STUDIES ON THE EFFICACY OF *BEAUVERIA BASSIANA* (BALSAMO) VUIL. AND *METARRHIZIUM ANISOPLIAE* (METS.) SOROKIN FOR THE CONTROL OF THREE COCONUT PESTS, *TIRATHABA RUFIVENA* WALKER, *PROMECOTHECA CUMINGII* BALY, AND *PLESISPA REICHEI* CHAPIUS UNDER LABORATORY CONDITIONS[1]

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The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuil. isolated from rice planthopper, *Nilaparvata lugens* was found to effect higher mortality on *Tirathaba rufivena* Walker, *Promecotheca cumingii* Baly, and *Plesispa reichei* Chapius than *Metarrhizium anisopliae* (Mets.) Sorokin isolated from *Oryctes rhinoceros* L.

The *B. bassiana* isolate needs further evaluation under field condition.

INTRODUCTION

Biological control has been an integral part of pest management recommendations of the Philippine Coconut Authority (PCA). Utilization of parasitoids as biocontrol agents of major pests of coconut has been carried out and proved to be successful in some pest species. For example, *Argyrophylax basifulva* Bezzi, a tachnid fly from Fiji introduced against *Tirathaba rufivena* Walker was found to establish successfully in the field after 5 yr.

In some cases where the use of parasitoids is not practical the use of entomopathogens solely or in combination with parasitoids might prove useful.

Beauveria bassiana (Balsamo) Vuil. and *Metarrhizium anisopliae* (Mets) Sorokin naturally infect some coconut pests in the field in sporadic cases. These microorganisms were tested on *T. rufivena*, *Promecotheca cumingii* Baly, and *Plesispa reichi* Chapius under laboratory conditions preparatory to tests under field situations, possibly as mycoinsecticides.

The Target Pests

The coconut spikemoth, *T. rufivena*, is considered one of the major pests of coconut attacking the spike, hence the name. The pest normally attacks early bearing palms from 3 to 5 yr old. It was also observed that palms with slow opening spathes are usually infested as congested spikelets favor the development of the immature stages of the pest. The young larvae feed on male flowers and, as they grow older, attack the female flowers or nutlets, causing premature nutfall. The pest reduces yield up to as high as 32% (Taylor 1930).

In the Philippines, the pest was reported in Macrohon, Southern Leyte as early as 1979 (PCA-Annual Report 1980). Eloja and Abad (1982) found five chemical insecticides to be effective against *T. rufivena* applied at weekly intervals for 4 to 6 wk depending on the magnitude of infestation. These include diazinon, triazophos, trichlorphon, pirimiphosmethyl, and azinphos-ethyl. Later, Gallego and Abad (1985 and 1987) reported parasitoids associated with the pest. These were *Apanteles tirathabae* Wilkinson (larval) and *Venturia palmaris* Wilkinson (larval-pupal) parasitoid, and *A. basifulva*, which was introduced to the Philippines from Fiji.

The coconut leafminer, *P. cumingii*, is considered a major pest of coconut. The adult feeds on leaf tissues leaving a threadlike tunnel on affected leaflets while the larva feed and develops on tunnels made between the upper and lower epidermis of coconut leaflets. In severe outbreaks of the pest in Quezon, Batangas, and Laguna provinces, reduction in yield was as high as 85 % 1 yr after initial infestation (Aldaba 1931). In the early 1980s, severe infestation was reported in Northern Mindanao (Gallego et al. 1982). Five hymenopterous parasitoids were identified to affect the pest. These include *Sympiesis sp.*, *Cirrospilus* sp., *Pediobius anomalus* Ferr., *Cotterellia* sp., and *Achrysocharis promecothecae* Ferr.

The establishment of leguminous cover crops under coconut is said to indirectly control the coconut leafminer. The covercrop flower serves as source of food for the adult parasitoids which in turn control the pest.

The two-colored hispid beetle, *P. reichei*, is an important pest of coconut in the nursery and young field plantings. Both adult and larva of the pest gnaw long incisions in the epidermis and superficial tissues of unopened leaves. When the pest is abundant, these incisions are made close to one another so that the whole damaged portion of the leaflets dies, shrivels, and can easily be torn by the wind. Damage was observed to be pronounced during periods of low rainfall. Heavily infested palms lose vigor while

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growth is delayed (Gallego and Abad 1985b). In prolonged infestations the palms may be killed (Lever 1969).

MATERIALS AND METHODS

B. bassiana and *M. anisopliae* were cultured purely *in vitro*. The former was cultured in a commercially prepared Sabrouraud dextrose agar (SDA), while the latter was grown in potato dextrose agar (PDA). The test insects (*T. rufivena, P. cumingii,* and *P. reichei*) were mass-reared in the laboratory using their host plant.

Effect of Three Rates of *B. bassiana* on the Mortality of *T. rufivena*

The experiment was a completely randomized design (CRD). It involved three rates (10,20,30 mg/100 mL water) of *B. bassiana* against *T. rufivena* replicated three times with 30 sample insects per replicate and with the same number of samples in the control. The fungal suspensions were each misted on the test insects using an atomizer.

