UPM Inaugural Lecture Series

INSECT DISEASES :
Resources for Biopesticide Development

Prof. Dr. Ahmad Said Sajap

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Ahmad Said Sajap

Insect Diseases : Resources for Biopesticide Development

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INAUGURAL LECTURE

PROF. DR. AHMAD SAID SAJAP

Insect Diseases : Resources for Biopesticide Development

28 August 2004

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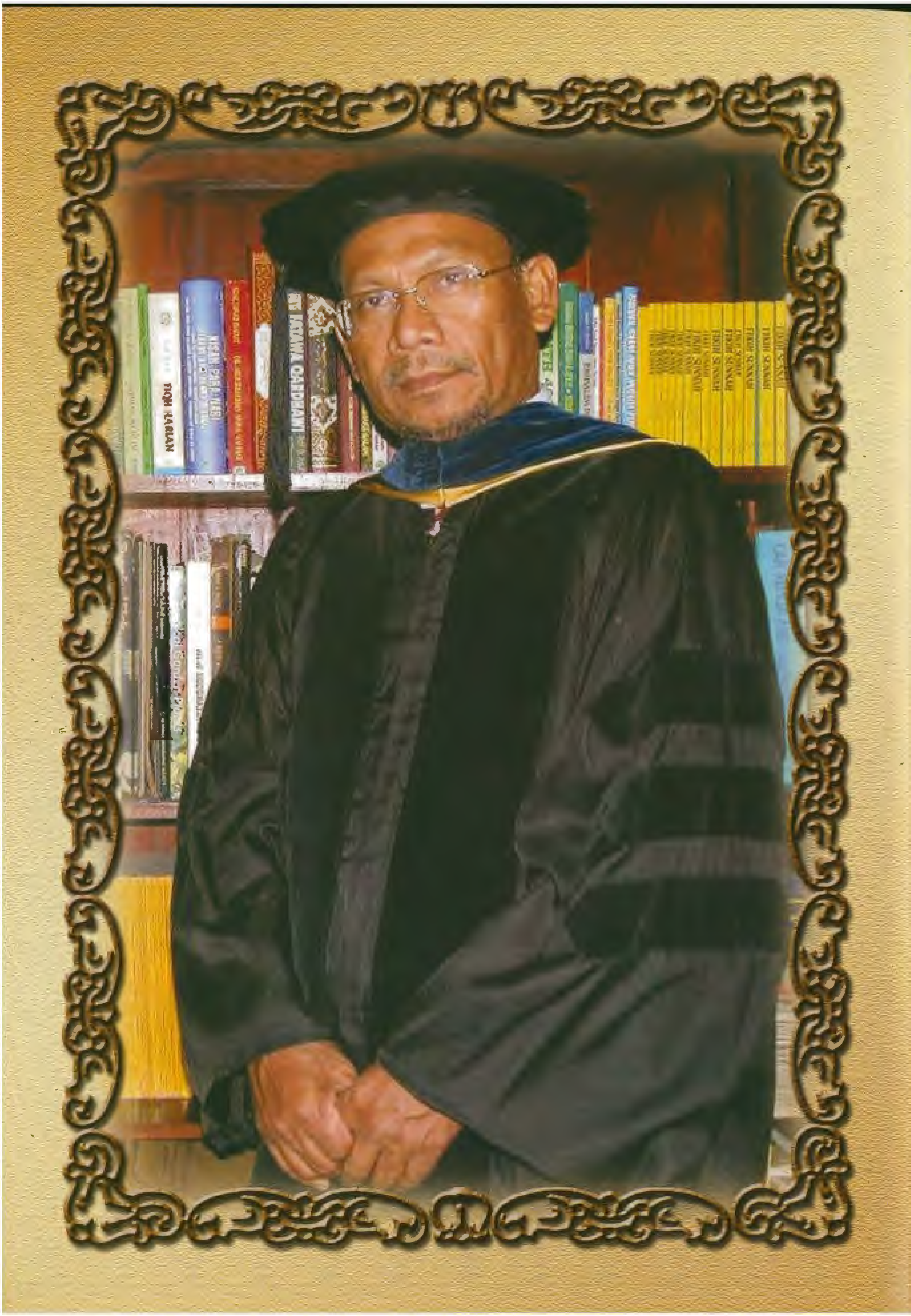

AHMAD SAID BIN SAJAP

He was born 13 July 1951, at Bukit Badong, Selangor. After completing the Malaysian Certificate of Education and Senior Cambridge in 1969, he attended the College of Agriculture, Serdang in 1970 and obtained a Diploma in Agriculture in 1973. He took undergraduate studies in Entomology at University of California at Davis and earned a Bachelor of Science with Honours in 1975. He continued his graduate studies at the Department of Entomology, Iowa State University at Ames and awarded a Master of Science in 1977. In 1987 he received a Ph.D. from the same university.

Professor Ahmad Said began his career as an assistant research officer at the FELDA Research Station, Sungai Tekam, Pahang in 1973. In 1977, he was an Agriculture Officer with FELDA and resigned to join the Faculty of Forestry, UPM as a lecturer in 1978. He was promoted to Associate Professor in 1993 and Professor 9 years later.

Apart from teaching and doing research, he was also an administrator in the faculty for more than seven years. He was an Acting Head, Department of Forest Management in 1983 and Head, Department Forest Management from 1989 to1991. He was the Deputy Dean of the Faculty from 1992 to 1994, 1996 to 1998 and 2002. Presently, he is the Deputy Director of the Rain Forest Academy.

He was a member in the editorial committee for PERTANIKA Journal of Tropical Agricultural Science, Malaysian Forester published by the Forestry Department, Peninsular Malaysia and Newsletter for the Malaysian Plant Protection Society. In 1988, he was awarded a fellowship by The Association of Commonwealth University for an academic exchange program at The International Centre of Insect Physiology and Ecology, Nairobi, Kenya.

