The potential of indigenous entomopathogenic fungi for the management of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: yponomeutidae) in Ghana

P. E. Anaisie[1]; V. Y. Eziah[2] and E. O. Owusu[3]

[1]African Regional Postgraduate Programme in Insect Science (ARPPIS), University of Ghana, Legon.
[2].Crop Science Department, University of Ghana, Legon.
[3]Department of Animal Biology and Conservation Sciences.

One major constraint to increased and sustainable production of cabbages in Ghana is its infestation by insect pests particularly the diamondback moth (DBM) *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). The control of this insect in Ghana relies heavily on the use of synthetic insecticides to which the insect has developed resistance. Efforts are therefore being made to look for alternative control strategies and their use within integrated pest management programmes. This study determined the potential of indigenous entomopathogenic fungi for the management of the DBM in Ghana. Three fungi, *Aspergillus* sp, *Penicillium* sp and *Fusarium* sp were isolated from field collected cadavers of DBM and identified. *Bacillus thuringiensis* was used as standard reference product. Bioassays were done using topical application and leaf disc assays. Third-instar larvae were treated with different concentrations of these fungi and the results showed that all three fungi induced significant levels of mortality in the larvae. *Aspergillus* sp, *Penicillium* sp and *Fusarium* sp induced 86.7% - 96.7%, 66.7% - 76.7% and 66.7% - 70% mortality, respectively. The study showed that the fungi isolates have potentials as biological control agents in the management of the diamondback moth in Ghana.

Keywords: *Plutella xylostella,* entomopathogenic fungus, biological control, *Aspergillus* sp, *Penicillium* sp, *Fusarium* sp.

INTRODUCTION

Cabbage, *Brassica oleracae* var*. capitata* L., is the most important in terms of production and consumption in West Africa among the cruciferous vegetables (Talekar and Shelton, 1993). One major constraint to the increased and sustainable production of cabbage is its susceptibility to infestation by insect pests. The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae), is one of the most destructive insect pests of cruciferous crops and requires globally US $1.0 billion in estimated annual management costs (Talekar and Shelton, 1993). This crucifer specialist may have its origin in Europe (Hardy, 1938; Carter, 1984) but now found wherever its host plants exist.

Outbreaks of the DBM in South-East Asia sometimes cause more than 90% crop loss (Verkerk and Wright,1996). In India it was first reported in 1914 where it causes annual losses of about US $16.0 million (Mohan and Gujar, 2003). It is very destructive pest in Pakistan especially Southern Sind (Hyderabad and Karachi) where cruciferous vegetables are grown throughout the year and growers are sometimes compelled to plough down their standing vegetable crops due to severe infestations (Abro *et al*., 1988). The situation in Africa and for that matter Ghana is not different. The high demand for cabbage has led to vegetable growers resorting to monoculture and intensive cultivation which has in turn altered the natural ecosystem and thereby, making the diamondback moth a major pest (Kumar, 1986).

Control of the DBM has generally involved synthetic insecticides. However the increasing concerns over environmental pollution, human-health risks, and insect

resistance as well as effects on non-targeted organisms have stimulated the search for alternative control strategies and their use within integrated pest management (IPM) programmes.

Entomopathogenic fungi may play a significant role in the regulation of many insect pests. Mycopathogens such as *Beauveria bassiana* and *Metarhizium anisopliae* infect many insects under a wide range of environmental conditions. Some notable successes have been achieved using fungi to control major pests such as desert locusts, tsetse flies, and Colorado potato beetle (Anderson *et al*., 1988). Entomophthoralean fungi such as *Zoophthora radicans* is a common natural enemy of the DBM and contributes to the natural regulation of the DBM population (Pell *et al*., 2001). In open field trials in the USA, *B. bassiana* significantly reduced the numbers of the DBM larvae when used alone (Vandenberg *et al*., 1998). This study was, therefore, undertaken to explore the potential of indigenous entomopathogenic fungi isolates for the management of the DBM in Ghana.

MATERIALS AND METHODS

Collection and Rearing of DBM populations

Field populations of the DBM were collected from five cabbage farms in parts of Accra and reared in separate laboratories. The conditions in the laboratory were maintain at 28 ± 1°C with relative humidity of 75-80% and a photoperiod of 12h: 12h (L: D). The adult moths were sustained on cotton wool impregnated with 50% (v/v) sugar solution. Larvae were fed on 7-8 weeks old insecticide-free potted cabbage plants. The insecticide-free potted cabbage plants were also provided as oviposition substrate for the adult DBM.