Comparative Effects of *B. bassiana* and *M. anisopliae* against *T. rufivena, P. cumingii*, and *P. reichei*

The experiment was arranged in a completely randomized design (CRD) using the two fungi (*B. bassiana* and *M. anisopliae*) against the three insect pest species (*T. rufivena, P. cumingii*, and *P. reichei*) replicated three times with 15 sample larvae per replicate. Two-week old axenic cultures of the fungi, each at 30 mg were suspended in 100 mL of water and sprayed on test insects using an atomizer. The treatments were:

T. rufivena treated with *B. bassiana* and *M. anisopliae*
P. cumingii treated with *B. bassiana* and *M. anisopliae*
P. reichei treated with *B. bassiana* and *M. anisopliae*

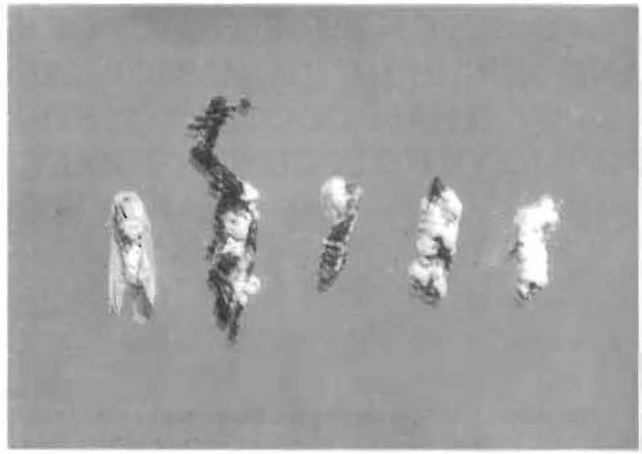

FIGURE 1. Different stages of *T. rufivena* infected with *B. bassiana.*

Data Gathered

a. Spore counts of *B. bassiana* and *M. anisopliae* per 100 mL of water (using a hemacytometer under low power objective of a compound microscope).
b. Percentage mortality, using the formula:

$$\% \text{ mortality} = \frac{\text{no. of dead insects}}{\text{total no. of test insects}} \times 100$$

c. Days to mortality after treatment application
d. Days from death to appearance of mycelial growth

RESULTS AND DISCUSSION

Effect of Three Rates of *B. bassiana* on the Mortality of *T. rufivena*.

a. Spore count

TABLE 1. Effect of different concentrations of *B. bassiana* on *T. rufivena**

Treatment	Spore Count/ 100 mL	Mortality after Treatment: Days	Mortality after Treatment: Percent	Appearance of Mycelial Growth after Death (days)
10 mg/30 mL	[illegible]19.5c	10.0c	32.1a	3.7b
20 mg/30 mL	765.9b	13.4b	21.7a	4.1ab
30 mg/30 mL	1,127.5a	10.4c	36.7a	5.2a
Control		30.0a	1.0b	0.0c
Stat. Significance				
S.E. (Treat. Mean)	29.1	0.06	0.33	0.07
LSD 0.05	64.0	0.15	0.75	0.16
0.01	87.7	0.22	1.09	0.23
C.V. (%)	7.9	4.1	21.5	9.4

* Means of four replicates; means with the same letter(s) are not significantly different at 5% level. Analysis based on transformed values (log x + 1).

The number of spores for the three rates of *B. bassiana* differed significantly. Spore count increased with increase in concentration (Table1).

b. Percentage mortality

Percentage mortality of *T. rufivena* did not vary among the three rates used but were all significant over the control.

It was observed that *B. bassiana* infected the larvae through the cuticle. An early sign of infection was cessation of feeding which was usually noticeable 5 to 8 days after treatment application. The insect then became sluggish and died. Later, white mycelial growths colonized the body of the insect (Fig. 1).

Percentage mortality of the different treatments ranged from 10% to 53.9%. The treatment with the highest percentage mortality was 30mg/100 mL water (36.7%), followed by the 10 mg/100 mL water concentration with 32.1%. The 20 mg/100 mL rate had the least mortality with 21.8% (Table 1). Mortality in the control (1.0)% was suspected to be caused by mechanical rupture of cuticle during food replenishments as no fungal growth was noted on the dead insects.

c. Days to mortality of *T. rufivena* larvae after treatment application

T. rufivena larvae died ranging from 9.2 to 15.4 days after treatment application. The lowest and highest concentrations (10 mg and 30 mg, respectively, per 30 mL water) yielded the shortest average periods for the test insects to die (10.0 and 10.4 days, respectively) from treatment application. The 20 mg/100 mL water concentration gave the longest period to mortality with 13.4 days which was still significant over the control (Table 1).

Most of the insects in the control pupated and reached the adult stage.

d. Appearance of mycelial growth of *B. bassiana* on larvae after death of insects

An inverse relationship was observed between mycelial growth of *B. bassiana* and inoculum concentrations.

