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Nosema bombycis INFECTION IN HIGHLAND POPULATIONS OF DIAMONDBACK MOTH (DBM), *Plutella xylostella* AND ITS PARASITOIDS, *Diadegma semiclausum* AND *Cotesia plutellae*

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ABSTRACT

A study was conducted to determine the prevalence of *Nosema bombycis* infection in the highland populations of diamondback moth (DBM) and its two parasitoids, *Diadegma semiclausum* and *Cotesia plutellae*. Insects were collected from two vegetable farms in the highland area (Sri Juliana Farm, Brinchang, and MARDI, Cameron Highlands, Pahang). Results showed that *Nosema* spores were abundant in DBM and its two parasitoids. There was a significant ($p < 0.05$) difference in the means of prevalence rate, spore concentrations and infection intensities between DBM and its parasitoids at both locations. Although results from both location was somewhat vary, the parasitoids seemed to have lower rate of *Nosema* infection (prevalence), spore concentrations and infection intensities than its host, DBM. The potential of *N. bombycis* to be used as a biological control agent of DBM are discussed.

INTRODUCTION

There are about 2.2 x 10^6 hectares cruciferous (Cruciferae) vegetables grown worldwide and about half occurs in Asia (Talekar & Shelton 1993). The members of family Cruciferae are essentially thrive in temperate climates and one of the most cultivated crucifers is cabbage (*Brassica oleracae var. capitata*), and in the tropic it is grown in both highland and lowland areas. In Malaysia, it was reported that there are 5600 hectares of land planted with cruciferous vegetables (Calderon & Hare 1986). In 2002, Cameron Highlands produced 92% of the local production of 206,102 metric tons of cruciferous vegetables. Production was good until intensive mono-cropping gave rise to the high insect pest population especially the diamondback moth (DBM), *Plutella xylostella* (L) (Lepidoptera: Plutellidae), the most important insect pests for crucifers. In Malaysia, the earliest record of DBM was in September 30, 1925 in Frasier's Hill, then in 1934 in Cameron Highlands (Ooi 1986). High infestation incurred by the DBM led to the intensive utilization of insecticides. The use of excessive insecticides including *Bacillus thuringensis* (B.t.) apparently led to resistance development of DBM (Talekar & Shelton 1993). These insecticides also posed other related problems such as polluting the environment, damage of non-target organisms and the presence of insecticide residues into the food chain (Loke *et al.,* 1997; Ooi 1986). Currently, natural enemies which have been used to control DBM population are gaining significance (Cheng 1988; Tabahsnik *et al.,* 1990). Among them are hymenopterans, *Diadegma semiclausum* Hellen, an ichneumonid, and *Cotesia plutellae* Kurdjumov, a braconid. Unfortunately, these parasitoids were found to be infected with a naturally occurring insect pathogen, *Nosema bombycis* Naegeli (Idris *et al.,* 1997). In Malaysia and the United States, it was reported that rearing of DBM as well as its parasitoids infected with *Nosema* caused much concern (Idris & Grafius 1999). Knowing the distribution and prevalence of *Nosema* sp. in DBM populations could be a good indication whether or not this microscporidium has a potential to be used as a biological control agent against DBM, as for *N. locustae* currently used against grasshoppers in USA. The evidence of the presence of *Nosema* sp. in *D. semiclausum* and *C. plutellae* could help

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explain the host-parasitoid relationship between DBM and its two main parasitoids. However, the prevalence of *Nosema* in DBM's main parasitoids may indicate that the microsporidium can have a negative effect in the parasitoids. This research was undertaken to study the presence of *Nosema* sp. in the highland populations of DBM and to determine the differences in prevalence rate, intensity of infection and spore concentration between field populations of DBM and its two major parasitoids, *D. semiclausum* and *C. plutellae*.

MATERIALS AND METHODS

Collection sites

Sampling was done on the 2 ha field of cabbages interplanted with chili of Sri Juliana Farm in Brinchang, Cameron Highlands, Pahang, Malaysia. The field was boarded by forested, residential and business establishment areas with daily temperature of 18 – 27ºC. Sampling was also conducted at 10 ha vegetables (cabbages, chilli, bell pepper and Chinese lettuce) field of the Malaysian Agricultural Research and Development Institute (MARDI) Station, in Tanah Rata, Cameron Highlands, Pahang, with daily temperature ranging from 14 – 23ºC. About 50% of the MARDI's Station surrounding area is forested while the other half consists of residential areas, schools and business establishments.