His duty as a scientist is rather incomplete if he does not participate actively in NGOs and other organizations that serve the public. He was the founding Secretary and later the Chairman for the National Natural Science Quiz for Secondary Schools, one of the Judges in the Discovery Quiz for Secondary Schools, organized by Discovery Channel, TV3 and Ministry of Education and Sony Science Education Award- SSEA, organized by Sony Corporation (M) Sdn.Bhd. and Ministry of Education. He was the Chairman for Technical Paper Committee and a member in the Main Organizing Committee of the 14th Commonwealth Forestry Conference that was held in 1993 in Kuala Lumpur.

In the professional society, he was a Council Member, the Vice President and finally in 1998/99 the President of The Malaysian Plant Protection Society (MAPPS). He was elected as the Chairman of the Asia-Pacific Congress of Entomology Secretariat during its meeting in Taichung, Taiwan in November 1997. This Congress of Entomology is under the auspices of a Standing Committee that is represented by prominent entomologists from 12 member countries: Australia, Canada, China, Japan, South Korea, India, Indonesia, Malaysia, The

Philippines, Taiwan, Thailand and United States of America. As the Chairman, he was responsible in coordinating meetings for the Standing Committee and organizing the 4th Asia-Pacific Conference of Entomology that was held in Kuala Lumpur in August, 2001. Presently, he is the Vice-Chairman of the Asia Pacific Congress of Entomology Standing Committee (2001-2005), MAPPS Council Member and Member in Board of Trustee of the MAPPS-Education Trust Fund.

As a researcher, Professor Ahmad Said involves in ecology and biological control of insects affecting forest trees and timber structures. His training as an insect pathologist has led him to search for natural enemies, particularly disease- causing microorganisms associated with those insect pests. His current research interest is in the development of viral and fungal-based biopesticides for controlling armyworms and termites. He was also involved in international collaborative research. One of them was a joint project between Forest Research Institute Malaysia (FRIM), Universiti Putra Malaysia (UPM) and National Institute for Environmental Studies of Japan (NIES) under the Global Environment Research Program (Grant No. E-2). This project involved studies on insect communities in selected tropical rainforests. Another one was funded by Winrock International Institute for Agricultural Development under the F/FRED program, which focused on entomopathogens associated with jumping lice of *Leucaena* and insect communities in an agro-forestry ecosystem. Related to research, he was appointed as a host scientist to Commonwealth Agriculture Bureau International (CABI)/North Korean Scientist working on nucleopolyhedrovirus in October 1997. He was made the Chairman of the National Committee on Multipurpose Trees (MPTS) in 1992/93 and represented Malaysia in the Regional MPTS Research Committee. His involvement in working with termites has led him to lead as the Chairman of the SIRIM Working Group for drafting Malaysian Standard on Subterranean Termite Control.

Over the years he has authored, co-authored a total of 135 papers; 58 in journals, 70 in proceedings/seminars, 7 in newsletters and handbooks, and edited 8 proceedings. In 1996, he was awarded an excellence service award by the university. The research conducted by his group has won gold and silver medals at the University and silver at the I-Tex 2004.

INSECT DISEASES: RESOURCES FOR BIOPESTICIDE DEVELOPMENT

ABSTRACT

The over dependence on chemical pesticides for pest control has led to negative effects on environment and health, and to pest resistance and resurgence. These problems may be solved through Integrated Pest Management (IPM), which usually involves reduction in chemical pesticide use and its replacement by a range of control methods that are environmentally accepted and economically feasible. One such component is the use of microbial insecticides, or biopesticides. Biopesticides have been strongly proposed for use in IPM systems because they can reduce pesticide risks. In general, they are less harmful than chemicals, highly selective for a very narrow range of target pests, and often effective in small quantities. The potential for growth for the biopesticide market looks positive against the backdrop of recent developments with regards to the following: the need for environment-friendly pest control technologies, rising costs of chemical pesticides, and the need to exploit local biodiversity towards the generation of new sources of economic activity and income generation. Biopesticides are expected to be a substantial component in pest management programs in Malaysia in future. With regard to research and developmental activities, our laboratory is undertaking both fundamental and applied research, in the areas of isolation, identification and bioefficacy of microorganisms. Among the microorganisms that have been isolated and screened are baculoviruses, nucleopolyhedrovirus (NPV) and granulovirus (GV), and entomopathogenic fungi, *Beauveria*, *Metarhizium* and *Paecilomyces*. They all have the potential to be developed for biopesticides. *Spodoptera litura* NPV (SpltNPV), in particular, is very effective for controlling armyworms in cruciferous vegetables thus it will have an important role in IPM programs in the near future.

INTRODUCTION

The rapid economic development in Malaysia over the last few years has inevitably resulted in heavy usage of its resources ranging from forestry, land and water to fossil fuels and minerals. Concomitant to this rapid development are increasing environmental problems - and agriculture, envisioned to be next focus of growth, is generally perceived to be one of the sectors currently contributing to this state of affairs. However, specific priorities and mechanisms had been formulated and underscored on processes that encourage sustainable agriculture in recent government policy documents, such as the Third National Agricultural Policy (NAP3). Amongst the specific objectives of NAP3 is one that underlines the need for the conservation and utilization of natural resources on a sustainable basis. Thus, sustainable agricultural systems directed at conservation of biodiversity and other natural resources and the environment have been given serious consideration in the areas of policy formulation and implementation. The exploitation of natural flora and fauna as sources of biological control agents and their eventual use in an integrated pest management (IPM) aimed at conservation are therefore central to the promotion of sustainable agricultural systems. Today IPM is a widely accepted strategy to reduce over dependence on chemical insecticides for managing insect pests.

Biological control agents have been used by farmers long before the advent of chemical pesticides. In the early twentieth century such approaches, however, were abandoned for almost total dependency on chemical pesticides to increase agricultural productivity. With high demand on agricultural produce and frequent resurgence of many invasive insect pests, there is a tendency for farmers to use excessive chemical insecticides to protect their crops. Over the years these unsustainable practices have led to serious environmental degradation and health problems. This is a dilemma currently haunting human population. Looking to the future, biological control agents offer a potential solution to the dilemma from both the environmental and the industrial viewpoint. It was for this reason many researchers began to revive their interest in biological control research and the outcomes in many ways lead to the development of biopesticides as an integrated pest management tool rather than an alternative to chemical pesticides.