Mycelial growth of *B. bassiana* appeared earliest on those treated with 10 mg/30 mL suspension (mean = 3.7 days after larval death). This was followed by those treated with 20 mg/30 mL water (mean = 4.11 days), and latest on the highest concentration (mean = 5.2 days).

This result suggests that as the spores become too crowded, mycelial growth is inhibited due to resource competition or other unknown factors which are beyond the scope of this experiment.

Comparative effect of *B. bassiana* and *M. anisopliae* against *T. rufivena, P. reichei,* and *P. cumingii*

a. Spore count of *B. bassiana* and *M. anisopliae*

The number of spores between the two fungal pathogens varied significantly. *B. bassiana* had higher spore count than *M. anisopliae*. *B. bassiana* spores ranged from 1,111 to 1,188 (mean = 1,127) while those of *M. anisopliae* ranged only from 31.6 to 49.0 (mean = 36.4) at the same concentration of 30 mg/100 mL water (Table 2). This could be due to the difference in the spore sizes of the fungi, the sporulating habit of the fungi on the culture media used, the environment in which the organisms were grown, and/or other unknown factors.

b. *T. rufivena*

Mortality of *T. rufivena* was significantly higher on insects treated with *B. bassiana* (mean = 55.5%) than *M. anisopliae*-treated insects with 13.3% (Table 2). Mortality in the control and *M. anisopliae*-treated insects was largely attributed

FIGURE 2. Adult *P. cumingii* infected with *B. bassiana*.

FIGURE 3. Adult *P. reichei* infected with *B.bassiana*.

TABLE 2. Percentage mortality of *T. Rufivena, P. cumingii,* and *P. reichei* treated with *B. bassiana* and *M. anisopliae**

Treatment	Spore Count/ 100 mL	*T. rufivena*	*P. cumingii*	*P. reichei*
B. bassiana	1,127.5a	55.5a	57.7a	100.0a
M. anisopliae	36.4b	13.3ab**	0.0b	8.9b
Control		4.4b	4.33b	2.2b
Stat. Significance				
S.E. (Treat. Mean)	12.7	0.43	0.51	0.65
LSD 0.05	32.8	1.06	1.26	1.59
0.01	31.4	1.61	1.90	2.40
C.V. (%)	4.4	28.4	54.1	48.2

* Means of four replicates; means with the same letter(s) are not significantly different at 5% level. Analysis based on transformed values (log x + 1).

** Mortality was not due to the fungus but to mechanical injury.

to mechanical injury during handling and in some cases due to contamination by *M. anisopliae.*

There was no significant difference in the length of time the test insects died after treatment application with either *B. bassiana* or *M. anisopliae.* Similarly, the time of appearance of mycelial growth after the test insect died did not vary between the two treatments.

c. *P. cumingii*

Mortality of *P. cumingii* was significantly higher on insects treated with *B. bassiana* (Fig. 2) at 57.7% than the other treatment (Table 2). In fact, there was no mortality on insects treated with *M. anisopliae* within the 12-day period after inoculation. Although untreated insects had a 4.3% mortality, this was not significant over the *M. anisopliae* treatments. Again, it was highly suspected that mortalities on the control were caused by handling, stress under captivity, some cases of cross-contaminations, and other factors.

TABLE 3. Length of time the *P. cumingii* and *P. reichei* died after treatment with *B. bassiana* and *M. anisopliae**

Treatment	Days to Mortality	
	P. cumingii	*P. reichei*
B. bassiana	12.1b	5.9b
M. anisopliae	30.0a	13.4ab
Control	25.0a	28.3a
Stat. Significance		
S.E. (Treat. Mean)	0.14	0.326
LSD 0.05	0.432	0.798
0.01	0.518	1.208
C.V. (%)	7.9	22.2

* Means of four replicates; means with the same letter(s) are not significantly different at 5% level. Analysis based on transformed values (log x + 1).

The length of time the test insects died after treatment application with *B. bassiana* was 12.1 days (Table 3). Mycelial growth of the fungus was noted 3 days after death.

d. *P. reichei*

A 100% mortality of *P. reichei* was obtained with *B. bassiana* treatment (Table 1, Fig. 3). Mortalities in the *M. anisopliae*-treated and control insects did not differ significantly. In addition, it took only 5.9 days for the test insects to die from *B. bassiana* treatments and 13.4 days from *M. anisopliae*

SUMMARY AND CONCLUSION

Beauveria bassiana isolated from rice planthopper *Nilaparvata lugens* was found to be more effective as compared to *Metarrhizium anisopliae* isolated from *Oryctes rhinoceros* L. against *Tirathaba rufivena, Promecotheca cumingii,* and *Pesispa reichei* under laboratory conditions. The reasons that can be forwarded with regard to these findings are: the host-specific nature of the fungi, the fungus strain, the environmental conditions with which they were tested, and other unknown factors.

The effectiveness of *B. bassiana* under field conditions needs further evaluation.

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