PATHOGENICITY TEST ON *BEAUVERIA BASSIANA* (BALSAMO) AGAINST OIL PALM BAGWORM *(Metisa plana Wlk)*

KEY WORDS : *Beauveria bassiana*: Biological control; Pathogenicity; *Metisa plana* and *Elaeis guineensis*.

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The pathogenicity of Beauveria bassiana *(*Balsamo*) Vuillemin towards bagworm,* Metisa plana Wlk. *second and fourth instars of* Metisa plana Wlk. *was confirmed. The larvae were sprayed with different concentrations of conidia suspension and exposed at a temperature of 28±1°C and a relative humidity of 65%-75% and 85%-100% ('ambient' and 'humid' respectively). The percentage mortality, lethal concentration (LC_{50}) and the leaf area damaged (LAD) were calculated.* B. bassiana *was capable of infecting both stages of* M. plana. *The percentage mortality increased with increasing concentration of conidia suspension. The LC_{50} of* B. bassiana *for second instar was 4.2 x 10^4 conidia/ml under humid, and 1.5 x 10^6 conidia/ml under ambient moisture conditions. For fourth instar the LC_{50} was 2.4 x 10^6 conidia/ml under humid and 2.5 x 10^7 conidia/ml under ambient moisture conditions. Second instar of* Metisa plana Wlk. *was thus more susceptible to the pathogen than fourth instar. Increased humidity enhanced the pathogenicity of* B. bassiana *and lowered the feeding activity of* M. plana.

INTRODUCTION

The bagworm, *Metisa plana* Wlk. (Lepidoptera: Psychidae), has been one of the most important occasional pests in plantations of the oil palm (*Elaeis guineensis* Jacq.) in Malaysia since 1956 (Wood, 1968). During the period 1981 to 1985, more than 10 000 hectares of oil palm were seriously attacked by bagworm (Basri *et al.*, 1988). To

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Original Manuscript Received : 6 October 1992.
Revised Manuscript Received: 22 November 1992.

date, spraying with narrow spectrum or trunk injection systemic insecticides have been the only available means of control for this pest (Wood *et al.*, 1974; Chung, 1988). Frequent usage of insecticides, and over-reliance on them, have eliminated some of the bagworm's natural enemies and developed insecticide resistance.

Selective use of insecticides has been practised to conserve natural enemies to a certain extent and to delay insecticide resistance. Other avenues of control which can be exploited include augmentation of natural enemies like parasitoids, predators and microorganisms. The use of microorganisms, particularly the entomopathogenic fungi, as biological control agents for *M. plana* is still undeveloped.

A white, powdery fungus was observed growing on cadavers of *M. plana* collected from an estate in the coastal region of Selangor. This fungus was isolated and with the co-operation of the International Mycological Institute in London, identified as *Beauveria bassiana* (Balsamo) Vuillemin.

Earlier investigations had proved the pathogenicity of *B. bassiana* to a wide range of insect pests (Ferron, 1978, 1981; Hussey and Tinsley, 1981). In Malaysia, *B. bassiana* was applied to control the coconut moth, *Levuana iridiscens* B. B. (Lever, 1948). It was also parasitic to the coconut leaf miner, *Promecothena cuminggi* in Province Wellesley, Penang (Ding, 1975). *Beauveria bassiana* was infective to the major pest of cocoa, *Helopeltis theobromae* at an estate in the coastal region of Selangor (Lim *et al.*, 1989). In 1989, Desmier de Chenon *et al.* reported the infectivity of *B. bassiana* to *M. plana*, but no study was carried out on the extent of pathogenicity.

This paper gives an account of the pathogenicity of the indigenous isolate of *B. bassiana* as a biocontrol agent for *M. plana*.

MATERIALS AND METHODS

Source of eggs

Eggs of *M. plana* still within pupal bags were collected from an estate in Selangor. Each pupal bag was placed individually on oil palm leaflets placed in plastic vials measuring 4.5 cm in diameter and 8.5 cm in height. The eggs hatched a week later in a controlled environment room.