BIOPESTICIDES

Biopesticides are defined by the U.S. Environmental Protection Agency (EPA) as certain types of pesticides derived from such natural materials as animals, plants, microorganisms and certain minerals. The EPA categorizes biopesticides into three major classes, (i) microbial pesticides- pesticides that contain microorganisms (for example a bacterium, fungus, virus, or protozoan) as the active ingredient, (ii) plant-incorporated protectants (PIPs)- pesticidal substances that plants produce from genetic material that has been incorporated into the plant and (iii) biochemical pesticides- naturally occurring substances that control pests by nontoxic mechanisms. However, microbial pesticides based on entomopathogens or entomopathogenic nematodes are presently the focus of many researchers. They are effective for controlling pests that have developed resistance to chemical pesticides, leave little or no toxic residues and pose no threat to beneficial insects and non target organisms, in contrast to broad-spectrum conventional pesticides. When used as a component of IPM programs, biopesticides substantially reduce the use of chemical pesticides, while crop yield remain high. Biopesticides may be used complimentarily with other management tools rather than as an alternative to chemical insecticides. In a resistant management program, biopesticides can be applied to break to the continuous of spray of chemical insecticides and thereby maintain a susceptible pest population in the agro-ecosystem. In situation where the pathogen present in the pest habitat is insufficient to prevent economic damage by the pest, biopesticides may be applied to augment the pathogen inoculums in the environment. Increasing the pathogen load theoretically should increase disease prevalence in the pest population. Biopesticides can be a better input in protected cultivation such as in glass houses, where environmental parameters can be manipulated so as to promote a condition conducive for growing of the crops and infectivity of the selected biopesticide.

The application of microbes for control of insect pests were proposed by distinguished pioneers such as Louis Pasteur and Elie Metchnikoff (Steinhaus, 1975). In the late 19th century, many researchers experimented the use of fungi as microbial control agents. However, the use of microorganisms for controlling insects came into practice only after the development of the bacterium, *Bacillus thuringiensis* Berliner in 1911 by Berliner. Since then, many *Bacillus* species and subspecies have been used for managing insect pests of agricultural crops, forest trees, stored products and arthropods of medical importance. Today, bacterial, fungal and viral-based biopesticides are on the verge of making noteworthy contributions to improving the productivity of agriculture and forest while maintaining the healthy ecosystem.

PATHOGEN RESOURCES

Tropical rain forests are by far the richest reservoir of biodiversity. The forests sustain very diverse flora and fauna including microbes. Microbial diversity in the tropical rain forest is immense and only a small fraction of microbes having been identified. Their roles in nature are also indispensable. This microbial diversity serves as bioresources for the betterment of future generations. New technologies are emerging that enable us to use these microbes to increase agricultural productivity without jeopardizing the environment. A group of microbes that are of interest are those that acts as natural mortality factors in insect species. These microbes, represented by viruses, bacteria, fungi, protozoa and nematodes, could cause diseases that are fatal to insects. Many of these pathogens are natural enemies to many insects including those of economically important pests. These pathogenic organisms must have played an important role in regulating natural population of their host insects. Over the years some pathogens have been isolated, screened against selected pests and applied in field studies.

Bacteria

Among many microorganisms that have been exploited and intensively studied for their insecticidal properties are bacteria. Bacteria are microscopic prokaryotes. There is no well-defined nucleus or organelles. They have a structurally distinct cell wall. They can be spore forming and non-spore forming bacteria. Entomopathogenic bacteria grow independently in the insect haemocoel. They must be ingested by the host insects to cause an infection. They normally rupture the gut wall and then invade the haemocoel. The bacteria that are pathogenic to insects are species from the genus *Bacillus*. *Bacillus* is aerobic, unicellular, usually bacilliform and spore forming bacteria. Infection by *Bacillus* occurs after ingestion of the bacteria. There are several species of *Bacillus* which are now being used in biological control. They are used for controlling of a broad range of crop and forestry pests, and several medically important arthropods. One of them is *Bacillus thuringiensis (Bt)*. *Bacillus thuringiensis* produces a parasporal crystal, which is bipyramidal in shape, and a thick- walled endospore. The crystal, produced during sporulation, is an endotoxin which dissolves in alkaline gut fluids and releases toxin peptidases. This toxin causes muscle paralysis in the alimentary tract that subsequently stops the larval host from feeding. The toxin attacks the membrane of the gut epithelial cells resulting in swelling, vacuolization and lysing of the cells. This breakdown of the epithelial cells facilitates spore germination within the gut lumen. The bacteria multiply in the larval hosts and induce a lethal septicemia.

Over the past decade, with the advancement in biotechnology, researchers have manipulated the *Bt* genes to optimize their effectiveness as biopesticides. Recently, the genes that produce insecticidal toxins have been incorporated into agricultural crops and forest trees in an effort to produce plants that are resistant to some phytophagous insect pests. *Bt* corn, for example, which produces its own insecticide, contains a toxin gene from *B. thuringiensis* represents the first group of genetically modified product released into agricultural system. Even though, transgenic crops offer some advantages, an extensive planting of these crops raises concerns with regard to the development and exacerbation of resistance (Hokkanen and Deacon, 1994; Lacey et. al. 2001)

Currently, in Malaysia, as is the case in many countries, products based on *B. thuringiensis* occupy more than 90% of the biopesticide market especially in vegetables, oil palm plantation and fruit production systems (Sivapragasam and Sajap 2002). Besides the availability of commercial strains (mainly *B. t. var. kurstaki, B.t. var. aizawai* and *B.t. var. israelensis*), a number of local bacteria, particularly *B. thuringiensis*, have been isolated from soils or naturally infected insects.

Viruses

Another group of microorganisms with potential to be developed as biopesticides are viruses. Viruses are biological macromolecules that have the ability to multiply within living cells. They are immobile obligate intracellular parasites and they cannot respond to

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stimuli. Viruses are important mortality factors in many insect populations and there are at least ten groups of viruses known from insects. One of those viruses are baculoviruses. Baculoviruses are pathogenic only to insects mostly of the order Lepidoptera, Hymenoptera and Coleoptera. Their host specific action allows the control of the target insects without adversely affecting non-target beneficial insects or the ecosystem.

