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Effectiveness of *Cordyceps fumosorosea* Wettable Powder Formulation against *Metisa plana* (Walker) and Its Side Effects on *Elaeidobius kamerunicus* in Oil palm Plantation

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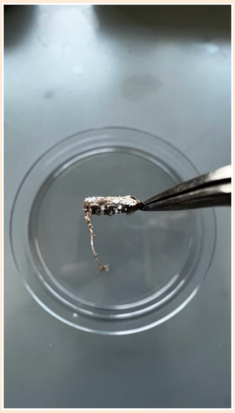
Development of mycoinsecticides with *Cordyceps fumosorosea* as an active ingredient is established as an alternate way to control the *Metisa plana* population while reducing chemical insecticide dependence. Three mycoinsecticide formulations (SS6, SS7, and SS8) with dispersing and wetting agents were developed as wettable powder formulations in this trial. SS8 demonstrated the best wettability, suspensibility, and dispersibility with viability at 10^7 (CFU)/mL even after three months of storage. However, SS7 developed with *C. fumosorosea* as an active ingredient was found to effectively reduce the bagworm population by more than 95%. The application of all mycoinsecticide formulations in the infested oil palm area was able to reduce the *M. plana* population by more than 95%, 30 DAT. The formulations also show no significant increase in mortality of the oil palm pollinator, *Elaeidobius kamerunicus*. This finding indicates that the *C. fumosorosea* tested has potential for managing bagworms without harming pollinators on oil palm plantations.



Keywords: Bagworm, biological control, *Cordyceps fumosorosea*, entomopathogenic fungi, pollinator.

Introduction

In Malaysia, three major bagworm species are documented as the most significant pests affecting oil palm plantations, namely *Metisa plana* Walker, *Pteroma pendula* Joannis, and *Mahasena corbetti* Tams.[1)] Both *M. plana* and *P. pendula* can cause severe yield losses, up to 33–47%, with *M. plana* being the most economically significant and important defoliator.[2)] Since the 1990s, more severe bagworm outbreaks have been reported,[3)] and the infestations have seriously affected the oil palm yield due to delayed and incorrect control strategies.[4)] During the outbreaks, the bagworm larvae devoured an enormous amount of the photosynthetic leaf areas of oil palm trees,[5)] thus affecting productivity in both the number and size of fruit bunches.[6)] Without proper control, severely attacked palms can suffer high crop loss, which further affects the livelihoods of oil palm planters, especially the smallholders. Currently, chemical control using conventional synthetic insecticides is the main solution against bagworm infestation. However, most of these insecticides are detrimental to non-target organisms such as the oil palm pollinator *Elaeidobius kamerunicus* Faust. Recent interest in eco-friendly entomopathogenic fungi has provided an alternative to conventional insecticides.[7–9)] Entomopathogenic fungi specifically infect and kill insects and other arthropods, which makes them suitable to be used as biological insecticides.[10)] The fungi could be produced economically by submerged fermentation and fungal propagules comprised of conidia, mycelia, and blastospores and formulated similarly to conventional insecticides.[11)]

There are many ways of formulating entomopathogenic fungi, and the most common is as a wettable powder (WP). WP is a basic dry solid pesticide formulation that is finely ground, mi-

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cronized into powder form, and typically applied as suspended particles in water. The ideal WP would be quickly wetted when dispersed in water and form suspensions having relatively high solid contents that are low foaming and do not exhibit tendencies toward sedimentation with age.[12] The recent development of inert WP ingredients such as a dispersant and wetting agent has provided more options in the formulation of entomopathogenic fungi, particularly in the aspect of good wettability and infectivity of the fungi. A WP could provide long-term storage stability, good miscibility with water, and convenient application due to its compatibility with conventional spraying equipment.[13] *Cordyceps fumosorosea* (Wize) (formerly *Isaria fumosorosea*) (Hypocreales: Cordycipitaceae) is a well-known fungal species which belongs to a large group of biocontrol agents.[14,15] Strains of *C. fumosorosea* have also been proven to be pathogenic against various insect species,[16] including diamondback moths,[17,18] whiteflies,[19] bagworms,[20] and red palm weevils.[21] Thus, the aims of this experiment are to prepare a physically and biologically stable wettable powder formulation of *C. fumosorosea* mycoinsecticide, evaluate its field efficacy against *M. plana*, and determine its side effect on *E. kamerunicus.*