Indoor Insect Rearing

Leaflets from number 17 fronds of *tenera* oil palm were cut, surface sterilized with 70% ethanol, and rinsed twice with double deionized water. The lower third of each leaflet was inserted through an opening made in the stopper of a plastic vial and submerged in distilled water in the vial. The plastic vials were then placed individually in sterilized transparent plastic cylinders measuring approximately 13.5 cm in diameter and 25.5 cm height.

Test insects

Ten first instars were transferred into sterilized plastic cylinders containing the leaflets as described above. Starting from the second instar, the number of larvae was reduced to five per leaflet. The leaflets were replaced every week until the larvae pupated. The cylinders containing the larvae were kept in a laboratory with an average temperature of 24° ± 3°C and 65%–75% relative humidity. A total of 30 active second instars (L2) and larval instars (L4) were selected at random and weighed. The mean weights of L2 and L4 used in this study were 2.17 ± 1.37mg and 6.99 ± 2.69 mg respectively. The weights ranged from 0.2–5.3 mg and 3.8–12.7 mg for L2 and L4 respectively.

Inoculum

Subcultures of *B. bassiana* were maintained on potato dextrose agar plates for one month. They were incubated in the dark, at 28 ± 1°C.

Preparation of Conidia Suspension

An average of 10 to 15 ml of sterilized distilled water containing 0.02% Tween 20 was added into culture plates. The fungal spore suspensions so obtained were pooled in a conical flask and agitated for 30 minutes to render homogeneity. An aliquot was then filtered through sterilized glass wool to separate the mycelia from dissociated conidia. The concentration of stock conidial suspension was determined using the improved standard haemocytometer method with the aid of a phase-contrast microscope. Five concentrations of conidial suspension ranging from 5.625×10^2 to

5.625 x 10^6 conidia per ml were prepared.

Bioassay

Prior to treatment, the active L2 and L4 were placed on leaflets as described above. Five concentrations of conidia suspension, namely C1 = 5.625 x 10^2, C2 = 5.625 x 10^3, C3 = 5.625 x 10^4, C4 = 5.625 x 10^5 and C5 = 5.625 x 10^6 conidia per ml and control (Co) were sprayed on to the leaflets with the larvae. Each treatment was done in triplicate. The larvae were sprayed with sterile distilled water containing 0.02% Tween 20 in the control.

Incubation

The treated larvae were maintained at room temperature at two relative humidity levels: 'ambient' (65%-75%) and 'humid' (85%-100%). To obtain the humid condition, an additional plate containing moist cotton wool was placed in the respective cylinder. To maintain the humidity, an average of 10–15 ml of distilled water was added to the plates every two days.

Data recording and processing

Larval activity was observed daily for fifteen days and mortality was recorded. The data on mortality were compared using Duncan multiple range test (SAS, Institute, 1985). The LC_{50} and LC_{90} were determined using probit analyses (Finley, 1971; Wigley and Kalmakoff, 1977).

Measurement of Leaf Area Damage (LAD)

The areas damaged by larvae on both sides of the leaflets were cut out and measured, using a leaf area meter (AT Delta-T, England). The mean areas damaged were statistically analyzed as just described for larval mortality. The percentage reductions in LAD were then calculated using the following formula:

$$\% \text{ Reduction LAD} = \frac{(\textit{LAD control} - \textit{LAD treatment}) \times 100}{\text{LAD control}}$$

Histopathology

Dead larvae obtained from each treatment were fixed in 4% buffered glutaraldehyde and washed with 0.1 M sodium cacodylate buffer for 10 minutes. The specimens were post fixed in osmium tetroxide and rinsed twice with double distilled water. They were then dehydrated in a series of graded acetone and infiltrated with agar araldite in a vacuum oven (Hot pack) at 45°C for two hours and at 60°C overnight. The embedded specimens were sectioned (semithin), using a microtome (Reichert Jung, Ultracut E). The semithin sections were fixed on cleaned glass slides and stained with lead. Photomicrographs were taken using a phase-contrast microscope (Jenaval, Carl Zeiss).