In Malaysia, a baculovirus was first isolated from armyworm, *Spodoptera litura* in an epizootic in Kelantan during the search for natural enemies associated with the pest (Sajap, 1995). The virus was propagated in the laboratory using armyworm larvae. These naturally diseased armyworms were apparently infected with two viruses. Through SEM and TEM studies, we confirmed the viruses to be nucleopolyhedrovirus (NPV) and granulovirus (GV). These two viruses were characterized by Lau (2002). The *Spodoptera litura* NPV (SpltNPV) is typically polyhedral shaped between 1.0 – 1.83 µm in diameter and embedded with multi-virions. These virions are rod-shaped and measure 100- 280 nm in diameter and 320- 410 nm in length. Each virion contains nucleocapsids measuring 46-60 nm wide and 300 – 350 nm long. The nucleocapsids are enveloped within a multilayer membrane. The *Spodoptera litura* GV (SpltGV) possesses virions that are singly occluded into small occlusion bodies called granules. SpltGV granules, about 0.2-0.3 µm wide and 0.45 – 0.55 µm long, and contain single virion, about 60-73 nm wide and 245-267 nm long. The nucleocapsid, singly occluded within an envelope, is approximately 54-60 nm wide and 287 – 410 nm long.

The infection cycle of a baculovirus starts with occlusion bodies being ingested by an insect larva. The alkaline midgut conditions in the host, dissolves the occlusion body, releasing the viral nucleocapsids into the gut lumen. These nucleocapsids then initiate a primary infection in the columnar cells of the midgut epithelium. A similar pattern of infection also occurred in both viruses, SpltGV and SpltNPV (Lau, 2002). The rate of infection by SpltGV, however, was relatively slower than SpltNPV. SpltNPV replicates only in the nucleus while SpltGV replicates in the nucleus and the cytoplasm of infected cell. Figures 1 and 2, respectively illustrate some stages of SpltNPV and SpltGV infections in the host tissues. Even though SpltGV replicates both in the nucleus and the cytoplasm of infected cell, the effect on the host larva was less lethal as compared with that of SpltNPV. The LD_{50} values recorded from a bioassay test conducted on the neonates were 9.04×10^2 and 1.26×10^4, for SpltNPV and SpltGV, respectively. In some instances, SpltGV infected larvae lived longer than normal larvae. This impaired development was observed in larval growth of *Adoxophyes honmai* (Lepidoptera: Tortricidae) infected with granulovirus (AdhoGV) (Nakai et.al. 2002). They believe that AdhoGV-infected larvae are prevented from pupating because Juvenile Hormone Esterase (JHE) activity is suppressed and Ecdysteroid Glucosyl Transferase (EGT) expression inactivates ecdysteroid in the haemolymph. Consequently the larvae failed to pupate and died in the final instars.

SpltNPV is very pathogenic to its host, *S. litura* larva. The infectivity of SpltNPV, however varies with the developmental stages of the larvae and the virus concentration to which the larvae were exposed (Bakir, 2001). It could cause 100% mortality to second instars within 3-7days when they were exposed to different virus concentrations. The virus killed younger larvae faster than older larvae and high concentration killed faster than low concentration (Table 1).

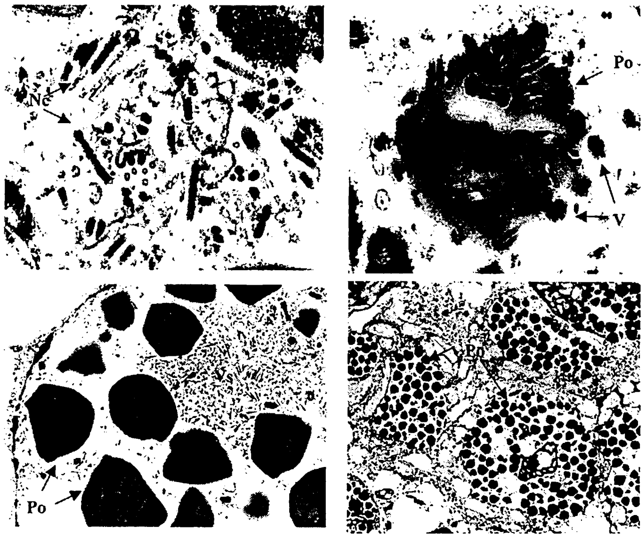

Figure 1: Some stages of SpltNPV development in infected *S. litura* cells.
Nc = Nucleocapsids, V = Virions, Po = Polyhedra, Vs = Virogenic Stroma

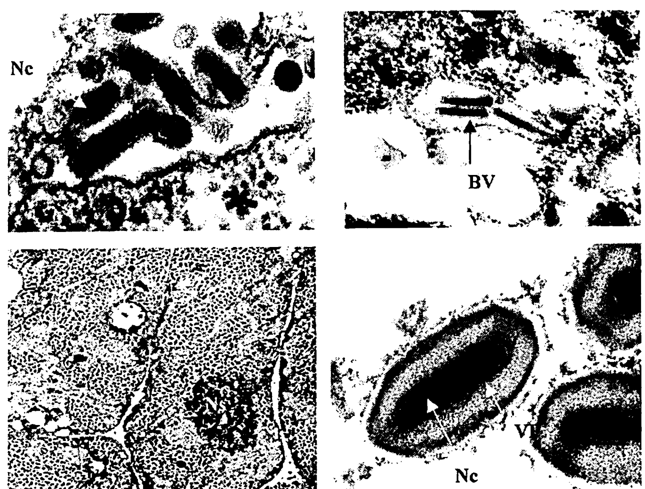

Figure 2: Some stages of SpltGV development in infected *S. litura* cells.
Nc = Nucleocapsid, VP = Virus Particle, Ns = Nucleolus,
BV = Budded Virus

