FUNGAL PATHOGENICITY AND PROFILE CUTICLE DAMAGE CAUSED BY ENTOMOPATHOGENIC FUNGUS *Metarhizium anisopliae* INFECTION AGAINST TO *Oxya japonica* (ORTHOPTERA: ACRIDIDAE)

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Abstract

Resistance is one of impacts that arrising from the use of synthetic insecticides, and it's became a major problem in agriculture. This encourages the application of integrated pest management including through biological control method. One of the biological agent known great potential in controlling the pest population is *Metarhizium anisopliae*. Entomopathogenic fungus is known as natural enemies of insects. The necessary information about the pathogenicity of the biological agent is very important in developing the *M. anisopliae* applications. Based on this, the research was conducted to find out the infected pathogenicity of *M. anisopliae* against *Oxya japonica* (Orthoptera: Acrididae). The Research was used a completely randomized design with single factor, the concentration fungal infection, and the parameters was letal time of *O. japonica*. The results was showed that concentration fungal infection was effected the letal time of *O. japonica* significantly ($P < 0.05$), which is the shortest time of death occurred at the highest concentration fungal infection. Observed symptoms of fungal infection against to *O. japonica* was showed through changes in morphology and behavior of *O. japonica* infected. Furthermore, the profile longitudinal incision of Histologic cuticle was observed, there was cuticle degradation in the area of hyphae penetration. The hyphae was growth through the cuticle tissue forming mycelium that filled hemocoel. Its growth was resulted in damage to the structures of hemocoel inner tissues

Keywords: *Metarhizium anisopliae, Oxya japonica, pathogenicity, letal time, concentration fungal infection, profile cuticle damage*

Introduction

Pest resistancy to chemical insecticides has become major problem in agriculture. To overcome this problem, an integrated pest management was introduced with biocontrol as a part of the pest control methode. One of the biocontrol agent that exhibit a great potensive to control insect pest population is *Metarhizium anisopliae*. This entomopathogenic fungus is a natural enemy of insect (Prayogo et al., 2005). The information of fungus pathogenicity and insect immune response are the key factors in developing indigenous *M. anisopliae* as biocontrol agent. The necessary information about the pathogenicity of the biological agent is very important in developing the *M. anisopliae* applications. Based on this, the research was conducted to find out the infected pathogenicity of *M. anisopliae* against *Oxya japonica* (Orthoptera: Acrididae).

Materials and Methods

The material of research was used 5th instar of *O. japonica*, it was infected by the spore suspension of *M. anisopliae* that was cultured in BPTP Propinsi Jawa Barat. The research methode

was used Completely Randomize Design, with dosage infection as single factor, there are 0 (control), 1.5×10^2, 1.5×10^3, 1.5×10^4, 1.5×10^5 spore/individual

Histologic preparation performed by *O. japonica* infected for 24 hours, 48 hours, 72 hours and 96 hours, to determine the growth and development of fungal. Samples were obtained from infected animals that were fixed in advance in Bouin fixative solution. Further washing using 70% alcohol, which replaced two interval of 1 hour and then allowed soaked in 70% alcohol for 12 hours. The next stage is done dehydrated with a solution of NBA series, as follows: Alcohol 80%: NBA (3: 1) for 1 hour, Alcohol 96%: NBA (3:1)) for 1 hour, Alcohol 100%: NBA (3:1) for 15 minutes, the last in the NBA pure 12 hours. Furthermore, the infiltration with liquid paraffin in the oven at 56 ° C. Infiltration is done three times, each for 30 minutes, 60 minutes and 90 minutes. Sample test insects which have been infiltrated with paraffin then planted in a block of paraffin and sliced using a microtome brand "American optical" with a thickness of 6µm. The incision is affixed to a glass slide with adhesive albumin meyer, then stained by the method of hematoxylin-eosin (Utari, 2000), which has been modified as follows: Xilol for 30 minutes, Alcohol 100% for 3 minutes, Alcohol 96% for 3 minutes, Alcohol 80% for 3 minutes, Alcohol 70% for 30 minutes/until the yellow color lost tissue, hematoxylin for 3 minutes, water flows for 30 seconds, Alcohol 70% for 3 minutes, Alcohol 80% for 3 minutes, eosin for 2 minutes, Alcohol 96% for 3 minutes, Alcohol 100% for 3 minutes, the last in Xilol for 3 minutes. Mixture which has been tainted by entelan then closed with coverglass.

Results and Discussion

Pathogenesis *M. anisopliae* infection process begins with the spore form of contact between the cuticle *O. japonica*. Fungus spore can penetrate the cuticle and was internal replicated into haemocoel of *O. japonica*. However, it was decreased the consumption, less activation and fitness of insect. The infection of *M. anisopliae* was showed that death occurs within 14 days, the body less haemolimph and turn to dark, mycelium with green spore was covered the cadaver of insect at reduced humidity.