RESULTS

The percentage cumulative mortalities of L2 and L4 of *M. plana* exposed under the two moisture conditions are shown in *Figures 1* and *2*. Fifteen days after treatment (DAT), the L2 exposed to ambient moisture conditions showed 80% and 60% cumulative mortality at fungal concentrations of 5.625 x 10^6 and 5.625 x 10^5 conidia per ml respectively. This result was significantly effective at an α value of 0.05. No significant differences were observed for any inoculum concentration less than C4 (5.625 x 10^5 conidia per ml), *i.e.* they were equally ineffective. Under humid conditions, *B. bassiana* caused 100% mortality at 5.625 x 10^6 conidia per ml even at 9 DAT, and 93% mortality at 5.625 x 10^5 conidia per ml at 15 DAT.

The extrapolated lethal times 50 (LT_{50}) for L2 subjected to an inoculum concentration of 5.625 x 10^6 conidia per ml were 6 and 10 days, under humid and ambient conditions, respectively. The LT_{90} for the most concentrated inoculum was achieved under humid conditions within approximately a week for the L2.

Likewise, under humid conditions *B. bassiana* induced greater mortality for the L4. The cumulative mortality at 15 DAT was 81% under humid conditions as compared with 60% under ambient conditions with an inoculum concentration of 5.625 x 10^6 conidia/ml (*Figure 2*). The results obtained with the highest level of inoculum under both moisture conditions were significantly effective for L4 at an α value of 0.05.

The LT_{50} for L4 subjected to an inoculum concentration of 5.625 x 10^6 conidia per ml were 9 and 15 days under humid and ambient conditions,

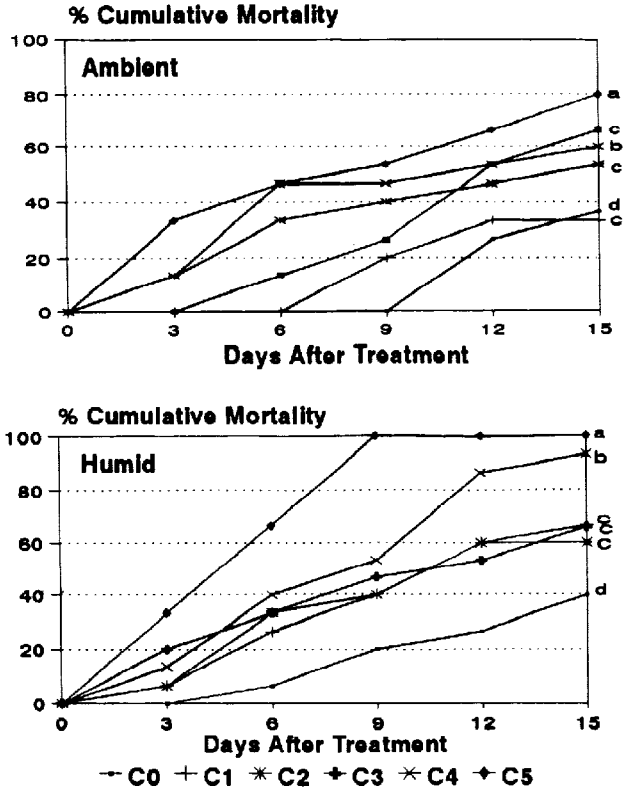

Figure 1. The percentage cumulative mortality of M. plana *L2 treated with* B. bassiana *with the same letters are not significantly different at P=0.05. For the meaning of C0, C1, O2, etc., see text (Bioassay)*

respectively. However, the highest concentration of inoculum used for L4 was insufficient, under both moisture conditions to obtain the LT_{90} in less than fifteen days.

