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Preliminary Evidence on the Resistant Development of *Nosema* Spp Infecting Diamondback Moth Larvae on Fumidil-B

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Introduction

The diamondback Moth (DBM), *Pluetefia xylostella* L., is the major pests of crucifers and it has been the subject of numerous researches because of its economic impact on the cabbage industry. The usual method employed in controlling this pest is by spraying insecticide including the biological one, *Bacillus thuringiensis* (Bt) (Talekar and Shelton, 1993). Lately, the use of natural enemies has been emphasized as a result of pesticides resistant problem developed by DBM (Cheng, 1988; Tabashnik *et al*., 1990). The use of parasitoids, *Diadegma* eipp. and *Cotesia* spp., has shown a promising result in controlling DBM in Guatemala (Biever *et al*., 1992). The use of insect pathogens including microspordia (example-*Nosema* spp.) in controlling DBM has yet been reported, as it still in the laboratory studies (Wilding, 1986). However, Idris and Grafius (1999) reported that infection by *Nosema* sp. (most probably *N. bombycis)* has caused major concern in the rearing of DBM and its parasitoids in Malaysia and United States. The use of predatory arthropods in biological control necessitates that the predators are pathogen-free to avoid the risk of introduction of pathogens that could destabilized the natural fauna. Cultures of insects and probably other invertebrates, reared at high density for experimental use, can be decimated if microsporidia is introduced by a few infected individuals. Although many Nosema-infected individuals are successfully surviving, the result of any study using them may be invalid and cause misleading the fact. This is because the protozoan may enhance the toxicity effect of Bt and other insecticides on DBM larvae (Manasherob *et al*., 1994).

The usual techniques being employed in reducing microsporidial infection in the laboratory are heat treatment, selection, pairing of healthy individuals and adding Fumagillin or Fumidil-B into the artificial diet (Steinhaus, 1949; Geden *et al*., 1995). However, there is a great possibility of resistance to Fumidil-B with prolonged use of this antibiotic in rearing DBM. For example, Idris and Sajap (2001) reported that as high as 70% of DBM egg samples collected from DBM culture of the Malaysian Agriculture Research and Development Institute (MARDI) contained *Nosema* spores. This is in spite of the fact that these samples were raised on artificial diets treated with Fumidil-B. The objective of our study was to investigate resistant development of *Nosema* spp. infecting DBM culture in which their artificial diet was incorporated with fumidil-B, a conventional antibiotic recommended for controlling microsporidian diseases (Steinhaus, 1949; Katznelson and Jamieson, 1952).

Materials and Methods

The *Nosema* infected-DBM eggs and untreated artificial diet were provided by MARDI (Malaysian Agriculture Research and Development Institute). DBM eggs were allowed to hatch for 3 days. Five different concentrations of Fumidil-B (100, 200, 300 and 400 ppm) were prepared. A slice of 4 cm^2 and 0.1 cm thick artificial diet (without Fumidil-B) was soaked into a respective Fumidil-B solution for 15 minutes (to ensure diet being impregnated well by the antibiotic), air dried for 2 h and placed in a 15-cm diameter petri dish. Five first instar DBM larvae (3 h after hatching) were randomly selected and placed in a respective petri dish plus diet (25 larvae per treatment). Diet was changed daily. Larva mortality were recorded every other days started with 2 day after hatching until the day 10^{th} as most surviving larvae started pupating. The untreated diet (treated with distilled water) was used as a control treatment. Experiment was conducted in a laboratory condition. Each treatment was replicated four times. Percent mortality was calculated as the total number of larvae per replicate minus the accumulated dead larvae and divided by the total larvae x 100. Data were analyzed by one-way ANOVA and the treatment's means were separated by Fisher's Protected LSD test (Abacus Concept, 1991).

Results and Discussion

There was a significant difference in the percent of larval mortality among the treatments at all day's of data collection (Table 1). The highest accumulated larval mortality (92.5% at day 10^{th}) was observed when larvae fed untreated diet. There was no significant difference ($p = 0.001$) in the % mortality when larvae fed diets treated with 50 and 100 ppm antibiotic solutions. Although there was a significant different in percent mortality of DBM larvae fed on diets treated with 100 and 200 ppm Fumidil-B solutions, except on day 6, the accumulated percent mortality was still considerably high (36.3%). Percent mortality was significantly lower when larvae fed diet treated with 200 ppm Fumidil-B solutions on day 8 and 10 than those treated with 100 ppm Fumidil-B. However, the mortality was significantly lower when larvae fed diet treated with 300 ppm than that of 200 ppm Fumidil-B on each day of data collection. This indicates that the recommended 220 ppm Fumidil-B used in preparation of DBM artificial diet is unable to contain the development of the disease. Interestingly, further increase in Fumidil-B concentration (400 ppm) had significantly increased the percent mortality, indicating that the deleterious effect of Fumidil-B on DBM larvae as there were no *Nosema* spores observed from the dead and surviving larvae.

Results of this study showed that the *Nosema* spp-infecting colony of DBM at MARDI might have developed resistant toward Fumidil-B. The disease development could only be checked when diet is treated with Fumidil-B above 300 ppm but further study needs to be conducted. The resistance development may happen elsewhere but it may be unnoticed. However, *Nosema* problems are always overcome by restart a new colony and/or plus other measured as suggested by Steinhaus (1949). There may be other methods that are easier and/or cheaper. One of the methods is by using other

Table 1: Percent mortality (accumulative) of diamondback moth larvae fed artificial diet treated with various concentration of Fumidil-B

Concentration (ppm) of Fumidil-B	Days after treatments (+SE)				
	2	4	6	8	10
Untreated diet	45.4±5.6a	60.5±7.8a	70.3±8.5a	85.4±10.2a	92.5±10.2a
50	20.5±10.5b	25.7±7.3b	38.5±5.6b	52.6±6.3b	72.5±7.6b
100	23.3±11.3b	24.4±6.7b	35.6±4.7b	50.5±7.3b	69.4±8.2b
200	15.5±10.2c	18.3±5.6c	22.4±5.3b	30.5±5.4c	36.3±4.3c
300	1.5±2.4d	2.6±4.2d	3.5±5.6c	6.3±3.3d	10.9±3.8d
400	0d	10.5±1.5d	15.5±1.5c	17.5±1.5d	21.5±1.5d

Means in column with same letters are not significantly different (Fisher's Protected LSD, $p>0.05$) antibiotics

For example, Haque *et al*. (1993) reported that Albendazole is very effective against development of *Nosema bombycis* infecting *Spodoptera frugiferda* and *Heliocoverpa zea* cell *in vivo* and *in vitro* and there was no deleterious effect of this antibiotic on the growth and ability of *H. zea*. This antibiotic is much cheaper and easier to buy from any pharmacy than Fumidil-B that is more expensive and difficult to get. Others antibiotics that could potentially replace FumidilB are those commonly used in veterinary medicine to combat protozoan diseases.

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