Materials and methods

1. *Preparation of a wettable formulation of* Cordyceps fumosorosea

Cordyceps fumosorosea (voucher number TSJ772C) coded as BSB01 was obtained from Bukit Senorang, Kemayan, Pahang, Malaysia (3.14°N, 102.38°E). The fungus was cultured on submerged propagules using Czapek–Dox Broth (BD Difco™, USA)[22,23] with a yeast extract medium for conidia production. This medium was selected due to its ability to be fully colonized by *C. fumosorosea* resulting in a thick milky suspension where the medium could produce at least 1×10^8 CFU mL^{-1}. Suspended submerged fungal propagules were filtered using a vacuum pump, then dried and stored in the refrigerator at −2°C. The inert ingredients in the preparation of the wettable powder formulation were polyacrylic acid sodium (PAAS) and lignosulfonic acid sodium (Lig) as dispersants and sodium 1-naphthalenesulfonate (SNS), sodium xylenesulfonate (SX), and sodium cumenesulfonate (SC) as wetting agents. These dispersants and wetting agents were selected based on their effect on *C. fumosorosea* fungal growth by exposing the conidia to an 8% concentration of the inert ingredients. The selected ingredients were found to give germination greater than 70%. *Cordyceps fumosorosea* WPs comprised of the compatible wetting and surfactant agents were prepared by constructing a dispersant–wetting agent mixture called a surfactant system through a pseudoternary diagram. The quantity of the inert ingredients mixed in the preparation of the WP formulation was based on Knowles.[24] Three surfactant components (dispersant A, dispersant B, and a wetting agent) were mixed at a ratio of 80:10:10, 10:80:10, 10:10:80, 10:40:50, 50:40:10, 40:10:50, and 30:30:40, for a total of 0.8 g of surfactant. The mixed surfactant was suspended in 100 mL of World Health Organization (WHO) standard hard[25] water for 30 min before 90% of the upper part of the suspension was removed using a vacuum pump. Sediment left in the container was dried in an oven at 50°C until a constant weight was obtained. The suspended material percentage was calculated and plotted in the ternary diagram to find the stable suspension region. A point was selected in the mutual stable region for every surfactant system.

2. *Evaluation of wettable powder formulation*

A wetting time test was conducted in which wettability was determined by adding 5 g of the formulated powder sample to a 250 mL beaker containing 200 mL of standard hard water (28±1°C), 342 ppm, based on the $CaCO_3$ concentration. A stopwatch was started as soon as the formulated powder came in contact with the surface of the hard water. The timer was stopped after the powder was fully wetted. The experiment was conducted according to CIPAC method MT 53.3. The wettability result comprised 25% (25A) of the final score.

A suspensibility test was conducted according to CIPAC method MT177. A 250 mL measuring cylinder was filled with 250 mL of WHO (342 ppm) water. Two grams of the formulated wettable powder was transferred to the measuring cylinder. The cylinder was inverted 25 times (one inversion/second). The mixture was allowed to stand for 30 min at ambient temperature. The top 90% (225 mL) of the formulation suspension was pipetted out of the cylinder with the aid of a vacuum pump attached to a glass pipette, leaving behind 10% (25 mL) of the formulation. The remaining content of the measuring cylinder was transferred into a small, pre-weighed Pyrex dish with the aid of water. The dishes were placed in the oven for at least 24 hr at 50°C, and the content of the dish was weighed. The dishes were dried until a constant weight was obtained. The suspensibility result comprised 25% (25B) of the final score.

Foam formation and sedimentation were evaluated according to CIPAC method MT 47. An Imhoff sedimentation cone was filled with 2.5 g of the formulated powder, and the sedimentation height was checked. Then 2.5 g of the formulated powder was added to WHO standard hard water (342 ppm) inside a 100 mL measuring cylinder. The cylinder was sealed with a stopper and inverted, with 30 complete inversions in 1 min. The foam (measured in milliliters) and sediment level were noted immediately after inversion and again after remaining undisturbed for 30 min. The foam volume and sedimentation rate were calculated according to the satisfactory rate. The foam and sedimentation results comprised 25% (25C) of the final score.

A dispersibility test was conducted based on the Mitsui and Takada method,[26] with some modifications. A powder sample weighing one-half of the specific gravity was used in an apparatus for mixing powder and water in a vacuum, the powder was stirred at 10^{-4} mmHg for 1 hr, and then it was dipped completely in distilled water without coming into contact with air. The dispersion liquid of the powder sample was put into a 30 mL sedimentation tube, and water was added to make it 50 mL in volume. It was shaken 25 times by hand and then was left standing.