Data for the leaf area damage (LAD), caused by the L2 and L4 *of M. plana* are shown together in *Figure 3*. For L2, the LAD under high humidity in the control trial was 5.31 ± 0.88 cm^2; this was slightly more than, but not significantly different from the LADs in the other treatments. On the other hand, under ambient conditions, the highest level of *B. bassiana* (C5: 5.625 x 10^6 conidia per ml) significantly reduced the LAD (*Figure 3*).

In the case of L4, the LAD for the control under ambient condition was 20.92 ± 5.39 cm^2, significantly different from the LAD for C5, which was 13.36 ± 1.71 cm^2. Under humid conditions, the LAD in the control was 16.48 ± 3.58 cm^2, significantly more damage than with C4 and C5 (10.04 ± 2.54 cm^2 and 6.11 ± 2.74 cm^2 respectively).

Figure 4 shows that the percentage reduction in LAD caused by *M. plana* subjected to *B. bassiana* was generally higher for L2 (40%-70%) than for L4 (20%-50%). *Figure 4* also shows that for L2 under ambient conditions, treatment C5 was significantly effective in obtaining a high percentage reduction of LAD. On the other hand, under humid conditions both C4 and C5 effected a similar reduction of LAD. As for L4, none of the treatments under ambient conditions was effective, but under humid conditions C5, gave a 50% reduction in LAD, as also shown in *Figure 4*.

LC_{50} and LC_{90} probit analyses for *B. bassiana* as a biocontrol agent for *M. plana* are summarized in *Table 1*. As expected, the LC_{90} values were high under ambient conditions, 8.6 x 10^{19} and 3.6 x 10^{10} conidia/ml for the L2 and L4 respectively. The LC_{90} values were much smaller, 1.6 x 10^{13} and 1.49 x 10^{14} conidia/ml, for L2 and L4 larvae subjected to humid conditions.

Under ambient condition, reasonable levels of *B. bassiana* were sufficient to obtain LC_{50}, *i.e.* 1.5 x 10^6 and 2.4 x 10^7 conidia/ml for L2 and L4 larvae respectively. Under humid conditions, the LC_{50} values decreased to 4.2 x 10^4 and 2.4 x 10^6 conidia/ml for L2 and L4 respectively.

Sections examined for histopathological effects revealed that *B. bassiana* was the aetiological agent responsible for the larval mortality. *Plate I* shows

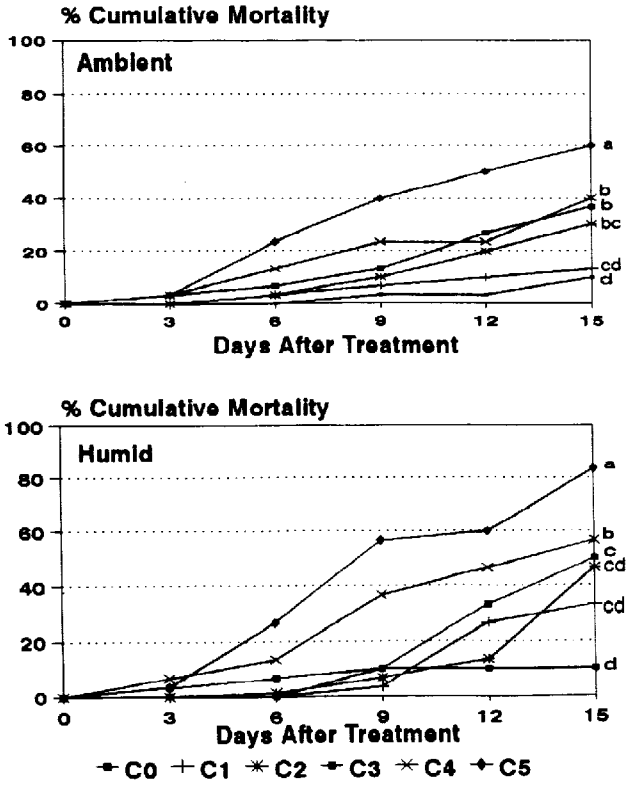

Figure 2. The percentage cumulative mortality of M. plana *L4 treated with* B. bassiana *with the same letters are not significantly different at P=0.05.*