Field Collection

Three insect collections (replicates) per site were done in 2 days per week per alternate month, i.e, in March, May and July 2004 for Sri Juliana Farm and in June, August and October 2004 for MARDI field station. Adult insects were caught using a sweep net. The 3rd and 4th DBM larvae and pupae were collected manually using forceps. DBM adults along with the pupa and larvae of DBM were killed and preserved in small bottles filled up with 70% alcohol and brought back to the laboratory for checking the presence of *Nosema* spores. The 3rd and 4th instars of DBM larvae were collected because more spores could be harvested from these instars compared to 1st and 2nd instars (Idris & Sajap 2003; Idris *et al.,* 1997). Data per month per insect species (or stages) were lumped together to become one replicate per month in three replicates (months) experiment.

Laboratory Preparation

Spore counting were made by crushing insects using mortar and pestle with 200 µl sterilized distilled water and homogenized using electric shaker. A drop of the homogenate solution was placed on a slide, smeared and fixed using 99.9% methanol and left to air-dried overnight. The slides were then stained with Giemsa solution (a mixture of 1 ml of Giemsa and 3 ml of phosphate buffer, pH 7.2) for 20 minutes (Idris *et al.,* 1997). Observations of the stained slides with *Nosema* spores were examined under compound microscope of 40x and 100x magnification. This procedure was done on every individual insect. The crushed insect solution (homogenate) that was placed in tubes (stored in the refrigerator at 4-5ºC if not in used) was then centrifuged at 3000 rpm at 10ºC for 10 min. The yielded supernatant was discarded and the pellet was re-suspended with sterile distilled water with the same volume of the supernatant. This dilution and centrifugation was repeated three times, pipetted 10 µl and poured into hemocytometer for spores counting. Three parameters measured were prevalence rate, spore concentration and prevalence intensity per insect per collection (month). The computation was done according to the method described by Cantwell (1970) as described below.

Data Analysis

To determine the prevalence of *Nosema* among the insect populations, below were the following formula that was used.

a. Prevalence Rate

$$\text{Prevalence Rate} = \frac{\text{number of individual with } \textit{Nosema} \text{ spores}}{\text{total number of individual insects}} \times 100$$

b. Spore Concentration

$$\text{Spore Concentration} = \frac{\text{total number of spores counted}}{\text{total squares counted (80)}} \times 4 \times 10^3 \times \frac{1000\ \mu l}{1\ ml}$$

c. Infection Intensity

$$\text{Infection Intensity} = \text{spore concentration (spore/ml)} \times 10\ ml = \text{total number of spores}$$

$$= \frac{\text{total number of spores}}{\text{total number of insect}}$$

The prevalence rate was calculated in the same manner as percent infection, which was described by Idris and Sajap (2003). The data was analyzed using one-way Analysis of Variance (ANOVA) where DBM or its parasitoids are independent variables and prevalence rate, spore concentration or infection intensity are dependent variables. The comparisons of means were made using Tukey's pairwise test, and all analysis was run on the MINITAB Statistical Program Release version 12.1 (1998). The data analysis was focused on the comparison of means of prevalence rate, spore concentration and infection intensity of insect species on each farm.

RESULTS AND DISCUSSION

The presence of *Nosema* spores in DBM populations, *D. semiclausum* and *C. plutellae* at Sri Juliana farm was indicated by the high prevalence rate of *Nosema* infection on the parasitoids (as high as 57.8%) (Table 1). The present results showed that the prevalence rate of *Nosema* infection among insects was low as compared to what was reported by Idris and Sajap (2003) (60–75%), but the difference between parasitoids and DBM was significant ($F = 3.55$, df. = 4 & 9, $p > 0.05$). DBM pupa had relatively lower percentage of infection than that of DBM larva even though the different was not significant (Table 1) and this tend to agree with that of reported by Idris and Sajap (2003). The infected DBM larvae might have failed to pupate and those survived might be able to limit the spore proliferation. Intriguingly, the percentage of infection was somewhat higher in DBM parasitoids, *D. semiclausum* and *C. plutellae* than the DBM adult and larvae. This indicates the parasitoids population in field is negatively affected by the *Nosema* infection on its host. This is somewhat unwanted as it shows that the parasitoids field population, as a result of their host (DBM) being infected, will be negatively affected and that its role as biological control agents of DBM.