The dispersion states after 1, 5, and 30 min and 1, 3, 6, and 24 hr were observed and evaluated. The dispersibility result comprised 25% (25D) of the final score. The selection of wettable powder formulation was based on the final cumulative score from each physical test conducted. The formulation's physical score was determined by using a mathematical formula, and the index weight given to the four parameters in the equation (wettability, suspensibility, foam and sedimentation, and dispersibility) was empirically chosen and modified from a similar model.[27)]

$$\text{Final Score} = \frac{25A + 25B + 25C + 25D}{100}$$

The evaluation score was modified from a patented method.[28)] Scores for each formulation were graded as follows:

Above 80 = A
0–79 = B
60–69 = C
50–59 = D
40–49 = E
Below 39 = F

3. *Field evaluation of the effectiveness and side effects of* C. fumosorosea *WP on oil palm plantations*

The experiment was conducted on 5-year-old oil palm trees (*Elaeis guineensis*) at Felda Lepar Hilir 1, Pahang, Malaysia (3.65°N, 103.08°E). In the test, which had 522 trees set up in a randomized complete block design (RCBD) received 5 treatments with 3 replications. The treatments included the three formulated WPs, a standard-practice flubendiamide (granule), and the control. The *M. plana* population was monitored pre- and post-census at 3, 7, 15, and 30 days after treatment (DAT). Frond number 17 was pruned from each sample palm, and the bagworms collected from each pruned frond were sorted, counted, and recorded according to their larval stages: 1.3–2.5 mm, 2.2–3.2 mm, 3.5–4.4 mm, 4.4–6.5 mm, 7.3–8.8 mm, 7.6–10.1 mm, and 9.3–11 mm for the first to seventh instar, respectively.[29)] Further study was conducted on the pollinating weevil *E. kamerunicus*, wherein the population was monitored pre- and post-census at 3, 5, 7, and 14 DAT. The experiment included 87 oil palm trees arranged in completely randomized design (CRD), and 5 treatments with 5 replications were administered. Five male inflorescences at anthesis stage were marked for each treatment using a sample population tapping technique. The sample population tapping was designed on a hard board clipped with A4-sized wax paper sprayed with sticky glue, and the board was held under the male inflorescence. The inflorescence was tapped three times with equal strength, and the adult weevils were counted for each treatment. Data collected were subjected to an analysis of variance (ANOVA) using Statistical Analysis System (SAS) version 9.4, and the means were compared using the honestly significant difference (HSD) test at a significance level of 0.05.

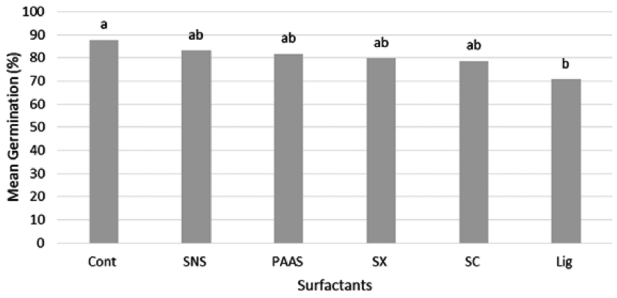

Fig. 1. Germination percentage of *C. fumosorosea* conidia against formulation surfactants.

Results

1. *Additive screening through fungal conidia germination*

Figure 1 shows that five of the surfactants selected achieved 70% germination or above and qualified for further testing. In this experiment, SNS, PAAS, SX, SC, and Lig emerged as qualified surfactants, and among those tested, only two of them were wetting agents SC and SX. The germination rate of the control was the highest among all treatments recorded, at an average of 87.79%. The percentage of germination for SNS, PAAS, SX, and SC differed significantly from that for the control.

2. *Wettable powder formulation*

The region where the formulation gives good suspensibility, dispersibility, and wettability is shown in Fig. 2. Five surfactant systems gave good physical stability to the formulation. The regions of these five surfactants were overlapped to find a mutual stable region. A point was selected in the mutual stable region after limiting the wetting agent to 15% of the surfactant system. This point was used for further mixing with an active fungal ingredient (Fig. 2). Submerged propagules produced from different ways of mass production were used as active ingredients.