Table 1. LC_{50} Values of NPV for Different *S. litura* Instars

Larval Instar	No. of Larvae Used	LC_{50} (PIBs/mL) (95% Confidence Interval)	Slope ± SE	Heterogeneity χ^2	*P*
1st Instar	712	$3.2x10^6$ ($1x10^4$ - $1.3x10^7$)	0.85 ± 0.31	3.27	0.20
2nd Instar	854	$1.1x10^7$ ($1.6x10^6$ – $2.2x10^8$)	0.25 ± 0.05	2.65	0.27
3rd Instar	712	$1.3x10^7$ ($5.4x10^5$ – $1.4x10^9$)	0.13 ± 0.03	1.29	0.73
4th Instar	738	$4.7x10^7$ ($1.3x10^7$ – $2.1x10^8$)	0.37 ± 0.05	5.3	0.15

LC_{50} values were calculated using cumulative mortalities (%) of larvae after 12 days of infection
Data fit probit model by χ^2 test at $P > 0.05$
Natural mortalities were included in the analysis for the first instar larvae
Pupated larvae were considered as escape from infection

SpltNPV, like other baculoviruses, is regarded as a safe and selective insecticide. It is specific to its host and it is not infective to Lepidopteran such as *Pteroma pendulla* and *Spirama retorta* (Sajap et. al. 2000). However, when an assassin bug, *Sycanus leucomesus* was fed on SpltNPV-infected larvae of *S. litura*, variable effects on its biology were observed (Sajap et. al. 1999). The predators that were fed with infected prey through out their lives appeared normal but they had significantly smaller head capsules and shorter tibial lengths than those fed with healthy prey. These individuals had their overall pre-imaginal survival rate reduced by 10%, pre-oviposition period prolonged to more than 12 days, longevity rate reduced by 10 to 19 days in the males and females, respectively, and fecundity reduced to 41%. The impaired developmental and reproductive biology of the predator could be due to the nutritive quality of the prey rather than infection by the virus. The abundance of virus particles in the prey's body reduced the assimilable proportion of proteins in haemolymph and fat body and caused nutritional imbalance to the predator. Consequently, the predator failed to accumulate enough protein reserves that are required for growth and reproduction.

Even though the impact of infected prey on the predator was rather significant, such impacts might not always occur in the field. Like many generalist predators, *S. leucomesus* do not necessarily feed on diseased prey through out their lives. They may consume any soft-bodied arthropods they encounter in the ecosystem. At the same time they defecate a substantial amount of infective virus in their faeces if they feed on infected preys. It has been shown that virus collected from the faeces defecated by predators fed infected preys caused 97% mortality on *S. litura* larvae within 10 days (Sajap et. al. 1999). The predator, therefore, could serve as a carrier or disseminator of the virus within the ecosystem.

There are several limiting factors when baculoviruses are exposed to the environment. Factors such as solar radiation, temperature, pH and moisture, influence their persistence in the environment. Studies in infectivity of SpltNPV in different pH suspensions and exposure to sunlight prior to ingestion and effects of rearing temperature on the mortality of infected larvae show that mortality significantly increased by 47% from 53% when the larvae were reared at 20°C, to 100% when they were reared at 30 and 35°C. There was a positive correlation between mortality and rearing temperature, indicating that the infectivity of the SpltNPV increased with the increase in larval rearing temperature as reflected by a significant reduction in LT_{50} values from 24 to 6 days. All virus suspension used in the experiment induced mortality to more than 90% except in pH 11, where it was 64%, and 100% mortality was recorded from pH 7. The LT_{50} value increased to about four folds when the pH of suspension was increased from 7 to 11. Even though mortality obtained from the larvae that had been fed with virus suspension of pH 3, 5 and 9 were not significantly different, infectivity of the virus was more adversely affected in alkaline than in acidic suspension. Like many baculoviruses, SpltNPV is affected by sunlight. Complete virus inactivation occurred after 12 hours of direct sunlight exposure (Table 2). Inactivation by sunlight primarily is caused by ultraviolet light (UV). The virus was completely inactivated when it was exposed to UV-A and UV-C for 360 hours (Bakir 2001). The deleterious effects of UV light, however, could be alleviated by adding UV protectant such as Tinopal, an optical brightener, into the virus preparation (Bakir, 2001; Shapiro and Doughterty, 1993).

Based on results form laboratory experiments, a small-scale field experiment using formulated virus preparations was conducted. Virus preparations were sprayed onto *Brassica juncea* (sawi bunga) that have been infested with *S. litura* larvae. The result shows that the virus could protect this leafy vegetable from damages caused by armyworms. No significant differences in yield between *Brassica* treated with virus+tinopal compared with that of *Brassica* treated with Nurelle®, an insecticide recommended for *Brassica* (Table 3). The result clearly shows that SpltNPV is a promising candidate for controlling armyworms in vegetable growing areas.

Table 2. Percentage of mortality of *S. litura* larvae infected with NPV that had been exposed to direct sunlight for different periods of time

Sunlight Exposure Periods (h)	Mortality (%) * (Mean ± SE)	Range	AR **
0	$100^a \pm 0$	0	1
1	$100^a \pm 0$	0	1
4	$60.84^b \pm 2.10$	56.67 - 66.67	0.61
8	$60.00^b \pm 1.36$	56.67 - 63.33	0.60
12	0^c	0	0
16	0^c	0	0
Shaded (16)	$100^a \pm 0$	0	1
Control	0^c	0	0

* Means followed by different letters are significantly different (DMRT, $P < 0.05$)

Table 3. Effects of SpltNPV application on *Brassica* Yield

Treatment	Dose/ha	Adjuvant Used	Yield (kg)/ha * (Mean ± SE)
Virus	$2x10^{12}$ PIBs	None	9640.25 [a] ± 814.67
Virus	$2x10^{12}$ PIBs	1% Crude Sugar	7925.75 [a] ± 818.73
Virus	$2x10^{12}$ PIBs	0.5% Tinopal	8699.75 [a] ± 1625.26
Nurelle-D	@ 10mL/9L water (45.90% w/w) + Cypermethrin (4.59% w/w)	a.i.: Chlorpyrifos	7434.75 [a] ± 516.64
Control			5222 [b] ± 846.62

$F = 5.92$, $P = 0.0072$, Mean = 7784.5 kg/ha
* Means followed by different letters are significantly different (DMRT < 0.05)
PIBs = Polyhedral Inclusion Bodies

Apart from baculoviruses in *S. litura*, granulosis and nucleopolyhedrosis viruses have been isolated from bagworms, rhinocerous beetles and nettle caterpillars of oil palm (Ramlah et al. 1996). Non-occluded NPV associated with *Oryctes rhinoceros* (OrV) is endemic in Malaysia. OrV causes chronic infection in the adult beetles and they essentially serve as reservoirs and disseminators of the virus (Zelany, 1973). Ramlee et. al. (2002) recorded three strains of OrV with varying virulence and a mass release of infected adults reduced frond damage from 12 to 4%. However, applying OrV alone in the field may not be enough to suppress the beetles below the economic threshold. It is, therefore, recommended that OrV may be applied in field together with other control method such as *Metarhizium anisopliae*, which is effective against the larvae.

