Combination Intervals Effects of *Mirabilis jalapa* Bioinsecticide and *Metarhiziumanisopliae* Entomopathogen Fungi Against *Spodopteraexigua* Larvae

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***Abstract*—The application of biological pest management by combining *Mirabilis jalapa* as bioinsecticide and *Metarhiziumanisopliae* entomopathogen fungi has been shown to be toxic against *Spodopteraexigua* larvae in the laboratory. However, knowing of the combination interval has not been established, when *S. exigua*was exposed *M. jalapa* followed by addition of *M. anisopliae*. There is a need to investigate where it carried out to study on the mortality of *S. exigua*, with combination intervals were 0 hours; 2 hours; 4 hours; and 6 hours with 48 hours of treatment. The result of treatments showed that the highest mortality level of *S. exigua* when *M. jalapa* was combined with the *M. anisopliae* entomopathogen fungi in a combination interval of 6 hours, resulting in mortality percentage of 87%.**

***IndexTerms*—Bioinsecticide, EntomopathogenFungi, Larvae, Mortality**

I. INTRODUCTION

S.exigua is commonly found in vegetable farms, especially on onion plants. The larval stage has high polyphagous activity so that these insects have potential as pest in agriculture causing losses on crops especially if the growth of S. exigua population is out of control. The percentage of crop damage caused by *S.exigua*reached 98.68%. These pest attacks can cause up to 100% losses[1].In tackling the problem, themajority of farmers still use synthetic insecticides.

The unwise use of synthetic insecticidescould create new problems such as insects' resistance. However, synthetic insecticidesin Indonesia, which is often used, especially in the onion crop from the class of organophosphorus, carbamates and synthetic pyrethroids with the frequency of 5-30 times per growing season had been reported to cause the resistance of *S. exigua*.Synthetic pesticides can harm non-target organisms and the accumulation of pesticide toxic residues in crops[2]. Also, there are problems in insect resistance to synthetic pesticides in many sectors, especially agriculture. Cases occurring in Indonesia such as S. exigua onion tree pests have been resistant to the insecticides of the type chlorfluazuron[3] and the settlements insects in some parts of Indonesia have been resistant to some synthetic pesticides [4], [5]. Conditions become more complicated because insects will inherit resistance to their offspring and make people use synthetic pesticides with larger doses. As a result, the environment is more polluted, and this causes many other problems including the decline of species diversity affecting the ecology of the food chain and the emergence of many diseases including cancer.

The application of biological pest management through biological control agent used the combination of *M. jalapa*and *M. anisopliae*entomopathogen fungiare becominga safe alternative in controlling *S. exigua*.*M. jalapa* is biological control agent containing antifeedant compounds in the form of beta-sitosterol. The RIP (Ribosome Inactivating Proteins) in *M. jalapa* called MAP (Mirabilis Antiviral Proteins) also contain repellent compounds[6], [7]. *M. anisopliae* attacks insects by way of penetration through the cuticle, and mycelium of the fungi causes the insect's body was hardened, rigid, and underwent mummification [8]. The combination of the two biological agents, i.e.,*M. jalapa* leaf extract bioinsecticides and *M. Anisopliae fungi*, is expected to increase the mortality of *S.exigua*.

II. RESEARCH METHODS

M. jalapa leaves were dried and macerated, then diluted into 4 series (b/v) of 0.1%; 0.2%; 0.4%; 0.8% and 0% (control) dilution. The application of *M. jalapa* on the tested larvae by dipping the *S. exigua*feed in a solution of *M. jalapa*.Infection was carried out by dripping 5µL of *M. anisopliae* spore suspension on the dorsal surface of the larvae. The concentration of *M. anisopliae* preparation was 2.59×10^7 spores/ml in 5µL volume (solvent media), so the concentration of spore preparations that must be prepared is 2.59×10^7 spores/ml x (5×10^{-3} ml) = 1.29×10^5 spores/tail. The larvae used were the early instar IV. The addition of M. anisopliae used combination Intervalswere 0 hours; 2 hours; 4hours; and 6 hours with 48 hours of treatment.