TABLE 1. THE PATHOGENICITY OF *B. bassiana* AGAINST *M. plana* EXPOSED TO AMBIENT AND HUMID CONDITIONS

Parameters	Second Larval Instar		Fourth Larval Instar	
	Ambient	**Humid**	**Ambient**	**Humid**
1. LC_{90} (conidia/ml)	8.6371×10^{19}	1.6126×10^{13}	3.55678×10^{19}	1.4852×10^{14}
Fiducial Limit (95%)				
–Upper Limit	1.7482×10^{32}	5.6216×10^{17}	3.3202×10^{27}	3.6478×10^{17}
–Lower Limit	4.2673×10^{7}	4.6226×10^{8}	3.8337×10^{11}	6.0440×10^{10}
2. LC_{50} (conidia/ml)	1.4684×10^{6}	4.2457×10^{4}	2.4943×10^{7}	2.4014×10^{6}
Fiducial Limit (95%)				
–Upper Limit	4.8335×10^{6}	2.2246×10^{5}	3.1695×10^{8}	9.9751×10^{6}
–Lower Limit	4.4613×10^{4}	8.1028×10^{3}	1.9629×10^{6}	5.7810×10^{5}
3. Regression of log (concentration) on probit mortality	Y=3.7169 + 0.2905X	Y=3.6551 + 0.4462X	Y=2.8128 + 0.3291X	Y=2.1094+ 0.5134X
4. Slope	0.2905	0.4662	0.3291	0.5134
5. SE Slope	0.1563	0.1556	0.1172	0.1179
6. X^2 Test	2.50	1.34	1.83	1.23

The data were obtained 10 days after treatment. Heterogeneity about regression at P=0.05 and df=3. Log Concentration, X=Probit Mortality. SE=Standard Error.