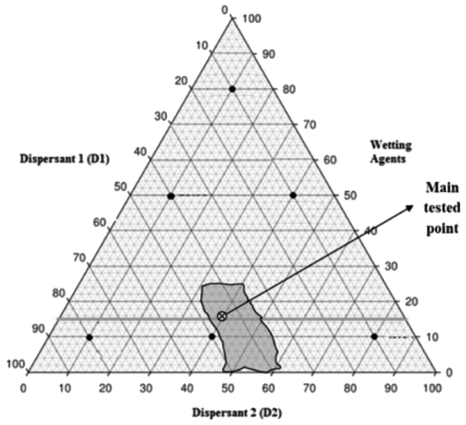

Fig. 2. Point selected from the mutual stable region for all surfactant system tested.

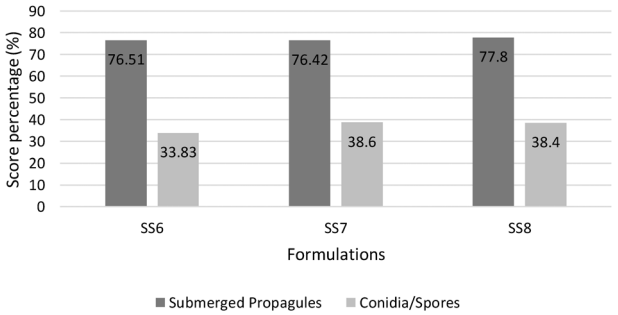

Fig. 3. Submerged propagules formulation and conidia formulation physical characterization cumulative score.

3. Physical test of wettable powder formulations

The selected point, point X, D1 : D2 : W = 52 : 33 : 15, was subjected to physical evaluation with the addition of fungal propagules to the formulation mixture. Figure 3 shows that all submerged propagule formulations showed good physical stability after going through all the physical tests. All the submerged propagule formulations with a score of 70% or higher qualified for further evaluation. SS8 showed the best wettability performance, followed by SS7 and SS6. In the suspensibility test, SS8 performed best, followed by SS6 and SS7. In the dispersibility test, SS6 had the highest score, with 24 out of 25, followed by SS7 and SS8 with 22 and 17, respectively. However, all surfactant systems performed similarly in the sedimentation test as sedimentation formed after suspending the formulation. The best formulation was SS7.

4. Storage stability

All the submerged propagule formulations were stored for a viability test performance within a specific period of time. The result of this experiment is shown in Fig. 4, where the submerged propagule viability for SS8 dropped for the first 3 days of the storage period. The formulation in SS8 had the highest CFU mL^{-1} in comparison with the other two formulations until 3 days after storage. The CFU mL^{-1} of *C. fumosorosea* in all three formulations became stable right after the fifth day. The viability performance for all three qualified formulations was still within $\pm 1.0 \times 10^7$ CFU mL^{-1} after 90 days or 3 months of storage.

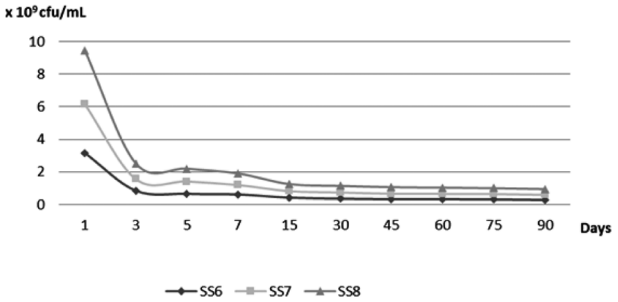

Fig. 4. *C. fumosorosea* submerged propagules formulations viability within three months of storage period.

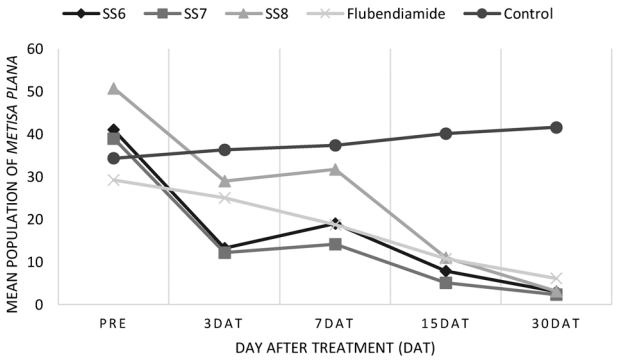

Fig. 5. Mean population of *M. plana* at each DAT application.

