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Nosema Disease of Diamondback Moth, *Plutella xylostella* (L.), in Malaysia

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Abstract: A survey on the incidence of *Nosema bombycis* infection on diamondback moth (DBM), *Plutella xylostella* (L.), was conducted in the cabbage fields of Cameron Highland (CH) and Serdang-Gombak (SG), each of them represent a high and lowland cabbage growing areas respectively, and laboratory culture of MARDI. Percent of *Nosema* infection (disease incidence) on the DBM larvae was much higher in CH (73.1%) than in SG (10%). The disease was prevalence in the highland cabbage fields or in low temperature environment. Although the percent of infection on DBM pupae showed a similar trend as for larvae, the difference was much smaller (13.3% and 8% in CH and SG respectively). This suggests that many infected DBM larvae in the CH had failed to develop and form DBM pupae. The infection intensity (number of spores per larva or pupa) and the range of infection intensity also showed similar trend as the percent of disease infection. More than 70 and 85% of DBM eggs and larvae, respectively, of the laboratory culture were infected by *N. bombycis*. The high percent of infection in the field population could be viewed as good news as *N. bombycis* may have a potential to be used as a biological control agent of DBM. But *N. bombycis* could indirectly kill parasitoid larvae within the infected DBM larvae of laboratory reared and field population. As such further study on impact of *N. bombycis* to DBM need to be conducted before any suggestion on it use can be made.

Key words: *Nosema bombycis*, diamondback moth, parasitoid, highland, lowland

Introduction

The *Nosema bombycis* Naegeli is well known as the causal agent of microsporidium disease of a silkworm larvae, *Bombyx mori* (Steinhaus, 1949). However, results of molecular studies had indicated that *N. bombycis* is also infecting insects other than *B. mori* (Canning *et al.*, 1999a). For example, microsporidium disease has been said to cause serious problems in a diamondback moth (DBM), *Plutella xylostella*, and its parasitoids rearing work in the laboratory (Dough and Shelton, University of Florida and Cornell University, USA, respectively; Hussan, MARDI, Malaysia; Mohamad Rani, University of Wales, UK – personal communication). In Malaysia, study on *N. bombycis* infecting DBM was conducted by Idris *et al.* (1997) and Canning *et al.* (1999b). Idris & Grafius (1999) reported that the frass, silk, regurgitate, exuviae and meconium were the possible sources of inoculum of infection on DBM larvae in the laboratory culture. Information on the prevalence or field incidence of *Nosema* infecting DBM in the field both in Malaysia and other countries is lacking. The prevalence of other *Nosema, such as N pyrausta* and *N. locustae*, in the field was reported by Andreadis (1982) and Henry (1972). These *Nosema* were found to have negative impacts on both insects host and it parasitoids (Siegel *et al.*, 1986). As such, it is important to study the prevalence of *N. bombycis* in the field as the two DBM's main parasitoids, *Diadegma semiclausum* and *Cotesia plutellae*, may be negatively affected by this disease (Ooi *et al.* 1992).

In this experiment the field incidence of *N. bombycis* infection on DBM larvae populations in the high and lowland cabbage growing areas in Peninsular Malaysia as well as in the laboratory culture. Result may provide us information on the intensity of *Nosema* infection on DBM population in the cabbage growing areas of different latitudes. The information could help us to formulate a more exact dosage of *Bacillus thuringiensis* (B.t), an environmental friendly microbial pesticide, for controlling DBM. This is because the protozoan may enhance the toxicity effect of B.t on DBM larvae (Manasherob *et al.*, 1994). If the *N. bombycis* prevalence is high then study on its impact on DBM's parasitoids would be recommended.

Materials and Methods

DBM field population: Two cabbages growing areas, Cameran Highland (CH) and Serdang-Gombak (SG), of different latitudes were selected. The CH represented a highland and SG for lowland area and locations was about 300 km apart. The latitude of CH is between 1400 - 1500 m, whilst the SG is between 20 - 30 m above the sea level. The ranges of daily temperature of CH and SG were from 15 – 25°C and 29 – 35°C respectively. Two days sampling on four randomly selected cabbage fields per location were carried out twice per month (biweekly interval) for two months (October and April 1998 and 1999 respectively). DBM larvae and pupae were collected from 10% of the cabbage plants per field randomly selected prior to sampling. The collected DBM larvae and pupae were put in plastic vials (one larva or pupa per vial) then placed in the cool container and brought back to the laboratory and kept in a freezer. Dead DBM larvae and pupae observed in the field was counted and categorized to *Nosema*-infected and non-*Nosema* infected individual.

DBM larvae and pupae were taken out of the freezer to study the percent and intensity of disease incidence. Each larva or pupa was crushed on a glass slide and the presence of *Nosema* spores were observed under the compound microscope with 40x magnification. The crushed body of individual DBM larvae or pupae were then put in a small test tube, added with 5 ml distilled water and shook using electronic shaker until it was thoroughly mixed to become a larval liquid. To get the value of infection intensity or range of infection intensity a total of 1 ml larval liquid was pipetted and poured on a hemocytometer and the number of spores per larva or pupa were counted under a similar microscope and magnification as mentioned above. In addition, spore size was measured using an image analyzer.

DBM of Laboratory Culture: DBM eggs and larvae were collected from rearing culture in MARDI, Serdang. The eggs were soaked in the clorox (Sodium hipochloride) to kill the

spore contaminating on oviposition substrate (foil) and outside the eggs. Twenty DBM eggs were randomly selected and detached per aluminum foil (used for DBM oviposition) on to a glass slide by using sterilized forceps and replicated it 10 times by taking egg samples at different week from MARDI. A drop of distilled water was added on the eggs after which they were smashed using a cover slip. The presence of *Nosema* spores was observed as mentioned above. As many as 20 larvae per instar were also crushed on the slide to look at the presence of *Nosema* spores.