Fungal spores penetrate the cuticle and internal replicate in *O. japonica* haemocoel. On the surface of an infected cuticle darker colored, this occurs in 1-2 days after infection (**Figure 1a**), the darker the color when grasshoppers eventually die (**Figure 1b**). Nutrients in haemolymph will be reduced until it is used by the growth of the pathogen, until finally there is death. *O. japonica* who die in dry conditions will experience a depreciation of body fluids, dry body stiff, then blackened (**Figure 1c**). The spread of hyphae and mycelium growth in *O. japonica* body and produced conidia with greeny spores (**Figure 1d**).

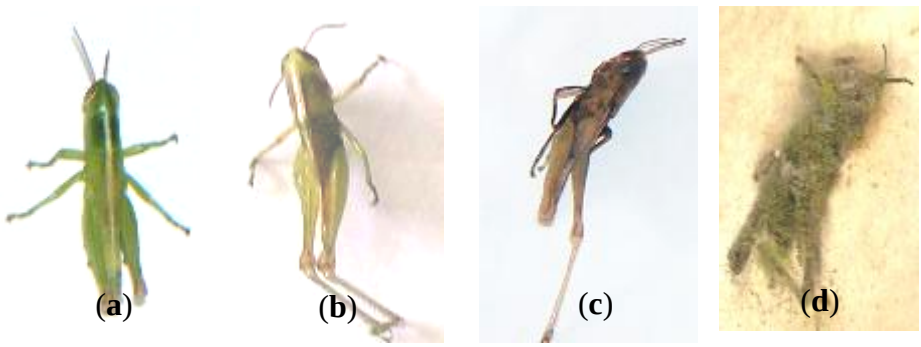

Figure 1. (a) *O. japonica* infected, at the beginning of the process occurs melanization in the area of infection (b) Early death of *O. japonica*, darkening the wider part of the body is infected (c) The cadaver of *O.japonica* shrinkage of body fluids, body dry, stiff and blackened (d) The cadaver of *O. japonica* growth by mycelium of *M. anisopliae* with fully spores.

The result of study showed that all dosages treatment of infection was led to 100% mortality of *O. japonica*. The mortality was no significant showed different in each dosages of treatment (**Figure 2**).

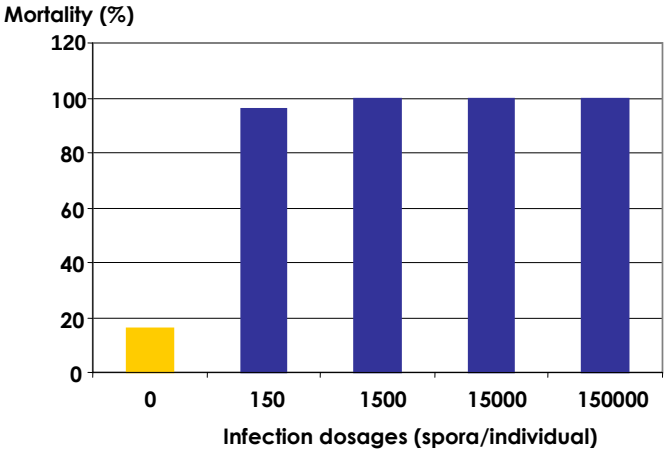

Figure 2. Mortality *O. japonica* infected by *M. anisopliae* at 5 infectious dose level.

The result showed that significantly the rate dosages infection of 1.5 x 10^2, 1.5 x 10^3, 1.5 x 10^4, 1.5 x 10^5 spore/individu were caused mortality (P<0.05). It also effected the average of death time significantly (P<0.05), that was shorter as the high infection dosage.

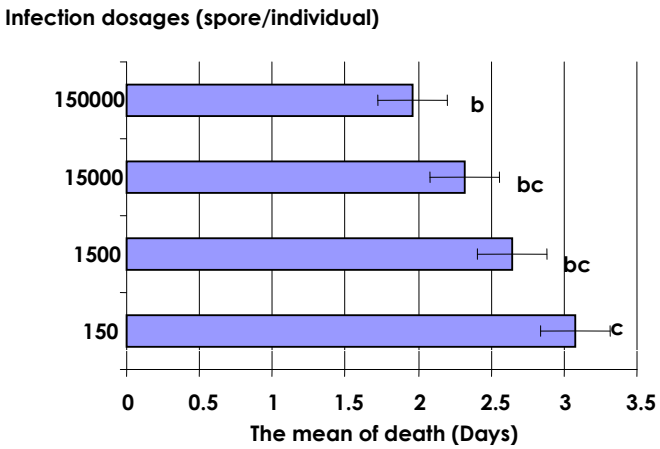

Figure 3. The average time of death *O. japonica* infected by *M. anisopliae* at 5 infectious dose level.

