0.5% and 1%, in PDA growth media caused the number of conidia of the entomopathogenic fungus *M. anisopliae* to be higher when compared to PDA media without chitin/control (Figure 1). The addition of chitin from 0.5% and 1% of *G. assimilis* resulted in the number of conidia which were not significantly different, they were 5.33×10^7 and 4.83×10^7 each, but significantly different from other treatments. The addition of chitin from *L. migratoria* and *O. rhinoceros* at concentrations of 0.5% and 1% resulted in the number of conidia that were not significantly different, they were 2.33×10^7; 1.83×10^7; 2.17×10^7; 2.5×10^7 respectively. The lowest number of conidia produced on PDA media without the addition of chitin was 1.5×10^7.

The observation showed that the higher the concentration of chitin added to the growth medium, the conidia growth inhibited. Chitin is a source of nutrients needed by fungi to build energy so that the conidia formed have better quality than fungi grown on media that do not contain chitin. Senthiraja *et al.* (2010) reported that chitin was a source of carbon and nitrogen that functions as nutrients and energy. Carbon and nitrogen contained in the growing media can increase mycelium formation and conidia germination (Nuryanti *et al.,* 2012; Kim *et al.,* 2014). Meanwhile, a growth medium that lacks protein will

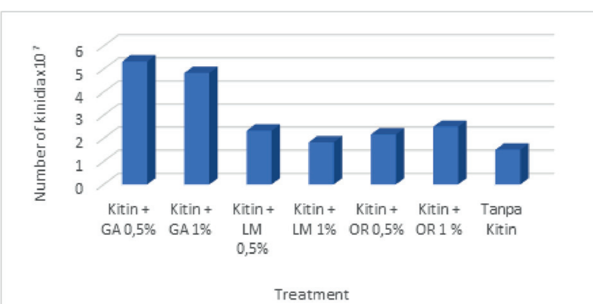

Fig. 1. Conidia production on three-enriched PDA growth media sources of chitin with different concentrations

cause lower conidia germination so that insect mortality was also low.

The results of analysis of variance showed that the entomopathogenic fungus *M. anisopliae* cultured on PDA media enriched with chitin had a significant effect on spore viability. Chitin enrichment in media was able to increase spore viability. However, the higher the concentration of chitin added to the PDA growth medium, the conidia viability of *M. anisopliae* decreased (Figure 2).

The highest Conidia viability of *M. anisopliae* was found in the addition of 0.5% chitin from *O. rhinoceros*, namely 56.17% which was not significantly different from 0.5% chitin from *L. migratoria*, which was 54.33%, followed by 1% chitin from *O. rhinoceros.* by 52.5% and *L. migratoria* 1% by 51%, but significantly different with the addition of 0.5% chitin

Fig. 2. Viability of *M. anisopliae conidia* on PDA growth media enriched with three sources of chitin with different concentrations

from *L. migratoria* and 1% from *G. assimilis* which were 45% and 43.5%, respectively. The lowest viability was found in PDA growing media without chitin (control), which was 40.67%. Nutrient limitations in PDA culture media make spore viability low. According to Prayogo (2017), PDA media has a protein content of only 2% and a mineral content below 1%. Meanwhile, insect flour contains chitin and protein so that the addition of insect flour in the media adds nutrients to the media which can provide more energy for spores to germinate. The more often entomopathogenic fungi were sub-cultured in media with low nutrients, the energy stored in the spores was low, which can reduce viability. The results of the analysis showed that the protein content in insect flour was high enough to support fungal growth. The highest percentage of protein content was found in insect flour from *L. migratoria*, which was 55.56%, while *G. assimilis* and *O. rhinoceros* were 42.87% and 42.43%, respectively. The results of this study showed that the addition of chitin was able to increase the viability of *M. anisopliae conidia* although only 15.5%. This result can be seen from the viability of conidia in the control treatment of 40.67%, while the viability of conidia in the chitin treatment ranged from 43.5 to 56.17%.

The results of several studies showed that nutrient-rich growing media had an effect on germination and viability of conidia of entomopathogenic fungi (Lopes *et al.*, 2013; Kim *et al.*, 2014). Production and germination of fungal conidia were influenced by sugar and nitrogen content in the growing media used (Mishra and Malik 2013; Liu *et al.*, 2012). Judging from the number of conidia formed and the viability of conidia from the addition of chitin, further studies are needed to explore the type of chitin and the right dose to obtain high quality conidia.

Mortality of Imago *Cylas formicarius*

The addition of chitin compounds from *G. assimilis*, *L. migratoria,* and *O. rhinoceros* to the growing media significantly affected the mortality rate of *C. formicarius imago* (Figure 3). The lowest mortality was found in the PDA media treatment without the addition of chitin flour, which was only 25.83%. The highest mortality was found in the addition of 0.5% chitin from *O. rhinoceros*, namely 74.17% which was not significantly different from 0.5% chitin from *L. migratoria*, namely 68.33%, but significantly different with the addition of 1% chitin from *O. rhinoceros* was 50.83% and chitin from *L. migratoria* 1% is 47.5%. The addition of 0.5% chitin from *L. migratoria* and 1% from *G. assimilis* caused mortality of 45% and 43.5%, respectively.

