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BIOEFFICACY OF ENTOMOPATHOGENIC FUNGUS (*Metarhizium anisopliae*) STRAIN MET-GRA4 AGAINST GOLDEN APPLE SNAIL (*Pomacea canaliculata*)

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Golden Apple Snails (GAS), *Pomacea canaliculata* is an invasive species in Malaysia causing millions of ringgit of damages to the paddy sector. Numerous strategies were implemented to develop greener molluscicides such as plant-derived extracts and biological control methods, however most are still in development. The utilization of biological control gained significant interest recently including entomopathogenic fungus. To develop a safer alternative to chemical molluscicides, nano-emulsion formulated conidia of *Metarhizium anisopliae* (strain Met-Gra4) were evaluated against GAS adults (n = 60) under laboratory conditions. The *M. anisopliae* (strain Met-Gra4) were cultured on rice media while pesticide free GAS adults were collected from UniSZA lake side were exposed to varying concentrations of conidia (10^5 , 10^6 , 10^7 and 10^8 conidia mL⁻¹) prepared in conidial suspension and emulsion-formulated forms for 24 hours and post-treatment observation for 14 days. Population of GAS exposed to emulsion-formulated conidia had significantly lower survival compared to conidial suspension (p < 0.05). The emulsion-formulated conidia exhibited LC₅₀ 6.7×10^5 conidia mL⁻¹ after 14 days, a lower concentration than conidial suspension with LC₅₀ of 6.7×10^6 conidia mL⁻¹ indicating higher efficacy. LT₅₀ of emulsion-formulated treatments demonstrated significantly faster 50% mortality than conidial suspension except the highest concentration (6.7×10^8 conidia

mL⁻¹) ($p < 0.01$). The increased efficacy in emulsion-formulated treatments is due to the delivery system provided by nano-sized particles facilitating the uptake of fungus into the snails more effectively. Histopathological analysis revealed that digestive tubules of hepatopancreas exhibited severe alterations particularly hyperplasia of K corpuscles in both *M. anisopliae* treatments ($p < 0.05$). Histopathological analysis of gonads unveiled that exposure to *M. anisopliae*, especially emulsion-formulated caused significant castration evident by gamete disintegration and necrosis of reproductive tubules in testis and ovary ($p < 0.05$ respectively). Histopathological condition index scores obtained from the same tissue samples further supported the histopathological findings confirming emulsion-formulated conidia treatment caused the most significant histopathological alterations ($p < 0.05$) among all treatments. Overall, the results highlight emulsion-formulated *M. anisopliae* as a potential bio-molluscicide for effective control of GAS, offering an environmentally sustainable solution to severe rice pests.

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**KEBERKESANAN KULAT ENTOMOPATOGEN (*Metarhizium anisopliae*)
STRAIN MET-GRA4 TERHADAP SIPUT GONDANG EMAS
(*Pomacea canaliculata*)**

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Siput gondang emas (GAS), *Pomacea canaliculata* merupakan spesies invasif di Malaysia menyebabkan kerosakkan mencecah jutaan ringgit kepada sektor padi. Pelbagai strategi dilaksanakan untuk mewujudkan racun siput yang mesra alam seperti ekstrak tumbuh-tumbuhan dan kawalan biologi tetapi kebanyakannya masih dalam perkembangan. Penggunaan kawalan biologi mendapat perhatian yang signifikan kebelakangan ini termasuk kulat entomopatogen. Bagi mewujudkan alternatif yang lebih selamat kepada racun siput kimia, formulasi emulsi-nano kulat *Metarhizium anisopliae* (strain Met-Gra4) telah diuji terhadap GAS dewasa dalam kondisi makmal ($n = 60$). Kulat *M. anisopliae* (strain Met-Gra4) telah dikultur menggunakan media beras manakala GAS dewasa yang bebas racun perosak telah dikutip dari persisiran tasik UniSZA telah didedahkan kepada pelbagai kepekatan konidia (10^5 , 10^6 , 10^7 & 10^8 konidia mL^{-1}) dalam bentuk suspensi konidia dan formulasi emulsi dalam keadaan makmal selama 24 jam and pemerhatian pasca-rawatan selama 14 hari. Kadar kelangsungan hidup bagi populasi GAS didedahkan kepada formulasi emulsi lebih rendah berbanding suspensi konidia ($p < 0.05$). Formulasi emulsi menunjukkan LC_{50} 6.7×10^5 konidia mL^{-1} selepas 14 hari, kepekatan lebih rendah berbanding suspensi konidia LC_{50} 6.7×10^6 konidia mL^{-1} , menunjukkan keberkesanan yang lebih tinggi. LT_{50} formulasi emulsi lebih pantas dalam mencapai 50% kematian berbanding

suspensi konidia kecuali pada kepekatan tertinggi (6.7×10^8 konidia mL⁻¹) ($p < 0.01$). Peningkatan keberkesanan pada rawatan formulasi emulsi disebabkan sistem penghantaran yang dibekalkan molekul bersaiz nano yang membantu pengambilan kulat yang lebih efektif ke dalam siput. Analisis histopatologi mendedahkan bahawa tubul pencernaan hepatopankreas menunjukkan perubahan teruk terutama peningkatan K korpusel dalam kedua-dua rawatan *M. anisopliae* ($p < 0.05$). Analisis histopatologi gonad mendedahkan bahawa pendedahan kepada *M. anisopliae*, terutamanya formulasi emulsi, mengakibatkan kesan pengembirian yang signifikan, jelas daripada penguraian gamet, dan nekrosis tubul pembiakan di testis dan ovari ($p < 0.05$, masing-masing). Markah indeks keadaan histologi yang diperolehi daripada sampel tisu yang sama selanjutnya menyokong dapatan histopatologi, mengesahkan rawatan formulasi emulsi menyebabkan perubahan histopatologi yang paling ketara ($p < 0.05$) berbanding semua rawatan lain. Secara keseluruhannya, hasil kajian menunjukkan bahawa formulasi emulsi *M. anisopliae* adalah racun siput yang berpotensi untuk mengawal GAS secara efektif dan memberi penyelesaian yang mesra alam kepada haiwan perosak padi yang teruk.