Isolation and Identification of fungi

Cadavers of DBM collected from the field were surface-sterilized with 5% sodium hypochlorite. The cadavers were then washed with distilled water and blotted with tissue paper. Cadavers were transferred onto Potato Dextrose Agar and incubated at 28°C with a photoperiod of 12h: 12h (L: D) for 7-10 days. Cadavers with fungal growth were also placed directly onto PDA and incubated under the same conditions. The sporulating fungi in various cultures were sub-cultured on PDA to obtain pure cultures as described by Poinar and Thomas (1984). Microscope slides of the fungi isolates were prepared and observed under the microscope. Identification of the isolates was done as described by Humber (1998) using the International Mycological Institute (IMI) Manual of Pathogenic Fungi and Bacteria (1983). The incidence rate of the isolates was also recorded.

Inocula preparation

The fungi isolates were inoculated onto PDA medium in Petri dishes and incubated at 28 ± 1°C, 75-80% RH and photoperiod of 12h: 12h (L: D) for 7-10 days. The harvested conidia were suspended in 25 mL Erlenmeyer flask containing 10 mL of sterile distilled water containing 0.05% Tween® 80. The inocula were homogenized by shaking thoroughly for 10 minutes. The conidial concentrations were determined by direct count using the Neuberger Haemocytometer as described by Lomer and Lomer (1996).

Bioassays for fungi isolates and Bt

Five levels (3.5×10^4, 3.5×10^5, 3.5×10^6, 3.5×10^7 and 3.5×10^8 conidia/mL) each of *Aspergillus* sp, *Penicillium* sp and *Fusarium* sp isolates were prepared and 0.5 µL of each inoculum was topically applied to each of the 3rd instar DBM larvae, each weighing between 2.5 and 3.4 mg. The treated larvae were transferred into clean, plastic 90 mm Petri dishes lined with filter paper and containing fresh cabbage leaf discs for feeding.

For *B. thuringiensis,* five levels (0.2, 0.1, 0.5, 0.025 and 0.0125 g/mL) (standard check) were prepared using sterile distilled water. Fresh cabbage leaves taken from insecticide-free potted cabbage plant were dipped into the prepared suspensions for 2 minutes and left to dry at room temperature. Ten 3rd instar larvae weighing between 2.5 and 3.4 mg each were transferred into clean, plastic 90 mm Petri dishes, which were each lined with filter paper and containing the treated cabbage leaves for feeding. In all experiments, there were 10 larvae per treatment with three replications. The control larvae were treated with sterile distilled water containing 0.05% Tween® 80. The experiments were maintained at 28 ± 1°C and 75-80% RH. Mortality counts were recorded daily for 3 days after treatment. Larvae that did not move or respond to prodding with a blunt probe and showed signs of mycosis were considered dead. Dead larvae showing signs of mycosis were transferred onto PDA and incubated under the same conditions. Sporulating fungi were re-isolated and identified.

Data Analysis

Mortality data were arcsine transformed and subjected to Analysis of Variance (ANOVA), using Genstat Statistical Software Version 9 release 9.2 (Lawes Agricultural Trust, 2007). Means were separated using the least significant difference (LSD). Probit analysis package (EPA Probit analysis ver. 1.5) was used to determine the LC_{50} values, slopes and 95% fiducial limits. Non-overlap of FL was used as criteria for significant difference between LC_{50}

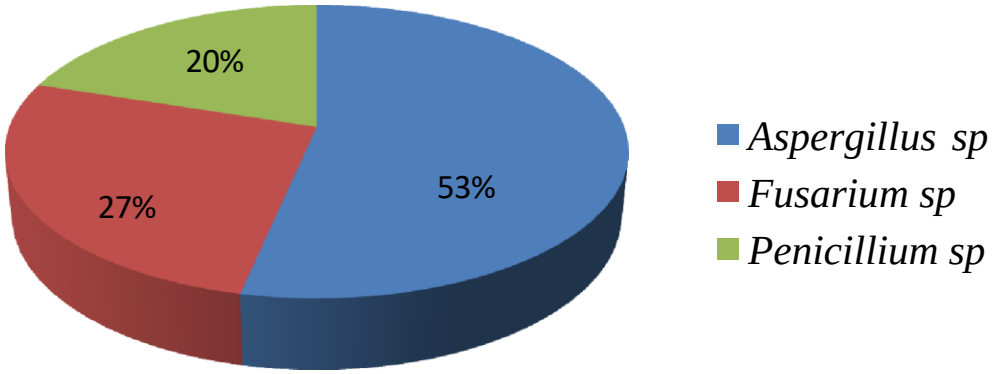

Figure 1 The incidence rate of isolated fungi from the DBM

Table 1. Fungi isolated from the DBM cadaver.