There was a significant ($F = 5.17$, d.f. = 4 & 9, $p = 0.039$) difference in the spore concentration among treatments (Table 1). DBM larva had the highest and *C. plutellae* had the lowest spore concentrations, $9.3 \times 10^5 \pm 1.1 \times 10^6$ and $7.8 \times 10^4 \pm 2.7 \times 10^4$ respectively, and the difference was significant ($P < 0.05$). However, the spore concentration in DBM larvae and another DBM parasitoid, *D. semiclausum* was not significantly ($P > 0.05$) different. As expected that the DBM pupae had the lowest *N. bombycis* spore concentrations as compared to the other two DBM stages. The survived DBM larvae that formed pupae were probably those tolerant to *Nosema* infection and that less spore produced. Although both *D. semiclausum* and *C. plutellae* are two main DBM parasitoids (Harcourt 1960 & 1963), *D. semiclausum* had more *Nosema* spores than *C. plutellae* (Table 1). In the field, compared to *C. plutellae*, the *D. semiclausum* might have contacted more DBM larvae than other DBM stages or other insect hosts. This is true because *D. semiclausum* is a more specialist DBM parasitoid which capable of recognizing its preferred host or host stages compared to *C. plutellae* (Harcourt 1986; Idris & Grafius 1993 & 1996, Ooi 1986).

The infection intensities of *Nosema* amongst treatments was also different significantly ($F = 6.73$, d.f. = 4 & 9, $p = 0.019$). DBM larva had significantly higher infection intensity than that of DBM adult and pupa and *C. plutellae* adult (Table 1). Intriguingly, the intensity of infection on DBM larvae and *D. semiclausum* adult was not significantly different ($P > 0.05$). As explained above that the *D. semiclausum* had contacted and parasitized more DBM larval individuals which exposed it to disease infected DBM as compared to *C. plutella*. Additionally, *D. semiclausum* is a major parasitoid of DBM (Ooi 1986, Harcourt 1986) compared to *C. plutellae* which may have many alternative hosts other than DBM larvae (Idris 1991). This could also mean that *Nosema* could be less adapted to *C. plutellae* than to *D. semiclausum*. However, *Nosema* spores may cause damage to parasitoids, which can affect the parasitoids behavior and fecundity and then negatively affecting *D. semicalusum* roles as biological control agents of DBM (Idris & Sajap 2001).

There was a significant difference ($F = 3.28$, df = 5 &11, $p < 0.05$) in prevalence rate of *Nosema* infection between treatments (DBM stages and its main parasitoids collected from MARDI station) (Table 2). The adult stage of *D. semiclausum* exhibited the lowest mean of prevalence rate at 37.8 ± 9.3 while DBM pupa had the highest rate at 69.3 ± 26.6, and these two prevalent rate were differed significantly ($P < 0.05$). However, the prevalence rate of infection on DBM pupae was not significantly

Table 1. Prevalence rate, spore concentration and infection intensity of *Nosema bombycis* infection on DBM, *D. semiclausum* and *C. plutellae* in Sri Juliana Farm, Brinchang, Cameron Highlands, Malaysia

Treatments		Prevalence Rate (% of infection) Mean ± SE	Spore Concentration (spore/ml) Mean ± SE	Infection Intensity (spore/insect) Mean ± SE
DBM	adult	40.0 ± 18.0ab	$2.8 \times 10^5 \pm 1.5 \times 10^5$ab	$3.1 \times 10^5 \pm 1.5 \times 10^5$bc
	larva[a]	38.0 ± 13.87ab	$9.3 \times 10^5 \pm 1.1 \times 10^5$b	$1.7 \times 10^6 \pm 0.8 \times 10^6$c
	pupa	16.7 ± 10.57a	$8.8 \times 10^4 \pm 1.2 \times 10^4$b	$2.9 \times 10^5 \pm 1.2 \times 10^5$bc
D. semiclausum		57.4 ± 31.51bc	$2.6 \times 10^5 \pm 2.2 \times 10^5$ab	$8.5 \times 10^5 \pm 1.0 \times 10^6$ab
C. plutellae		57.8 ± 28.92bc	$7.8 \times 10^4 \pm 2.7 \times 10^4$a	$1.4 \times 10^5 \pm 1.8 \times 10^5$a