III. RESULT AND DISCUSSION

S. exigua larvae are obtained in the Lempake Samarinda vegetable farm. Larvae are kept and fed mustard leaves at ± 27^{o}C and humidity ± 70%. An imago feed of ± 10% honey. The female imago eggs attached to the tissue moved with a brush to another container. Insect test used is instar IV stages. The selection of test insects is preferable to the physiological conditions of the insects. The newly chosen new instarIV from instar III due to the level of feed consumption the final instar IIIlarvae will be reduced when molting will occur. All the final instar larvae III transferred to a new container and the larvae that have become instarIV will leave an exuviae trace on the tissue. Physiologically, the condition of the digestive system ofinstarIV insects will be homogeneous and ready to be treated.

Fig 1: IV Instar *S. exigua*larvae

Based on the study results, thephysiological effect of antifeedant and toxic compounds from *M. Jalapa* is the decrease in value of the feed intake, weight and growth rate, as well as the time of becoming apupa. Physiological condition of larvae that are disrupted by the administration of bioinsecticides will affect the immune system of the insects, thereby allowing other microbial pathogens to invade into its body. In this study, *M. jalapa*bioinsecticides combined with *M. anisopliae* entomopathogen fungi.

For comparison, the test was done on mortality of *S. exigua* by administration of bioinsecticideswithout combinations. The results of the test after 48 hours of treatment showed that the mortality percentage of *S. exigua* which were treated only with *M. jalapa* was relatively low at 27%, whereas the mortality of *S. exigua* which were treated only with *M. anisopliae* was 20%.

The combiningsoursop leaves (*Annonamuricata*) with *Beauveriabassiana* fungi can also cause mortality of the second instar larvae of *Crocidolomiapavonana*, but it was still quite low due to the interval application of bioinsecticides which is two hours. Hence, a test was conducted to determine the most optimum *M. jalapa* and *M. anisopliae*combination interval. Data in Table 1 shows that the highest percentage of larval mortality was 87% in intervals of 6 hours.

Table 1 Combination Intervals of *M. jalapa* and *M. Anisopliae* on *S. exigua* Mortality

Combination Intervals (hour) (*M. jalapa* + *M. anisopliae*)	Larvae Mortality (%)
0	20
2	47
4	67
6	87

A high percentage of *S. exigua* mortality caused by the presence of *M. jalapa* extract affect the defense system of *S. exigua* bodies that play a role in the introduction of foreign particles. So that when applied *M. anisopliae* entomopathogen fungi, insects are no longer able to defend themselves against microbial pathogens attack, in this case,*M. anisopliae*.

The mechanism of action of *M. anisopliae* attack the insects with the adhesion and germination of spores on the insect cuticle. Spore germination largely depends on the humidity of the environment and temperature, lower light conditions and nutritional conditions. Spores attach to the host, forming a germination tube that functions in the penetration of hyphae [9]. In in-vitro, integral digestion by lipase, protease, and chitinase. Protease initiates degradation of the cuticle, preceding chitinase. Chitinase activity occurs mainly during fungus growth, conidial formation, and conidiophores sporulation. In general, when the integument is hard, thick, or soft, the germination tubes produce appressorium attached to the cuticle with a slimy substance. Appressorium forms a penetration plate, a penetrant or hypha tube, and a hypha body for invasion. Appressorium secretes proteases on the cuticle surface and penetrating hyphae in the cuticle. The protease enzyme releases monomers which can be metabolized by the germination tube to continue to grow into the integument [9]. After germination, the hyphae have penetrated the integument and entering hemocoel, producing the hypha body, essentially blastospores, and replicating through budding. Moreover,the fungi also produce toxins such as a destruxin compound which is known to participate in immunosuppression, activating calcium channels in insect muscles [10].

Statistical test analysis of variance in Table 2 shows that the combination of with several concentrations of M. jalapabioinsecticides and *M. anisopliae* significantly affect mortality against*S. exigua*.