The results showed that the addition of chitin was proven to increase the virulence of entomopathogenic fungi 68-187%. The addition of

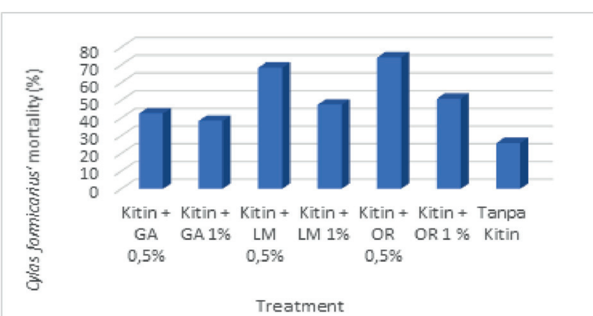

Fig. 3. Mortality of imago *C. formicarius* due to fungal infection of *M. anisopliae* growing on PDA media enriched with three sources of chitin with different concentrations

chitin of different types and concentrations resulted in higher quality conidia. This was indicated by the higher mortality of the test insects compared to the treatment of the control PDA growing media.

The speed of the fungus *M. anisopliae* to kill *C. formicarius* was influenced by the density of germinating conidia on the insect integument. The more conidia that germinate on the insect's integument will cause the integument to be damaged more quickly and body fluids will be depleted faster which causes the insect to die faster. According to Prayogo *et al.* (2017) the tissues and body fluids of insects that are attacked by entomopathogenic fungi will usually be completely absorbed by the fungus so that the insects die.

Conclusion

The addition of chitin sources in PDA growing media was able to increase conidia production, viability, and conidia virulence of the fungus *M. anisopliae*. This indicates that the nutrients contained in insect flour have an impact on the growth of the entomopathogenic fungus *M. anisopliae.* Chitin also affects the virulence of *M. anisopliae* in *C. formicarius* because it can increase the mortality percentage from 68-187%. Chitin in powder form derived from the insect *O. rhinoceros* with a concentration of 0.5% was a suitable concentration to increase the viability and virulence of the fungus *M. anisopliae,* besides its protein content of 42.43% was high enough to support the production of quality entomopathogenic fungi.

References

Bucarei, Lorena Barra, Pedro Vergara and Amparo Cortes, 2016. Conditions to Optimize Mass Production of *Metarhizium anisopliae* (Metschn.) Sorokin 1883 in Different Substrates. *Chilean Journal Of Agricultural Research versión On-line* ISSN 0718-5839. Chilean J. Agric. Res. vol.76 no.4 Chillán dic. http://dx.doi.org/10.4067/S0718-58392016000400008. Accessed on 23 February 2021

Camargoa, M. G., Allan, F. Marcianoa, Fillipe, A. Sáa, Wendell, M.S., Perinottoa, Simone Quinelatoa, Patrícia, S. Gôloa, Isabele, C. Angeloa, Márcia, C. A., Pratab and Vânia R. E. 2014. Evaluation of Control Tactics for Management of Sweetpotato Weevil (Coleoptera: Curculionidae). Veterinary Parasitology Journal. www.core.ac.uk. Accessed on 19 February 2021.

Capinera, J. L. 2018. Sweetpotato Weevil, *Cylas formicarius* (Fabricius) (Insecta: Coleoptera: Brentidae (=Curculionidae)). www.edis.ifas.ufl.edu. Accessed on 23 February 2021.

Chen. J. 2017. Evaluation of Control Tactics for Management of Sweet potato Weevil (Coleoptera: Curculionidae). https://digitalcommons.lsu.edu/gradschool. Accessed on 22 February 2021.

Hanti, L., Zach, S. and Seidi-Seiboth, V. 2012. Fungal Chitinases; Diversity, Mechanistic Properties And Biotechnological Potential. *Appl Microbiol and Biotechnol.* 93(2): 533-543.

Herlinda, S., Darmawan, K.A., Firmansyah, Adam, T., Irsan, C. and Thalib, R. 2012. Bioessay Bioinsektisida *Beauveria bassiana* dari Sumatera Selatan Terhadap Kutu Putih Pepaya, *Paraccocus marginatus* William dan Grana De Wilink (Hemiptera:Pseudococidae). *Journal Entomologi Indonesia.* 9(2): 81–87.