The mortality rate at the highest dose is 1.96 days shorter than the lowest dose that happens during the day 3,08. At the dosage of 1.5 x 10^4 spores, the mortality rate during the day 2,32, no more different than the average mortality in the treatment of 1.5 x 104 spores, that occurs at the day 2.64.

Infectious spores were needed time to penetrate the cuticle, the spread of hyphae and mycelium growth in hemosol, it produces toxins that was killed *O. japonica* (Boucias & Pendland, 1986). The achievement of the optimum amount of infective spores will expedite the process of infection. The higher the number of spores that are infected, it was expected the higher chances of pathogenicity and death in insects.

Disease in insects caused by a fungus called mycosis. Entomopathogenic fungus had an unique characteristic with their ability to penetrate the integument of insects which is the first barrier protection from the invasion of patogens. The development of the infection was performed by the profile of *O. japonica* cuticle Histologic preparations that have been infected by *M. anisopliae* in the period of 24 hours, 48 hours, 72 hours, and 96 hours post-infected. The parameters observed through the profile of hyphae growth, the conditions of infected cuticles and the growth of hyphae and mycelia

in the internal tissue of haemocoel. The profile of the *O. japonica* Histologic preparations was showed by photos presented in **Figure 4**.

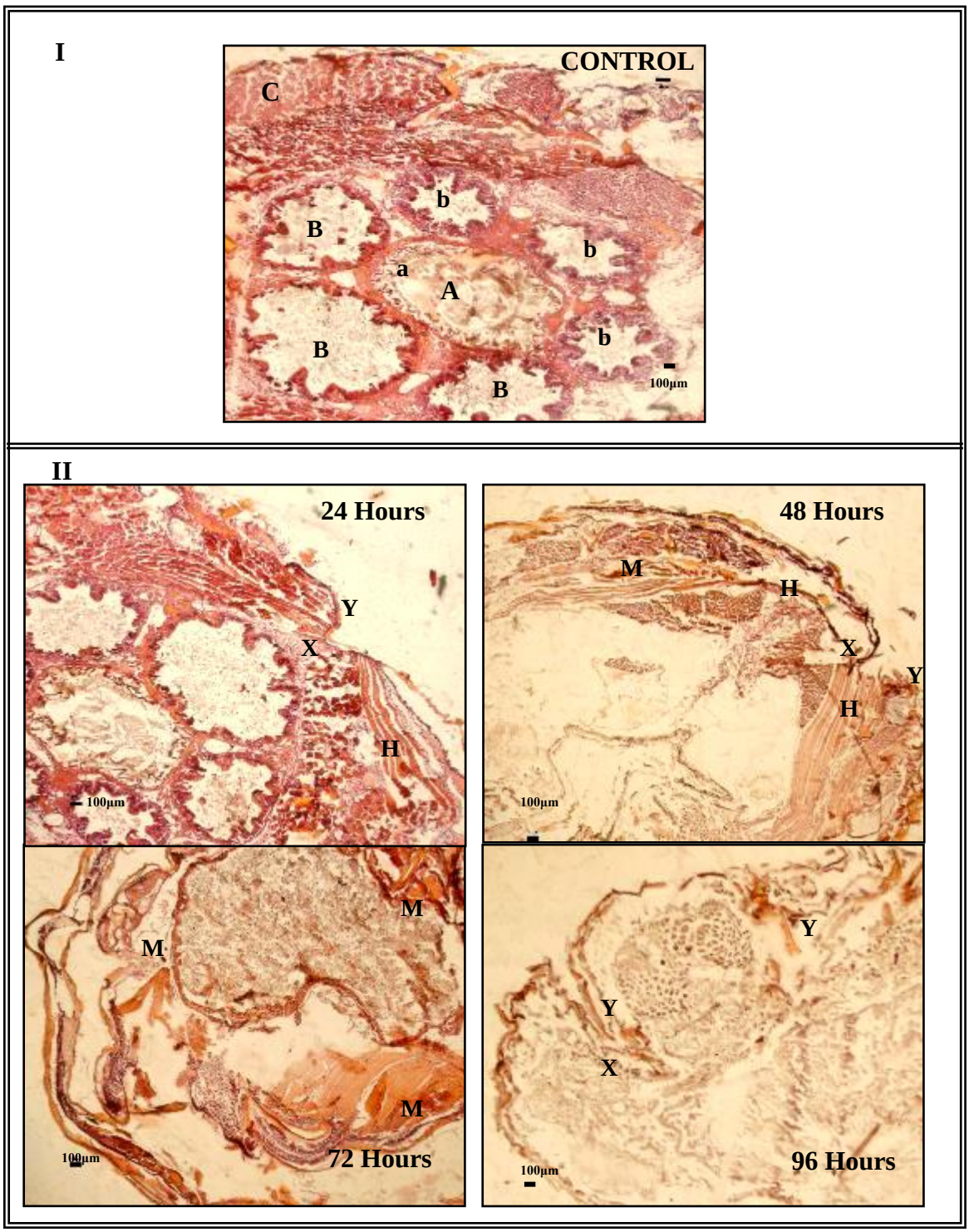

Figure 4. Histologic longitudinal preparations of *O. japonica* thoracic; (I). CONTROLS: The structure of the internal tissue in *O. japonica* haemocoel: A. Gastrointestinal cavity (gut), a. Peritrophic membrane, B.Trachea cavities, b.Trakeoli cavity, C.Muscle tissue (II). Histologic preparation of *O. japonica* tissue that have infected by spores of *M. anisopliae* in 24 hours, 48 hours, 72 hours and 96 hours post-infected (40X magnification) (X = area spore penetration, Y = cuticle degradation by penetration of spores, hyphae = H, M = mycelium

The profile of *O. japonica* Histologic preparation (control) was showed that the structure of internal tissue in good condition (**Figure 4 (I))** the longitudinal Histologic preparation was showed gastrointestinal tract (gut) in the central part haemocel, and the cavity digestion inner side of the trachea cavities around. The tissue around the cavity that stained bright red was the muscle tissue. The coloring looks bright, it was showed that the cells and tissues in good condition when the preparation was made.

The Histologic preparation was showed the infection after 24 hours (**Figure 4 (II)**), digestive tissue structures of *Oxya* as the same as control. The development of further infection at 48 hours of infection, which is characterized by the development and deployment of hyphae form mycelium in haemocoel. The tissue structure was damaged, cavity respiratory tract (trachea and trakeoli) was damaged too, the muscle tissue can still be identified even though equally damaged. The