5. Efficacy of formulations against the population of M. plana *and their effects on* E. kamerunicus

Table 1 shows that all wettable powder formulations were effective and as good as the positive control. At 30 DAT, the highest mean population of bagworms was with flubendiamide treatment, and the lowest was with formulation SS7. Both pre-census and at 30 DAT, there was a significant difference in the reduction of bagworm populations. For the study of treatment effects on the pollinating weevil population, Table 2 shows that there was a significant difference at 7 DAT. Generally, the control plot showed the highest mean population number, followed by SS8, SS6, SS7, and flubendiamide with the lowest. All in all, the population of bagworms was reduced below the economic threshold level (ETL), 10 larvae per frond, within 30 days of treatment application (Fig. 5). While there is a dip in the mean population of pollinating weevils until 7 DAT, the number recovers after that. The overall population was close to the original population number before treatment application (Table 2, Fig. 6).

Discussion

The time needed for fungal bodies to be mass produced might raise the total production cost. Jackson *et al.*[30] evaluated the production method targeting higher *C. fumosorosea* blastospore concentrations with shorter fermentation times to reduce blastospore production costs. In this study, submerged propagules took less time to fully colonize the liquid media compared to

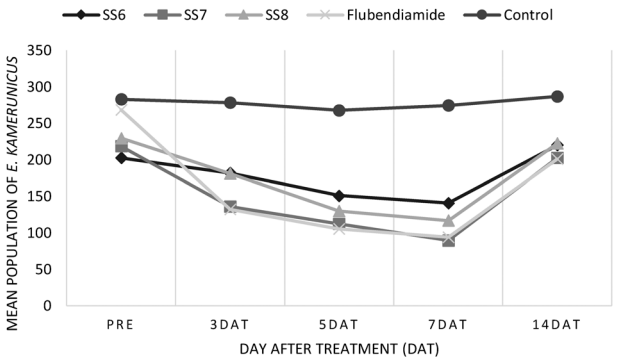

Fig. 6. Mean population of *E. kamerunicus* at each DAT application.

Table 1. Mean population of *M. plana* at each DAT application

Treatment	Pre-census	3 DAT	7 DAT	15 DAT	30 DAT
SS6	41.0±3.6b	13.2±3.8bc	19.0±7.1a	7.9±2.0b	3.0±0.8b
SS7	39.0±3.1b	12.2±2.3c	14.2±4.7a	5.1±2.0b	2.3±0.8b
SS8	50.8±2.8a	29.0±4.9a	31.7±6.2a	11.0±2.4ab	3.1±0.9b
Flubendiamide	29.2±1.8c	25.0±5.4ab	18.7±7.5a	10.7±3.1ab	6.1±0.8ab
Control	34.3±3.7c	21.4±4.5abc	18.00±4.4a	15.6±2.2a	2.4±0.6b
p value	0.0016**	0.0719	06707	0.2789	0.0013**

Note: Means followed by the same letter in the same column are not significantly different at 95% using Least Significant Difference (LSD)

the time taken by the fungus to fully colonize the solid substrate. The submerged propagules consist of several components, such as blastospores, submerged conidia, and macrosclerotia. Mass production of these propagules is easier and can be achieved within a shorter period of time by shaking the inoculated liquid culture medium.[31] The *C. fumosorosea* concentration produced in liquid media was approximately 1.9×10^8 CFU mL^{-1}. This result is still within the range produced with Jackson, Paris, and Catroux media, which was $1.4–5.5\times10^8$ propagules mL^{-1} after 3×10^6 propagules mL^{-1}.[32] Blastospores could germinate within 6–8 hr according to Vega *et al.*[33] Blastospores produced by deep liquid fermentation are more delicate than aerially produced conidia, but their infectability is said to be equal or better. This has been supported by the improvement in the speed of fungal germination with the addition of a nitrogen source, keratin hydrolysate, and the number of propagules developed in the media increased as well.[34] Mycoinsecticide formulations are candidates that still need to be screened, because different fungal species and strains pose different pesticides tolerance on germination and mycelia growth.[35] Furthermore, substances in the formulation also contain chemical that can affect the biological nature of the fungus.[36] Aerial conidia have an advantage when formulated with surfactants, as it has been demonstrated to be much more surfactant tolerance than any other fungal parts like blastospores, submerged conidia, or hyphae.[37,38] Studies have shown that dispersants can be used as microbial growth substrates.[39,40]

Previous studies have proven that biological activities are well known in the group of alkyls for p-hydroxybenzoic acids,[41] where they are common antimicrobial preservatives in pharmaceuticals, cosmetics, foods, and beverages. Generally, the antimicrobial effect of phenolic acid derivatives increases with the length of the alkyl chain.[42] Wraight and colleagues[43,44] found that the preparation of large-volume aqueous suspensions of *B. bassiana* and *C. fumosorosea* was greatly facilitated by the use of organosilicone wetting agents. Sodium lignosulfonate did not cause any harm to *Metarhizium anisopliae* conidia viability with a germination of 96.6%.[45] It was found that the surface activity and foaming property of lignosulfonate with a high molecular weight were superior to that with of low molecular weight,[46] so adding a small amount of sodium lignosulfonate to hammer-milled powder will greatly increase the suspensibility.[47] Therefore, the significant effect of slower fungal growth in sodium lignosulfonate–treated media shown in this study was probably caused by other factors.