Organism	Growth morphology	Colour	Phialides	Spores
Aspergillus sp	Fast growing and heavy sporing	Dirty green	Typically radiate	Typically globose to subglobose
Fusarium sp	Sparse to abundant mycelium, wrinkle in old cultures	White or peach with purple tinge	Simple lateral Phialides	Oval, ellipsoidal cylindrical to straight micro and macroconidia
Penicillium sp	Rapid growing, non-spreading and often wrinkled	Violet	Penicillate ending on Phialides	Mostly globose or ovoid

values (Finney, 1971).

RESULTS

In this study, 3 fungi species were isolated from cadaver of DBM and (Table 1) with incidence rates of 53% (*Aspergillus* sp), 27% (*Fusarium* sp) and 20% (*Penicillium* sp) (Figure 1). All three fungal isolates were pathogenic against the DBM larvae with mortality increasing in a dose-dependent manner. Also, within each population, there were significant differences (P= 0.5) in mortality at different levels of conidial concentration but there were no significant differences in mortality for each treatment across the five populations (Tables 4.2, 4.3, 4.4).

Aspergillus sp was highly pathogenic against 3rd instar DBM larvae inducing up to about 97% mortality. The concentration 3.5 x 10^8 conidial/mL induced the highest mortality in all the five populations. *Penicillium* sp isolate induced 20% - 77% mortality in the field populations (Table 4.3) while *Fusarium* sp isolate induced 23% - 70% mortality (Table 4.4).

The LC_{50} of *Aspergillus* sp for the DBM populations ranged from 6.5 x 10^5 - 13 x 10^5 conidia/mL. The Dzorwulu population had the highest LC_{50} value of 13 x 10^5 conidia/mL while the Operbea population had the lowest LC_{50} value of 6.5 x 10^5 conidia/mL. The LC_{50} of *Penicillium* sp for the DBM populations ranged from 55 x 10^5 - 170 x 10^5 conidia/mL. The Operbea population had the highest LC_{50} value of 170 x 10^5 conidia/mL while the Dzorwulu population had the lowest LC_{50} value of 55 x 10^5 conidia/mL. The LC_{50} of *Fusarium* sp for the DBM populations ranged from 35 x 10^5 - 120 x 10^5 conidia/mL. The Operbea population had the highest LC_{50} value 120 x 10^5 conidia/mL whiles the Haatso population had the lowest LC_{50} value of 35 x 10^5 conidia/mL. The LC_{50} values of the population were not significantly different since their 95% FL overlapped. The probit plots show very low slopes indicating that the populations were close to homogeneity (Table 5).

Table 2. Effect of *Aspergillus* sp on 3rd instar DBM larvae by topical application.

Concentrations (conidial/mL)	Mean ± SE % mortality at 72 hrs				
	Dzorwulu	**Haatso**	**Korle-Bu**	**Operbea**	**Uni. Farms**
Control	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
3.5×10^4	23.3 ± 0.33	30.0 ± 0.58	26.7 ± 0.67	33.3 ± 0.33	26.7 ± 0.33
3.5×10^5	40.0 ± 0.58	46.7 ± 0.88	40.0 ± 0.58	43.3 ± 0.88	43.3 ± 0.88
3.5×10^6	53.3 ± 0.33	53.3 ± 0.88	53.3 ± 0.67	56.7 ± 0.88	53.3 ± 0.88
3.5×10^7	70.0 ± 0.58	73.3 ± 0.67	76.7 ± 0.88	76.7 ± 0.88	76.7 ± 0.33
3.5×10^8	96.7 ± 0.88	86.7 ± 0.33	93.3 ± 0.33	93.3 ± 0.67	93.3 ± 0.67

LSD (P = 8.25)

Table 3. Effect of *Penicillium* sp on 3rd instar DBM larvae by topical application.