Fungi

Knowledge on fungal diseases of insects dated to a few centuries ago. For many years the Chinese have been using *Cordyceps*-infected caterpillar not for insect control but for treatment of many ailments, health tonic and aphrodisiac. A century ago, when insecticides were still unknown, entomopathogenic fungi had been proposed for used for insect control by scientists like Bassi, Pastuer and Metchnikoff. Their ideas and findings subsequently spurred many studies on these microbes in attempts to control insect pests especially in Europe and North America. Since then, more than 700 species of fungi have been reported (Hajek and St. Leger 1994).

Fungi, like virus, often act as one of the key mortality factors in natural insect populations. Most entomopathogenic fungi produce asexual spores or conidia as their reproductive units. Upon landing on cuticle of a susceptible host, the conidia germinate, the hyphae penetrate the cuticle and enter the insect's body. The host is normally killed by toxin secreted by the infecting fungus. Unlike other microbial control agents, fungi do not have to be ingested to cause infections. Thus they are able to infect almost all stages of their hosts.

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Among the Entomophthorales, we isolated *Conidiobolus coronatus* (Zygomycetes) from soil, and tested its pathogenicity to subterranean termite, *Coptotermes curvignathus*. The fungus could cause 100% mortality in worker termites within 12 hour after inoculation (Sajap et. al. 1997). Even though, *C. coronatus* is very pathogenic to *C. curvignathus*, the fungus is difficult to culture and its primary conidia are short lived. Thus it may not be a good microbial control agent for biopesticide development.

In the case of deuteromycetes, a number of fungi have been isolated and tested against some important insect pests. Of the major groups, *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces fumosoroseus*, most significant developments have occurred in *M. anisopliae*. *Metarhizium anisopliae* var. *major* is effective for controlling field population of *O. rhinoceros* (Ramlee et al., 1999). Application of *M. anisopliae* conidia into the breeding ground can result mortality up to 51% in the beetle's population at three months after treatment. This fungus is also infective on termite, *C. curvignathus* (Sajap and Kiranjeet, 1990) and flea beetle, *Phyllotreta striolata* (Priyatno, 2001). On termites, *M. anisopliae* isolates from soils caused 100% mortality within 3 to 7 days with LT_{50} values ranged from 1 to 3 days. Field trials, using trap, treat and release technique, conducted at two sites show that the selected strains could suppress termite population. Feeding activities ceased about two months after treatment. Figure 3 shows *M. anispoliae* and *M.anisopliae* - infected termite. Among three spcecies of fungi isolated from bagworms (Sajap and Siburat, 1992) *P. fumosoroseus* evidently is the most effective fungus for controlling the cabbage-heart caterpillar, *Crocidolomia binotalis* (Norzita, 2000) and DBM larvae (Ibrahim and Hashim 1998). Conidia formulated in palm oil recorded a mortality rate of more than 80% in cabbage-heart caterpillar.

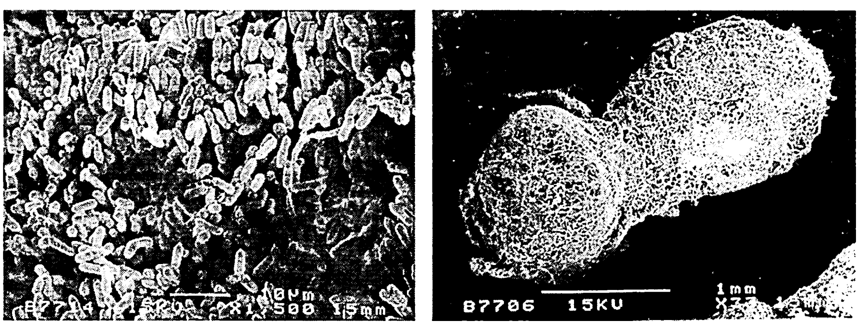

Figure 3: *Metarhizium anisopliae* and an infected termite

Nomuraea rileyi is a common entomopathogen associated with *Spodoptera* larvae in the tropical and subtropical regions. I managed to isolate *N. rileyi* from two places of different altitude and temperature, Cameron Highlands in Pahang and Telong in Kelantan, and studied their growth pattern and pathogenicity to *S. litura* . Both isolates were morphologically similar in their sizes of the conidiophores, conidia and hyphae (Fig. 4). However, they grew at different rates when exposed to certain temperatures (Table 4). The lowland isolate from Telong grew significantly faster and was more virulent at all given temperatures than the isolate from the highland of Cameron Highlands. Both isolates grew optimally at 25°C and caused 100% larval mortality ten days after inoculation (Sajap, 2000). Figure 4 shows conidiophores and conidia of *N. rileyi* and *S. litura* larva naturally killed by *N. rileyi.*