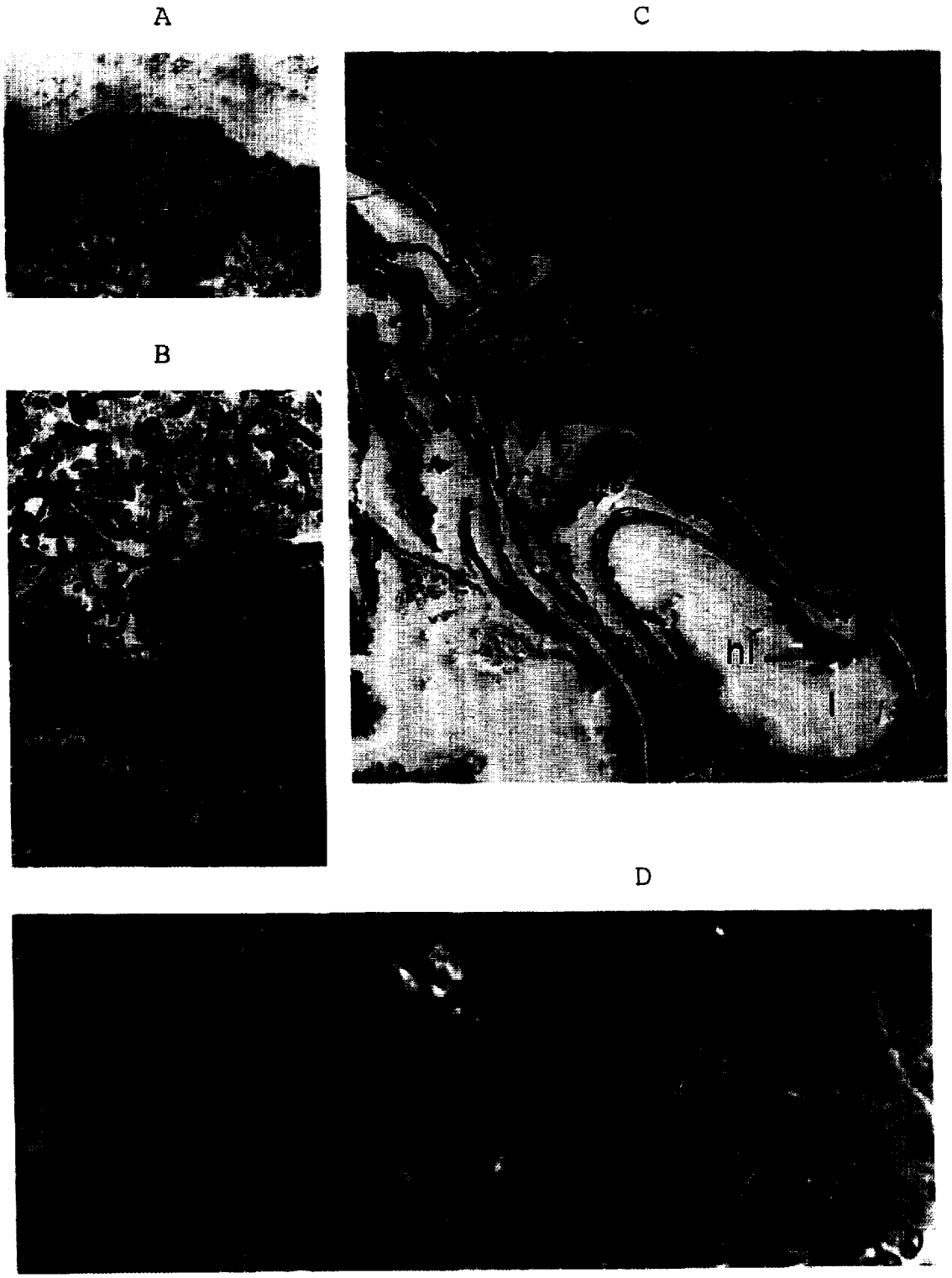

Plate 1. Photomicrographs of infected Metisa plana *fourth larval instar with magnification 500x (A), 1000x (B,C) and 2000x(D).* B. bassiana *in cuticle layer (bc), blastospores in interstitial tissue (bi), hypae penetrating gut lumen (hl), hypae penetrating muscle (hm), hypae penetrating the ganglion (hg), cuticle (c), muscle (m) and midgut lumen of insect (l).*

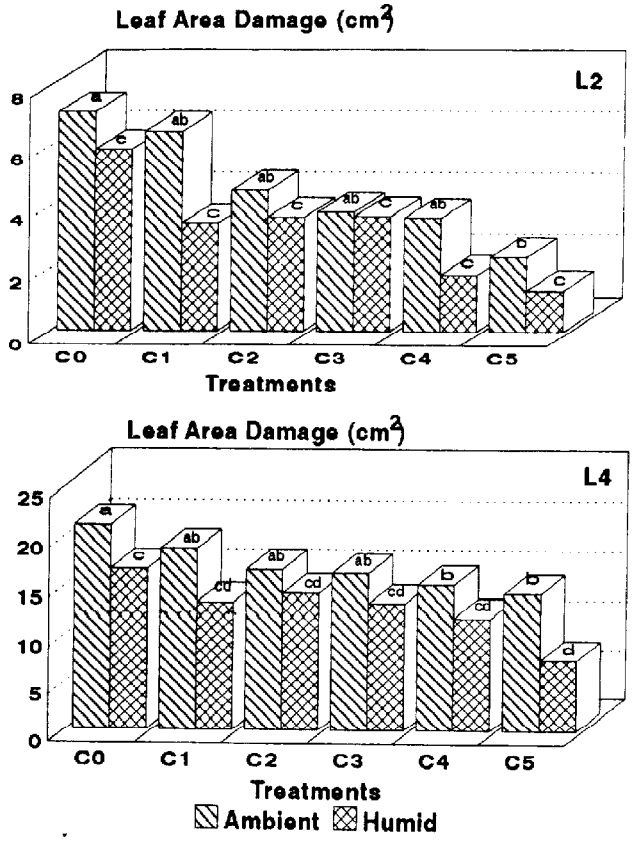

Figure 3. The leaf area damaged (LAD) caused by M. plana *L2 and L4 treated with various level of* B. bassiana. *LAD values with the same letters are not significantly different at P=0.05.*