Concentrations (conidial/mL)	Mean % mortality (± SE) at 72 hrs				
	Dzorwulu	**Haatso**	**Korle-Bu**	**Operbea**	**Uni. Farms**
Control	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
3.5×10^4	30.0 ± 0.58	26.7 ± 0.33	23.3 ± 0.33	23.3 ± 0.33	20.0 ± 0.58
3.5×10^5	33.3 ± 0.33	30.0 ± 0.58	33.3 ± 0.33	30.0 ± 0.58	40.0 ± 0.58
3.5×10^6	43.3 ± 0.82	36.7 ± 0.33	43.3 ± 0.33	43.3 ± 0.82	46.7 ± 0.67
3.5×10^7	60.0 ± 0.58	63.3 ± 0.82	60.0 ± 0.58	50.0 ± 0.58	56.7 ± 0.33
3.5×10^8	73.3 ± 0.33	76.7 ± 0.82	73.3 ± 0.88	66.7 ± 0.67	70.0 ± 1.16

LSD (P = 7.81)

Table 4. Effect of *Fusarium* sp on 3rd instar DBM larvae by topical application.

Concentrations (conidial/mL)	Mean % mortality (± SE) at 72 hrs				
	Dzorwulu	**Haatso**	**Korle-Bu**	**Operbea**	**Uni. Farms**
Control	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
3.5×10^4	23.3 ± 0.33	26.7 ± 0.33	30.0 ± 0.58	23.3 ± 0.33	23.3 ± 0.33
3.5×10^5	40.0 ± 0.58	36.7 ± 0.33	33.3 ± 0.33	30.0 ± 0.58	43.3 ± 0.88
3.5×10^6	46.7 ± 0.67	53.3 ± 0.88	43.3 ± 0.88	43.3 ± 0.67	50.0 ± 0.58
3.5×10^7	53.3 ± 0.88	63.3 ± 0.88	60.0 ± 0.58	56.7 ± 0.33	53.3 ± 0.88
3.5×10^8	66.7 ± 0.33	70.0 ± 0.58	66.7 ± 0.67	66.7 ± 0.67	66.7 ± 0.67

LSD (P = 7.94)

Table 5. Response of 3rd instar DBM larvae to *Aspergillus* sp, *Penicillium* sp and *Fusarium* sp by topical application

Population	N[1]	LC_{50} (Conidia/mL)	Slope ± SE	95% FL[2]
***Aspergillus* sp**				
Dzorwulu	150	13×10^5	0.53 ± 0.15	1.2×10^5 - 8.0×10^6
Haatso	150	8.7×10^5	0.39 ± 0.14	1.0×10^4 - 9.1×10^6
Korle-Bu	150	10×10^5	0.50 ± 0.15	7.0×10^4 - 6.8×10^6
Operbea	150	6.5×10^5	0.45 ± 0.15	1.8×10^4 - 4.7×10^6
University farms	150	11×10^5	0.45 ± 0.14	5.1×10^4 - 9.8×10^6
***Penicillium* sp**				
Dzorwulu	150	55×10^5	0.30 ± 0.13	5.4×10^4 – 7.1×10^9
Haatso	150	61×10^5	0.36 ± 0.14	2.9×10^5 – 4.0×10^8
Korle-Bu	150	69×10^5	0.34 ± 0.13	2.8×10^5 – 9.3×10^8
Operbea	150	170×10^5	0.29 ± 0.13	5.9×10^5 - 1.9×10^8
University farms	150	68×10^5	0.31 ± 0.13	1.5×10^5 – 4.4×10^9
***Fusarium* sp**				
Dzorwulu	150	89×10^5	0.27 ± 0.13	4.1×10^4 – 1.0×10^8
Haatso	150	35×10^5	0.29 ± 0.13	9.6×10^3 – 1.3×10^9
Korle-Bu	150	77×10^5	0.26 ± 0.13	1.6×10^4 – 1.0×10^8
Operbea	150	120×10^5	0.31 ± 0.13	4.4×10^4 – 1.0×10^{11}
University farms	15	69×10^5	0.26 ± 0.13	1.4×10^3 – 1.0×10^8

N1 = Total number of larvae
FL2= Fiducial limit

DISCUSSION

This study isolated and identified three fungi species from DBM including *Aspergillus* sp, *Penicillium* sp and *Fusarium* sp. The fungi isolated and identified from this study confirmed other studies. Thayib (1983) collected diseased larvae of cabbage head worm, *Crocidolomia binotalis* Zeller and observed from isolation and microscopic examination that the dead specimens were infected with some bacteria, and fungi from the genera *Aspergillus, Fusarium* and *Penicillium.* Not much has been in terms of the virulence of these pathogens in the management of DBM although they have been isolated from other insects and reported to be virulent against a number of insects.