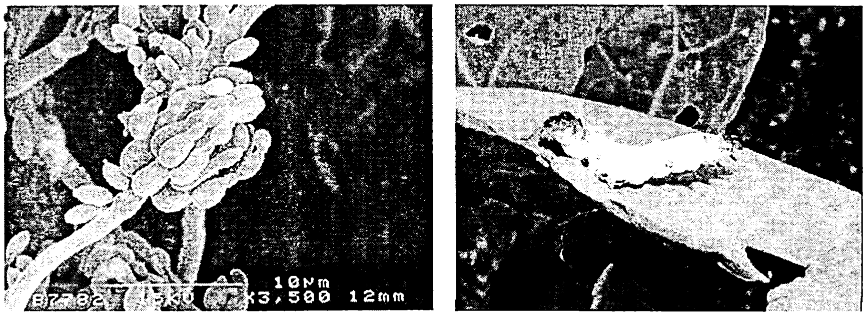

Figure 4: *Nomuraea rileyi* and an infected *S. litura* larva

Table 4. Colony sizes (mm) of *N. rileyi* isolates during four weeks of incubation at different temperatures

	7°C		25° C		32°C	
Week	**C. Highlands ($\bar{x} \pm$ s.e)**	**Telong ($\bar{x} \pm$ s.e)**	**C. Highlands ($\bar{x} \pm$ s.e)**	**Telong ($\bar{x} \pm$ s.e)**	**C. highlands ($\bar{x} \pm$ s.e)**	**Telong ($\bar{x} \pm$ s.e)**
1	2.0 ± 0.00	3.50 ± 0.29	2.00 ± 0.00	6.75 ± 0.62	3.5 ± 0.29	6.00 ± 0.24
2	2.0 ± 0.00	3.75 ± 0.29	8.75 ± 0.14	13.75 ± 0.75	7.12 ± 0.43	9.88 ± 0.13
3	3.12 ± 0.13	4.75 ± 0.48	14.88 ± 0.47	23.75 ±1.81	10.34 ± 0.38	16.75 ± 0.60
4	5.25 ± 0.25	6.88 ± 0.52	25.00 ± 0.71	33.00 ± 2.71	16.88 ± 0.72	28.13 ± 1.09

LSD = 2..02 mm, % = 0.05

The occurrence of *Hirsutella citriformis* infecting leucaena psyllid, *Heteropsylla cubana,* was first recorded in Malaysia in 1993 (Sajap, 1993). Leucaena psyllid is an exotic pest of *Leucaena leucocephala* and it was first reported in Malaysia in 1986 (Tho, 1986). At this time this tiny Homoptera became an instant problem of global concern especially in Southeast Asia where it caused devastating damage to the newly introduced multipurpose tree. A one-year survey on entomopathogens associated with this pest shows that even though the insect was relatively new in Malaysia, the psyllid was found to be susceptible to *H. citriformis.* The fungus controlled about 22% of the psyllid in natural population. Figure 5 shows an adult psyllid infected and killed by the fungus.

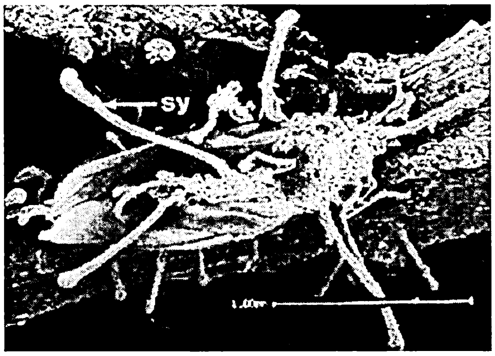

Figure 5: *Hirsutella citriformis* infecting an adult *Heteropsylla cubana*

Microsporidia

Microsporidia are unicellular and obligatory intracellular parasites formerly in the Phylum Protozoa. These parasites differ from traditional eukaryotes due to their lack of mitochondria and their addition of a specialized organ, the polar filament or tubule. Many of these microsporidia are responsible for natural mortality in many insects. Microsporidia usually have to be ingested to infect an organism, but once inside, they spread to various tissues and organs in the body, multiply, and cause the degradation of those tissues. Initial infection within the host begins with the extrusion of the polar filament from the germinating spore. The protoplasm, consisting of the cytoplasm, nucleus and other organelles, is injected into the midgut epithelial cells through the polar filament.

Although microsporidian infection does not entail in death, infected insects are very susceptible to various causes of death, including reduced feeding, inability to reproduce, and increased susceptibility to weather conditions, natural predation and diseases. They also show signs of infection by moving sluggishly and becoming smaller in size. Because the insects do not die rapidly after infection, they are able to pass on the microsporidia to the rest of the insect population. In addition of horizontal transmission, which occurs by ingestion of spores, many species of microsporidia are transmitted vertically from an infected female to her offspring, either on the egg surface or within the egg (transovarial). This route of transmission essentially ensures a continuous cycle of infection from one generation of the host to the next.

Among the insect pathogens, microsporidia are the least studied organisms in Malaysia because the effect on the host insects is very seldom lethal and the symptom of infection is ambiguous. Nevertheless, microsporidia are present in this country. Sajap (1995) reported an infection by a microsporidium, *Nosema* sp. in the larvae of *S. litura*. Infected larvae lost their vigour, became sluggish and ceased feeding. The colour of the body was relatively lighter than those of healthy larvae. Most of these larvae succumbed in the fourth or fifth instars. Some larvae underwent incomplete larval-pupal moulting and died in a bizarre prepupal form. Histological observation made on infected tissues revealed that fat body was the main site of infection (Fig. 6). In addition to the fat body, the reproductive tissues were also intensively infected. The gonads were lacking germ cells and instead they were filled with masses of spores. Other organs, such as Malpighian tubules, gut epithelium and nerve cords were also infected, but to a lesser extent. No infection occurred in the muscle even though some muscle fibres were in close proximity or coalesced with heavily infested fat body.

In addition to the armyworm, *S. litura*, microsporidian infection also persists in diamondback moth (DBM), *Plutella xylostella* (Fig. 7). Diamondback moth is a very serious pest of cruciferous vegetables such as cabbages and choy sam. Idris and Sajap (2001), while conducting a survey on natural enemies associated with DBM, found its larva and pupa were infected with *Nosema bombycis*. We noted that the microsporidian infection was more severe in Cameron Highlands (CH) than in the lowland areas of Serdang-Gombak (SG) (Table 5). The percentages of infected DBM larvae for two seasons were 71.3 and 66.8% in CH and 10.0 and 2.4% in SG. The mean intensity of infection in DBM larvae was 2.59×10^7 and 1.5×10^7 spores/larva in CH while in SG it was 1.4×10^6 and 2.0×10^5 spores/larva. The difference in the rate and intensity of infections in DBM collected from the sites with different altitude suggested that temperature may have influenced the severity of infection. Low temperatures in the highland may have prolonged larval development thereby increased probability of DBM larvae acquiring spores from *Nosema* contaminated leaves as they have longer feeding duration. High temperatures in the lowland area may have caused the insect growth rate to outpace the *Nosema* replication rate in DBM. As a result the intensity of infection of DBM larvae from the lowland was lower than those larvae from the highland.