Results and Discussion

DBM Field Population: The percent of infection (disease incidence) on DBM larvae in CH was much higher (> 71.3 %) than in SG (10 %) (Table 1). The mean intensity of *Nosema* infection (number of spores per larva) on DBM larvae was 2.59×10^7 and 1.33×10^6 spores per larva collected from CH and SG respectively (Table 1). However, the range of infection intensity on DBM larvae in CH (2.0×10^5 - 8.03×10^7) was wider than that of SG (3.0×10^6 - 2.70×10^7). The mean intensity of infection and range of infection intensity per pupae were also showed similar trend as for DBM larvae. However, the mean infection intensity of DBM pupae in CH was very much higher (6.7×10^6) than that of SG (2.6×10^6). Percent of dead larvae and pupae due to *Nosema* infection were also higher in CH than in SG (Table 2).

The *Nosema* infection was relatively severe in the highland than in the lowland. There is a possibility that the temperature had influenced on the severity of an infection. Low temperature in the highland (CH) may prolong larval developmental period (Ooi, 1986 and Salinas, 1986). This may have increased the success of disease infection as DBM larvae were exposed longer to *Nosema* spores. In laboratory studies, the infection rate of *Nosema portugal* on larvae of gypsy moth was observed to be higher when moth larvae were reared at low temperature (< 20°C) than at high temperature (> 30°C) (Bauer, L., Michigan State University - personal communication). Probably, at high temperature the insect growth-rate outpaces the microsporidium and as a result disease infection rate is higher in a highland than in the lowland.

Although light intensity of both locations was not recorded the latitude difference indicates that CH had relatively lower light intensity than that of SG. Low light intensity in the CH probably had killed less *Nosema* spores contaminating the plant leaf surfaces. As a result, DBM larvae may be ingested more spores while feeding on cabbage leaves as compared to DBM larvae at SG. Other insect pathogens were reported to be killed or less viable up on longer exposure to strong sunlight intensity as well as to high ambient temperature (Beegle *et al.*, 1981; Wilding, 1986 and Soares & Quick, 1992).

Unlike DBM larvae, the difference in percent of *Nosema* infection on DBM pupae between the CH and SG was smaller (13.3% and 8% for CH and SG, respectively) (Table 1). This is probably due to many highly *Nosema*-infected DBM larvae in CH had failed to develop and form DBM pupae (Table 2). However, further investigation should be conducted to find out the real causal agents of the mortality as fields in both locations were heavily sprayed with pesticides to control DBM.

The intensity and range of disease infection on DBM pupae were three times higher in CH than in SG (Table 1). Longer larval developmental period at low temperature in CH may attribute to longer time for *Nosema* to multiply and sporolate within the host (larva) body. It may also be due to a bigger size of DBM pupae in CH than in SG (Ooi, 1986). The high number of spores per DBM pupae (Table 1) in both locations indicates that DBM larvae may have had high tolerance to *Nosema* infection. Because of this they were able to pupate even with very high number of spores inside their body.

Laboratory Culture: There was 70 % of DBM egg samples were found to have *Nosema* spores and some of the samples had abundant of spores. This indicates that *Nosema* heavily infected DBM culture of MARDI. The result also suggests that *Nosema* is transovariolly transmitted by the DBM females through their eggs. Similar case was reported in other insects infected by *Nosema* spp (Andreadis, 1982; Sajap and Lewis, 1988). There were > 85% of DBM larvae inspected had *Nosema* spores. Although the number of spores per larvae was not counted the number of spores increased with instars and larger instar had the most abundant *Nosema* spores (Idris & Grafius, 1999).

High percent of *Nosema* infection as well as infection and range of infection intensity on DBM should be viewed as good news as *N. bombycis* could be used as biological control agent of DBM. The bad news is that *N. bombycis* could indirectly kill parasitoid larvae within infected DBM larvae in the field. In addition, it could become a major constraint in the laboratory rearing work of DBM and its parasitoids. This will indirectly

Table 1: Percent, intensity and range of *Nosema bombycis* infection on the field larval population of DBM, *Plutella xylostella* (L.) in the highland of Cameron Highland (CH) and lowland of Serdang-Gombak (SG) cabbage growing areas.

Sample (n = 400)	% infection[1]	Mean infection intensity (no. spores/larva or pupa) (± S.E.)	Range of infection intensity
Larvae (CH)	71.3	$2.59 (\pm 0.28) \times 10^7$	2.0×10^5 - 8.03×10^7
Larvae (SG)	10.0	$1.33 (\pm 0.15) \times 10^6$	3.0×10^6 - 2.70×10^7
Pupae (CH)	13.3	$6.70 (\pm 0.72) \times 10^6$	3.0×10^5 - 2.17×10^7
Pupae (SG)	8.0	$2.60 (\pm 0.41) \times 10^6$	5.0×10^5 - 7.02×10^6

[1] > 80% dead larvae and about 10% dead pupae in the field observed were probably caused by *Nosema*. This was based on the typical symptom of Nosema-infected larvae or pupae. Spore size: 5.072 μm (length) and 2.274 μm (width).

Table 2: Percent of dead diamondback moth larvae and pupae due to *Nosema* infection observed in the fields.

Locations	Percent of dead larvae	Percent of dead pupae
Cameron Highland	70.4 (n = 50)	65.6 (n = 40)
Serdang-Gombak	20.7 (n = 50)	12.3 (n = 10)

affect the integration of DBM parasitoids in an integrated DBM management. As such further study on impact of *N. bombycis* to DBM and its parasitoids need to be conducted before any suggestion on it use can be made.

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