Table 2 The Average Mortality of *S. exigua*Larvae after being Treated with Several Concentrations of *M. jalapa* and *M. anisopliae* with 6-hour Intervals

***M. jalapa*(%)+ *M. anisopliae*(spore/ml)**	***Mean of larvae mortality after 48 hour**
0 + 2.59 x 10 ^{7}	1.0 ± 0.70^{a}
0.1 + 2.59 x 10 ^{7}	1.4 ± 0.54^{ab}
0.2 + 2.59 x 10 ^{7}	2.0 ± 0.70^{bc}
0.4 + 2.59 x 10 ^{7}	2.8 ± 0.83^{c}
0.8 + 2.59 x 10 ^{7}	3.8 ± 0.44^{d}

* Mean ± SD, numbers followed by different letters indicate significantly different based on Duncan test (α = 0:05; n = 125)

The result of thestatistical test of variance analysis showed that combination of M. jalapa and M. anisopliaebiopesticide had asignificant effect on subsequent S. exigua mortality from Duncan test result, it was known that the highest treatment dose was significantly different from another treatment dose.

Based on the above analysis, biological pest management with the use of *M. jalapa*has a high potential as an insecticide in the future, especially if its application with M. *anisopliae*entomopathogenfungi, which is also known to have a high pathogenic, because its hyphae can cause the mummification of larvae.

IV. Conclusion

The combination of two biological control agents of *M. jalapa* and *M. anisopliae* significantly affects the mortality of larvae of *S. exigua*. The combination of*M. jalapa*and*M.anisopliae*entomopathogenfungi reached the highest mortality level of *S. exigua* in a combination interval of 6 hours, resulting in mortality percentage of 87%.

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References

[1] A. Negara, “Penggunaan Analisis probit untuk Pendugaan Tingkat Kepekaan Populasi Spodoptera exigua terhadapDeltametrin di Daerah Istimewa Yogyakarta,” *Inform. Pertan.*, vol. 12, 2003.

[2] M. Glover-Amengor and F. M. Tetteh, “Effect of pesticide application rate on yield of vegetables and soil microbial communities,” *West African J. Appl. Ecol.*, vol. 12, no. 1, 2008.

[3] A. Negara, “Resistensi populasihamabawangmerah Spodoptera exigua (Lepidoptera: Noctuidae) terhadapklorfluazuron,” *J. Entomol. Indones.*, vol. 2, no. 2, p. 1, 2017.

[4] W. Bestari, R. Rahayu, and N. Hariani, “Efektivitas Beberapa Insektisida Aerosol Terhadap Kecoak Blattella germanica (L.)(Dictyoptera; Blattellidae) strain VCRU-WHO, GFA-JKT Dan PLZ-PDG Dengan Metode Semprot,” *J. Biol. UNAND*, vol. 3, no. 3, 2014.

[5] W. R. Madona, R. Rahayu, D. Dahelmi, and N. Hariani, “Efektivitas Insektisida Komersial Terhadap Kecoak Jerman (Blattella Germanica L.) Strain VCRU-WHO, GFA-JKT dan PLZ-PDG dengan Metode Kontak (Glass Jar),” *J. Biol. UNAND*, vol. 4, no. 2, 2015.

[6] J. M. Vivanco, M. Querci, and L. F. Salazar, “Antiviral and antiviroid activity of MAP-containing extracts from Mirabilis jalapa roots,” *Plant Dis.*, vol. 83, no. 12, pp. 1116–1121, 1999.

[7] J. Duke’s, “Phytochemical and ethnobotanical databases chemicals and their biological activities,” *Melaleuca leucadendron*, 2009.

[8] N. Moazami, “Biopesticide production,” *Encycl. Life Support Syst.*, 2008.

[9] Y. Tanada and H. K. Kaya, *Insect pathology*. Academic press, 2012.

[10] A. Espada and M. M. Dreyfuss, “Effect of the cyclopeptolide 90-215 on the production of destruxins and helvolic acid by Metarhizium anisopliae,” *J. Ind. Microbiol. Biotechnol.*, vol. 19, no. 1, pp. 7–11, 1997.