profile of Staining Histologic preparation that compared to the control was changed, it means that tissue damage has occurred when the preparation was made. More severe tissue damage occurred after 72 hours and 96 hours infected, the tissue structure was not able identificated. The mycelium was grew and spreaded into haemocoel, so it can be assumed the tissue damage caused by the development of mycelium in hemosol. In addition, the development of mycelium was took some nutrients from the insect haemolymph. The fungal hyphae was produced mycotoxins such as destruksin to weaken the host tissue, based on this assumed tissue damage due to poisoned by destruxin.

The development of *M. anisopliae* infection was began with infective spores that was penetrated on the surface of cuticle. After 24 hours infected, the hyphae was penetrated into the epidermis tissue that cuticle below. At 48 hours later observed, hyphae form mycelium were developed and spread into into the inner tissue of haemocoel. Mycelium were grew and filled the entire tissue of haemocoel, it was showed by profil of Histologic preparation 72 hours post infected (**Figure 4 (II)**).

In the area of spore penetration, degradation of cuticle expanding along with the growth and development of hyphae, it was showed on the profile of Histologic preparation at 48 hours post-infected. The cuticle worst degradation occurs after infected, it was showed on the profile of Histologic preparation after 96 hours (**Figure 4 (II)**).

The development of fungi in the insect's body consists of three phases: (1) adhesion / attachment of spores and germination of spores of the cuticle, (2) penetration into haemocoel, (3) The growth and development of the fungus into haemocoel was produced mycotoxins such as destruxin to weaken the host tissue, and it was caused the death of insect (Tanada & Kaya, 1993).

Conclusion

The *M. anisopliae* infection against to *O. japonica* is was caused patogenecity the mortality of *O. japonica.* The spore infection of *M. anisopliae* in dosages of 1.5×10^2, 1.5×10^3 sp,1.5×10^4, 1.5×10^5 spora/individual, was led to 100% mortality that the mean of death was shorter as the high infection dosage. The mortality rate at the highest dose is 1.96 days shorter than the lowest dose that happens during the day 3,08. At the dosage of 1.5×10^4 spores, the mortality rate during the day 2,32, no more different than the average mortality in the treatment of 1.5 x 104 spores, that occurs at the day 2.64. Observed symptoms of fungal infection against to *O. japonica* was showed through changes in morphology and behavior of *O. japonica* infected. Furthermore, the profile of longitudinal histologic preparation of cuticle was observed, there was cuticle degradation in the area of hyphae penetration. The hyphae was growth through the cuticle tissue forming mycelium that filled hemocoel. Its growth was resulted in damage to the structures of hemocoel inner tissues after 24,72 and 96 hours infected.

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