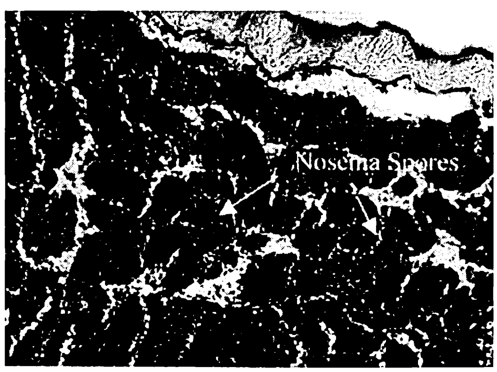

Figure 6: Fat body of *S. litura* larva infected with *Nosema* sp.

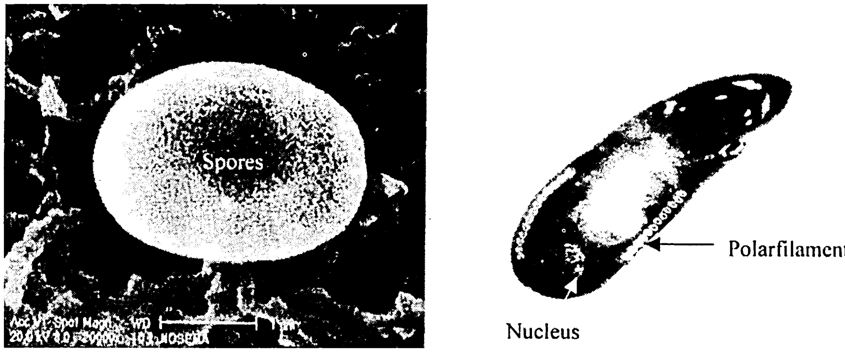

Figure 7: Spores of *Nosema bombycis*

Table 5. Percentage infection, mean infection intensity and range of infection intensity of *Nosema bombycis* in the larvae and pupae of the diamondback moth, *Plutella xylostella* from the highland (CH) and lowland (SG) cabbage-growing areas

Site	Season[1]	Sample	Percentage infection (n)[2]	Mean infection intensity ($\pm$SD)	Range of infection intensity
CH	I	larva	71.3 (20)	$259.3 \times 10^5 \pm 217.0$	$0.02 \times 10^7 - 8.03 \times 10^7$
		pupa	13.3 (10)	$67.2 \times 10^5 \pm 88.3$	$0.03 \times 10^7 - 2.17 \times 10^7$
	II	larva	66.7 (25)	$150.2 \times 10^5 \pm 217.3$	$0.01 \times 10^7 - 9.77 \times 10^7$
		pupa	12.5 (15)	$22.3 \times 10^5 \pm 35.1$	$0.01 \times 10^7 - 1.28 \times 10^7$
SG	I	larva	10.0 (20)	$14.0 \times 10^5 \pm 0.7$	$0.03 \times 10^7 - 0.27 \times 10^7$
		pupa	8.1 (14)	$26.5 \times 10^5 \pm 17.2$	$0.05 \times 10^7 - 0.078 \times 10^7$
	II	larva	2.4 (10)	$2.0 \times 10^5 \pm 0.1$	$0.01 \times 10^7 - 0.02 \times 10^7$
		pupa	7.0 (10)	$7.1 \times 10^5 \pm 0.1$	$0.01 \times 10^7 - 0.32 \times 10^7$

[1]October 1998 (l) and April (ll).
[2]n=10–20% of collected samples.

Even though, microsporidian infection in diamondback moth does not result in mortality, the infection can reduce the fecundity of infected female moths by at least 50%. The eggs laid by those females were loaded with the microsporidium. In severe infection, larvae may die before adult emergence and those that emerge may not oviposit. If infected females oviposit, their progenies are weak and more susceptible to other pathogens.

Being an endoparasite, *N. bombycis*, could pose a threat to parasitoids developing in the host larva. Like *Trichogramma nubilale*, an egg parasitoid, that was adversely affected by *Nosema pyrausta* (Sajap and Lewis 1988), *Diadegma semicalausum*, a larval parasitoid, was also affected by the microsporidium present in DBM (Idris et. al. 2001). They noted that some parasitoids were short-lived and their bodies were filled with *Nosema* spores. Thus if an organism such as *N. bombycis* is to be manipulated or placed in the agro-ecosystem in an effort to control DBM, one must first assess the impact of the microsporidium on nontarget beneficial insects, particularly its parasitoids.

CONCLUSIONS

Despite the vast amount of bioresources and their great potential for use in a sustainable agriculture and forestry, biopesticide development, commercialization and use in the developing countries including Malaysia has yet to be commended. This is evident from the research updates gathered from researchers and their agencies. Majority of the research are still in their early phase. Harris and Dent (2000) generally attribute the obstacles in the process of biopesticide development to lack of expertise, receive low funding, mainly from the public sector, and rarely involves the multi-disciplinary expertise needed to develop a biopesticide from start to finish. They believe that by removing those constrains successful development and application of biopesticides could be achieved. Biopesticides should be targeted for medium and long term effects rather than short-term as expected from conventional pesticides. There should be a greater recognition on the positioning of biopesticides in the overall pest management program because of their complimentary role. In short, the progress of research and development on biopesticides, like in many developing countries, is rather slow. Even though commercialization of the product is generally still far from reality, a product that stands to contribute to sustainable pest management will be made available in the very near future. A concerted and coordinated effort by various research and development agencies is therefore needed to take advantage of the long term benefits of biopesticides, a management tool in IPM systems.

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