that the mycelium mummified the larvae by penetrating throughout the cross-section of the midgut. Complete colonization of whole organs and tissues resulted in absolute systemic structural and functional disruption of the insect larvae.

DISCUSSION

The data collected in this study prove that the mortality of L2 and L4 *of M. plana* increase with the concentration of *B. bassiana*. Similar observations were made by Aeschlimann *et al.* (1985) on *Sitona discoides* and Magalhaes *et al.* (1988) on *Coleomegilla maculata* and *Eriopis connexa*. Under ambient condition the effective inocula used for the L2 were 5.625 x 10^5 and 5.625 x 10^6 conidia/ml. These levels of inoculum significantly killed 60% and 80% of the insect population, at 15 DAT at an α value of 0.05. Propagule concentrations of 5.625 x 10^4 and below were ineffective for L2. Increased humidity increased the pathogenicity of *B. bassiana*, as seen in the mortality for L2 under humid conditions which was 100% at DAT. Extrapolated, LT_{50} values for L2 under ambient and humid conditions were 10 and 6 days respectively. This observation implies that the biocontrol agent would be better applied during a wet season. Increased water activity increases the germination of the propagule and hence the rate of fungal colonization (Griffin, 1981).

Leaf area damage caused by L4 was greater than that by L2. The L2 were smaller feeders than the L4, which resulted in insignificant differences of LAD between treatments, as shown in *Figure 3*. *Figure 3* suggests that the L4 are voracious eaters significantly controllable by treatment with *B. bassiana* at a conidial concentration of 5.625 x 10^6 conidia/ml, preferably during a wet season. *Figure 4* shows that *B. bassiana* as a control agent for *M. plana* gave a higher percentage reduction of LAD in L2 than in L4. Significant LAD reduction with L2 was achieved

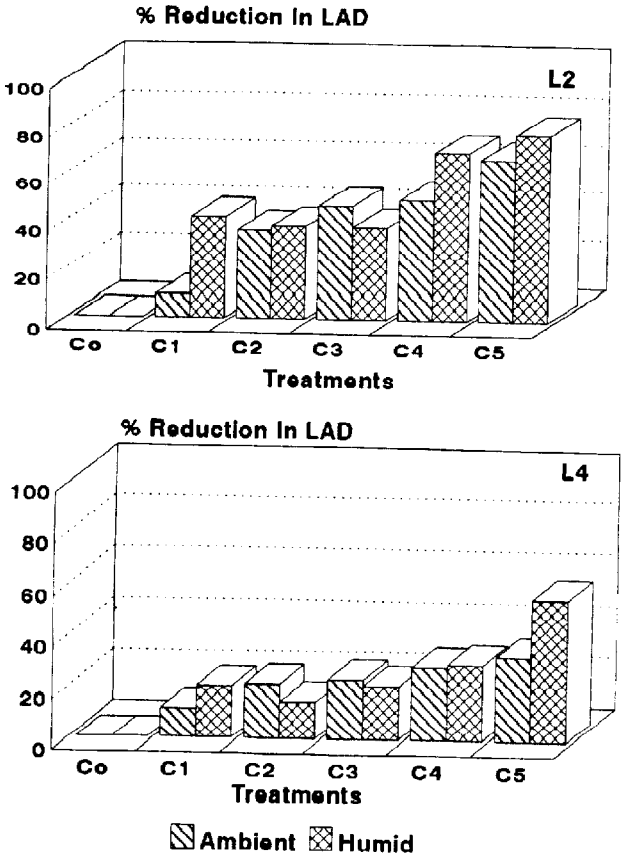

Figure 4. The percentage Reduction of leaf area damaged (LAD) caused by M. plana *L2 and L4 treated with various level of* B. bassiana.

even with treatment C4 under humid conditions. This suggests that the application of *B. bassiana* is more effective when the *M. plana* is at the earlier larval stage. Percent LAD reduction is significant for L4 only at C5 under high humidity, and it was less by 24% than in the case of L2.