Istiqomah, N. and Fatimah, S. 2014. Pertumbuhan dan Hasil Cendawan Tiram Pada Berbagai Komposisi Media Tanam. *Ziraaah.* 39(3) : 95–99

Kalshoven, L. G. E. 1981. Pest of Crop in Indonesia. PT Ichtiar Baru-Van Hoeve. Jakarta

Kim, J.S., Lee, S.J., Skinner, M. and Parker, B.L. 2014. A Novel Approach *Beauveria bassiana* Granules Applied to Nursery Soil for Management of Rice Water Weevils in Paddy Fields. *Pest Manag Sci.* 70(8).

Lapinangga, N. J., Jemrift, H. H. Sonbai, and Yosefus, da Lopez, Eksplorasi Cendawan Entomopatogen Isolat Lokal Untuk Mengendalikan Hama *Cylas formicarius* pada Ubi Jalar. National Strategic Research Report. Source of Funding DRPM DIKTI.

Liu, L., Z., Rulin, Y., Laying, L., Changcong, Z., Di, and H., Junshen, 2012. Isolation and Identification of *Metarhizium anisopliae* from Chilo Venosatus (Lepidoptera: Pyralidae) cadaver. *African Journal of Biotechnology.* 11(30). http://www.academicjournals.org. Accessed on 12 February 2021

Lopes, A.M., Joao K.G. and Rubik, D.F. 2013. Pre-treatment of lignocellulosic biomass using ionic liquids: Wheat straw fractionation. *Bioresource Technology*. 142: 198-208

Mishra, A. K., Pandey, B., Tyagi, C. Chakraborty, O., Kumar and Kain, A. K. 2015. Structual and Functional Analysis Of Chitinase Gene Family In Wheat (*Triticum aestivum*). *Journal of Biochemistry and Biophysics.* 52 : 169-178

Mishra, S., Kumar, P. and Malik, A. 2013. Effect of Process Parameters On The Enzyme Activity of a Novel *Beauveria bassiana* Isolate. *Int J Curr Microbiol Appl Sci.* 2(9) : 49-56.

Nastiti, D. Indah, Rina Rachmawati and Silvi Ikawati, 2018. Pengaruh Penambahan Tepung Cengkerik Terhadap Kerapatan Dan Viabilitas Spora Jamur Entomopatogen *Lecanicillium lecanii* Zimm (Hypocreales: Clavicipitaceae). *Jurnal HPT Tropika.*

Nuryanti, N.S.P., Wibowo, L. and Azis, A. 2013. Penambahan Beberapa Jenis Bahan Nutrisi pada Media Perbanyakan untuk Meningkatkan Virulensi *Beauveria bassiana* terhadap Hama Walang Sangit. *Jurnal Hama dan Penyakit Tumbuhan Tropika.* 12(1): 64-70.

Pramesti, N. R., Himawan, T. and Rachmawati, R. 2014. Pengaruh Pengkayaan Media dan Suhu Penyimpanan Terhadap Kerapatan dan Viabilitas Konidia Cendawan Patogen Serangga *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordypitaceae). *Jurnal HPT.* 2(3) : 42-50

Pramesty, N.R. 2014. Pengaruh Pengkayaan Media dan Suhu Penyimpanan Terhadap Kerapatan Dan Viabilitas Konidia Jamur Patogen Serangga *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales : Cordycipitaceae). *Jurnal HPT.* 2(3).

Prayogo, Y., Afandi, A., Puspitarini, R.D. and Rachmawati, R. 2017. Penambahan Senyawa Kitin Untuk Meningkatkan Virulensi Cendawan Entomopatogen *Beauveria bassiana* dalam Membunuh Serangga Hama. *Buletin Palawija.* 15(1) : 31-4.

Rosmayuningsih, A., B.T., Rahardjo and R., Rachmawati, 2014. Patogenitas Jamur *Metarhizium anisopliae* Terhadap Hama Kepinding Tanah (*Stibaropus molginus*) (Hemiptera:Cydnidae) dari Beberapa Formulasi. *Jurnal HPT.* 2(2) April 2014 ISSN: 2338 – 4336. 28-37.

Senthiraja, G., Ariand, T., Durairaj, C., Raguchander, T. and Samitappan, R. 2010. Chitin-Based Bioformulation of *Beauveria bassiana* and *Pseudomonas fluorescens* for Improved Control of Leafminer and Collar Rot in Groundnut*. Crop Protec.* 29(9).

Shahid, A. A., Abdul Qayyum, Rao, Allah Bakhsh and Tayyab Husnain, 2012. Entomopathogenic Fungi As Biological Controllers: New Insights Into Their Virulence And Pathogenicity. *Biol. Sci., Belgrade.* 64(1): 21-42. http://www.thejaps.org.pk. Accessed on 24 February 2021

Teja, K.N. and Rahman, S.J. 2016. Characterisation and Evaluation of *Metarhizium anisopliae* (Metsch.) Sorokin Strains for Their Temperature Tolerance. Journal Mycology An International Journal on Fungal Biology. 7(4): 171-179.