a = 3rd and 4th instars of DBM larva were pooled as one; In column, means with the same letter are not significantly different at $P > 0.05$ (Tukey's test).

Table 2. Prevalence rate, spore concentration and infection intensity of *Nosema bombycis*. infection on DBM, *D. semiclausum* and *C. plutellae* in MARDI Station, Tanah Rata, Cameron Highlands

Treatments	Prevalence Rate (%) Mean ± SD	Spore Concentration (spore/ml) Mean ± SD	Infection Intensity (spore/insect) Mean ± SD
DBM adult	56.89 ± 3.01 ^{ab}	1.21 x 10^6 ± 1.23 x 10^5 ^{bc}	3.53 x 10^5 ± 1.16 x 10^5 ^{a}
larva[a]	51.13 ± 20.21 ^{ab}	4.20 x 10^6 ± 3.68 x 10^6 ^{c}	1.64 x 10^6 ± 1.39 x 10^6 ^{bc}
pupa	69.29 ± 26.60 ^{bc}	1.58 x 10^6 ± 1.05 x 10^6 ^{bc}	1.85 x 10^6 ± 2.10 x 10^6 ^{bc}
D. semiclausum adult	37.77 ± 9.27 ^{a}	7.00 x 10^5 ± 3.12 x 10^5 ^{ab}	2.71 x 10^5 ± 7.03 x 10^5 ^{a}
D. semiclausum pupa	55.64 ± 7.98 ^{ab}	8.86 x 10^5 ± 3.36 x 10^5 ^{ab}	2.92 x 10^5 ± 1.17 x 10^5 ^{a}
C. plutellae adult	55.56 ± 9.62 ^{ab}	9.58 x 10^5 ± 7.53 x 10^5 ^{ab}	2.47 x 10^6 ± 2.98 x 10^6 ^{c}

a = 3rd and 4th instars of DBM larva were pooled as one; In column, means with the same letter are not significantly different at P > 0.05 (Tukey's test).

different with DBM adult and larvae, *D. semiclausum* pupae and *C. plutellae* adult (Table 2). It is interesting to note that adult *C. plutellae* and pupa of *D. semiclausum* had percent infection closer to the adult and larval stages of DBM. In contrast to what was recorded from Sri Juliana Farm (above), the prevalence rate of infection between the two parasitoids was not significantly different. This indicates that adult stage of *C. plutellae* might be feeding a lot on infected DBM larvae while the pupa of *D. semiclausum* might be infected through the larval feeding on heavily infected host.

There was a significant difference in spore concentrations (F= 3.82, d.f.= 5 & 11, p = 0.019) and infection intensities (F= 4.96, d.f.=5&11, p=0.043) between stages of DBM and its two main parasitoids (Table 2). The spore concentrations were significantly (P < 0.05) higher in DBM larvae than the spore concentrations in all other treatments. The highest spore concentration was shown in DBM larvae (4.20 x 10^6 ± 3.68 x 10^6 spores/ml) and lowest in *D. semiclausum* adult (7.00 x 10^5 ± 3.12 x 10^5 spores/ml) and the different was significant (P < 0.05). For infection intensity, *D. semiclausum* adult had the lowest with 2.71 x 10^5 ± 7.03 x 10^4 spore per insects while *C. plutellae* had the highest with 2.47 x 10^6 ± 2.98 x 10^6 spores/insect.