Relative humidity plays an important role in the survival and germination of *B. bassiana*. At high humidity, *B. bassiana* induced greater mortality than at ambient humidity, for both larval stages of *M. plana*. The optimum temperature for growth and sporulation of *B. bassiana* has been reported as between 25°C to 30°C (Lim *et al.*, 1989). The greatest survival of *B. bassiana* occurred in the range of 75%–100% RH and also in the range 33%- 53% RH, according to Lingg and Donaldson, (1981).

From the probit analyses (*Table 1*), it appears that the *M. plana* L2 can be more effectively controlled with *B. bassiana* than the L4. The result also indicated that with maturation, *M. plana* larvae became increasingly resistant to *B. bassiana*. A similar response has been reported with other Lepidopterous defoliators challenged with *B. bassiana* (McDowell *et al.*, 1990; Feng *et al.*, 1985). The LC_{50} values of *B. bassiana* for both *M. plana* larval instars subjected to both moisture conditions were higher than those reported with other Lepidopterans. For example, the LC_{50} of *B. bassiana* for *Trichoplusia ni* (Huhner) was 1.39 x 10^2 colony forming units (CFU)/cm^2 (Ignoffo *et al.*, 1982); on *Ostrinia nubilalis* (Hub.) it was 5 x 10^4 CFU/cm^2 (Feng *et al.*, 1985); and on *Elasmopalpus lignosellus* it was 12.1 CFU/cm^2 (McDowell *et al.*, 1990). It was slightly higher for other pests such as *Diuraphis noxia* (Feng and James, 1990) and *Pantorhytes plutus* (Prior *et al.*, 1988).

This phenomenon could be due to the presence of the bag in *M. plana* which possibly hinders direct contact of *B. bassiana* with the cuticle of the insect during the treatment. In naked larvae of other insects, infections were directly through the cuticle (Pekrul and Grula, 1979), integument (Broome *et al.*, 1976) and respiratory system (Clark *et al.*, 1968), *etc.* Although, the most effective infection was through the alimentary tract (Ferron, 1978), several factors are likely to influence *per os* infection so that several conditions must be met if it is to occur: spores must remain viable; gut pH must be suitable; the presence of other gut microflora must not interfere; there must be sufficient contact time within the gut to allow penetration; and the fungus must be able to penetrate the gut wall (Madelin, 1963). In the present study, viability and germination within the gut were not affected, meaning that the gut pH is favourable. The pH favourable for germination of *B. bassiana* is reported as 6.4 (Thomas *et al.*, 1987).

CONCLUSION

B. bassiana was shown to be a potential biological control agent against *M. plana*. The fungus effectively killed the smaller larval instar and the pathogenicity increased with humidity. The oral route of fungal entry was found infectious to the bagworm. It was found to be viable and proliferate within the gut, and penetrate the gut wall, ganglion and muscle of *M. plana* larvae. To increase infectivity in field trials, *B. bassiana* should be applied during a wet season and at the early instar of *M. plana*. Successful exploitation of *B. bassiana* for biological control of *M. plana* requires further study, particularly on inoculum viability and survival, efficacy, formulation, and mass production.

ACKNOWLEDGEMENTS

The authors would like to thank the Director-General of PORIM for his support and permission to publish this paper, Dr B C Suttan, Head of Taxonomic and Identification Service of CAB International Mycological Institute, London for confirmation of fungal species, Universiti Pertanian Malaysia for the use of transmission electron microscope. The authors also express their appreciation to all Entomology staff of PORIM for their support.

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