The performance of submerged propagule formulations was slightly lower than that of the blastospore formulations by Jackson *et al.*,[48] where no sedimentation occurs for up to 2 hr. This situation might be because the blastospore formulations developed have a smaller colloidal size than the submerged propagule formulations developed in this experiment. The weight of the colloid would affect settling where the powder made with a bigger colloidal size could not overcome the gravitational force that pull the powder to the base of the suspension forming sediments. Sedimentation and suspensibility are related to each other; therefore, particle size reduction can improve suspensibility. In spite of the presence of dry powder as a wetting agent, the conidia formulation could not penetrate the water surface tension. Formulation moisture content of can impact the spore survival during storage.[49] The same goes for any other propagules used in the formulation. The moisture content of a wettable powder formulation must be maintained at 5%, and product viability will deteriorate progressively if the moisture exceeds.[50] This experiment has strictly kept the moisture of the formulation below that percentage. The sudden decrease in formulation

Table 2. Mean population of *E. kamerunicus* at each DAT application

Treatment	Pre-census	3 DAT	5 DAT	7 DAT	14 DAT
SS6	202.6±36.6a	182.0±33.3a	150.8±23.0a	140.8±21.3b	219.8±11.6a
SS7	218.4±42.9a	136.2±32.8a	112.2±23.9a	89.6±23.6b	202.2±26.9a
SS8	229.6±32.1a	180.8±34.1a	129.8±6.7a	116.6±5.8b	222.0±28.5a
Flubendiamide	268.4±57.9a	131.8±31.4a	105.6±28.8a	94.0±27.1b	201.0±8.1a
Control	282.6±34.5a	218.0±44.8a	214.0±23.3a	204.0±4.8a	224.4±22.56a
p value	0.956	0.715	0.205	0.028*	0.498

Note: Means followed by the same letter in the same column are not significantly different at 95% using Least Significant Difference (LSD)

viability in the first 3 days of the storage period could be due to the desiccation effect. Relative humidity close to 100% is an ideal condition for entomopathogenic fungi to be effective biological control agents.

Treatment application in the field promises considerable outcome in controlling *M. plana*. All three formulations tested performed equally with the positive controls, which caused a reduction in the population of *M. plana*, starting gradually from pre-census and continuing until the last observation 30 DAT. At 3 DAT, the population reduction was quite high, without any significant difference among treatments. Stauderman *et al.*[51] explained that due the mode of action of entomopathogenic fungi, under very humid conditions, it usually takes 2 or 3 days to develop and penetrate their infectious mycelia through the insect cuticle, causing insect death by fungal toxins in a minimum of 3 days. Further, there was a highly significant reduction in the population of *M. plana* at 30 DAT, when all three formulations tested showed the lowest population as compared to both positive controls; the lowest population was recorded with formulation SS7. These results are in agreement with the finding of Loong *et al.*,[52] which demonstrated the efficacy of *C. fumosorosea* against Lepidopteran pests. In contrast, the efficacy of the formulations tested was different on *E. kamerunicus*, where there was a dip in the population of the pollinating weevil 7 DAT. The significant difference in population that can be observed on this day may be due to the fungi finally penetrating the cuticle layer.[53] From there on, the population started to recover, proving that the formulations are not harmful to beneficial insects. In conclusion, all tested submerged propagule formulations were able to control *M. plana* and had no side effects on *E. kamerunicus*. The ability of this wettable powder to adhere to the top of the bagworm's bag could be the key to success in preventing the outbreak of *M. plana*. Still, the best way to control the defoliating pest is *via* oral uptake. The contact between the propagules and the insect body could only cause mortality if the propagules were able to invade the pest body through the pores or any other opening. The formulations' time to lethality still needs to be improve, because the only way for mycoinsecticides to cause quicker infection is through the oral uptake of fungal propagules by the target pests.

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