In contrast to what was recorded at the Sri Juliana Farm (Table 1), the close prevalence rates of *Nosema* infection on *C. plutellae* adult and DBM adult and larval stages than adult *D. semiclausum* indicates that *C. plutellae* might have taken more of the spores from DBM compared to *D. semiclausum*. The lack of alternate hosts in the vicinity of MARDI field station might have forced more individual *C. plutellae* adults to parasitize DBM larvae more than usual and that more get infected with *Nosema*. Lower concentration of spores in parasitoids compared to all stages of DBM indicates that *N. bombycis* is comparatively effective in infecting DBM. This implies that *Nosema* can be a good biological control agent for DBM. In addition, spore concentration is a more accurate parameter since the rate of infection (prevalence rate) varies from one insect to another. A species can have a higher prevalence rate but this information does not necessarily reveal how much of the spores are present inside the bodies of insects that can cause death.

Both pupae and adult *D. semiclausum* and adult DBM have relatively lower infection intensities than larva and pupa of DBM and *C. plutellae* adult. This is not surprising as the pupae and adult have formed and emerged from larvae that are more tolerance to *Nosema* infection. Similarly, there is also a probability that *D. semiclausum* is more tolerance to the infection of *Nosema* than *C. plutellae* as indicated by significantly high infection intensity on the later than former. However, both parasitoids recorded at both locations seemed to be a good vector of *Nosema* disease in DBM as both are infected and carry considerably high number of spores (Table 1 & 2). Idris *et al.* (2001) reported that the microsporidian disease caused by *N. bombycis* was transmitted by *D. semiclausum* of which 100% had microsporidian spores. They also pointed out that the abundance of spores had resulted to the 41.3% mortality of DBM larva. In another study, Idris and Grafius (1999) stated that *D. insulare*, a closely related species of *D. semiclausum*, is also an effective vector for the transmission of *N. bombycis* spores to DBM larvae.

Results also indicate that even though both locations are highland areas they still vary in the mentioned parameters, especially spore concentration. It is also important to note that Brinchang and Tanah Rata where the Sri Juliana farm and MARDI station situated respectively, are belong to the Central Zone of this highland. This zone is considered the highest of all three zones which have an elevation of 1400-1500 meters above sea level and much cooler compared to Northern and Southern Zones (Arthurs 1996; Lim 1970; Taylor *et al.,* 1992). The Central Zone has a daily temperature range from of 14ºC to 27ºC. There is well

distribution of rainfall in the highland areas. It highly varies both within and between months of the year, ranging from 60mm–500mm per month and averaging about 2650 mm per year. The wet months are usually April, May, October, November and December, while other months are relatively drier (Syed *et al.,* 1996). The variation in results obtained between Sri Juliana farm and MARDI may have been influenced by the difference in rainfall distribution which influences the number of insects during the months that they were collected from each location as well as the exposure of hosts to the *Nosema* spores (less spores during rainy times). Rainfall may well be the most probable cause of variation in results since the collection in Sri Juliana was performed between March to July 2004, one month or two months of relatively rainy weather, while the collection in MARDI station was performed between June to October 2004 which are relatively drier months of the year. The dry months must have given the insects more mobility and freedom to do other activities such as reproduction and parasitism. In DBM alone, the sum of individuals collected from three periods in MARDI is 240 but only 77 individuals were collected in Sri Juliana. Another factor that may have affected the difference is the frequency of application of insecticides. In MARDI, the farmers use insecticides once in five days while Sri Juliana uses insecticide two days in a week or at least three times per week, according to the need. The intervals of insecticide application have a direct impact on DBM and its parasitoids. In another study, Ho and Ng (1970) assessed the effectiveness of Thuricide against the DBM that attacks cabbage and stated that application at 4-day intervals gave better control than spray intervals of seven and 10 days. This application interval of Thuricide might have indirectly and negatively affected the parasitoids.

CONCLUSIONS

N. bombycis is prevalent and abundant in the field populations of DBM, *D. semiclausum* and *C. plutellae*. It is also well distributed in highland areas of cruciferous vegetable farms. The spore concentrations between DBM and its parasitoids do not significantly differ between two locations in the highlands. Because both parasitoids species are also infected as a result of attacking hosts, an applying commercially produced *Nosema* in the field for controlling DBM need further detail study as the disease can also infect the parasitoids. As such the possibility of using a combination of parasitoids and *Nosema* in IPM for DBM is surely questionable. Only after detail study over years has been conducted than the possibility of *Nosema* to be commercially produced can be certained.

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