Balogun and Fagade (2004) isolated *Fusarium* sp, *Aspergillus flavus*, *A. niger* and *Penicillium* sp from *Zonocerus variegatus.* Also, Kaya and Okech (1990) have isolated *Aspergillus flavus*, *A niger*, *Penicillium* sp and *Fusarium* sp from pupae and adults of *Glossina pallidipes*. Norberg *et al*., (1999) also reported the isolation of *Aspergillus fumigatus*, *A. niger*, *Fusarium* sp and *Penicillium* sp from adults of *Musca domestica* while Costa and Oliveira (1998) isolated various species of *Penicillium* from mosquito vectors of tropical diseases.

In the current study, *Aspergillus* sp had the highest incidence rate of 53% followed by *Fusarium* sp 27% and *Penicillium* sp 20%. Sales *et al*., (2002) reported a high prevalence of the genus *Aspergillus* and *Penicillium* from cadavers of *M. domestica*. Gillian and Prest (1972) and Gillian et al. (1974) also reported *Aspergillus flavus* as the most prevalent in their studies.

In determining the effect of the fungi isolates on DBM, *Aspergillus* sp was found to be highly pathogenic against 3rd instar DBM larvae inducing 86.7% - 96.7% mortality. *Penicillium* sp and *Fusarium* sp were also pathogenic inducing 66.7% - 76.7% and 56.7% - 63.3% mortality respectively, at the highest concentration of 3.5×10^8 conidia/mL. The effectiveness of *Aspergillus* sp against insect species has been reported by Batra *et al*., (1973) and Schlein et al. (1985). The authors stated that *Aspergillus sulphureus* strain VGCN1ADP was effective against *Culex quinquefasciatus*, inducing 75% mortality while the strain CPRR8LP was highly effective against two mosquito species; *Culex quinquefasciatus* and *Aedes fluviatilis*, inducing 96% and 100% mortality respectively. Batra *et al*., (1973) and Schlein *et al*., (1985) also reported that two strains of *Aspergillus flavus* (VGCN9E and VGC2P) were effective against *Aedes fluviatilis* causing 100% and 62% mortality respectively. Studies have also shown that mosquito larvae are susceptible to infections by fungi such as *Aspergillus* sp (de Moraes *et al*., 2001), *Aspergillus flavus*, *A. parasiticus*, *Penicillium falicum* and *Fusarium vasinfectum* (Govindarajan *et al*., 2005). *Aspergillus ochraceus*, *A. kanagawaensis* and *A. sulphureus* strains have been reported to be effective against mosquito larvae inducing at least 80% mortality (Powell et al., 1994).

Rachana (2007) reported that, the fungus *Fusarium semitectum* Berk.and Ravenel was effective against the spider mite *Tetranychus neocaledonicus* causing 60% mortality under both laboratory and green house conditions. Balogun and Fagade (2004) have also shown that *Penicillium* sp were effective against *Zonocerus variegatus*, inducing 80% mortality.

The current studies also confirmed the efficacy of *Bacillus thuringiensis* against the DBM larva inducing 70% - 76.7% mortality at the highest concentration 0.2 g/mL. Several other studies (Varma and Gill, 1977; Asokan et al., 1996; Sannaveerappanavar and Viraktamath, 1997; Malathi *et al*., 1999) have shown that *B*. *thuringiensis* was potent against DBM. In Bajio region, Mexico, the use of *B. thuringiensis* resulted in over 50% control of the DBM (Talekar and Shelton, 1993). Similar successes have been reported in Philippines (Morallo Rejesus *et al*., 1996), Singapore (Ng *et al*., 1997) and in Jamaica (Ivey and Johnson, 1998).

There were differences among the LC_{50} values of *Aspergillus* sp, *Penicillium* sp and *Fusarium* sp for the five DBM populations. The DBM populations were more susceptible to *Aspergillus* sp than *Penicillium* sp and *Fusarium* sp. The differences in LC_{50} values also indicate the varying degrees of virulence of the various fungi isolates against the 3rd instar DBM larvae. The genus *Aspergillus* shows a great diversity of species. This genus does not have representatives that are exclusively entomopathogenic but has been the focus of several studies, mainly due to its high potential for biotechnology applications, as well as for its capacity to produce various secondary metabolites with entomopathogenic and/or entomotoxigenic potential (Nnakumusana 1985; Laakso and Gloer 1993; Powell *et al*., 1994; de Moraes *et al*., 2000).

Gupta et al. (1991) also isolated beauvertcin as an insect toxin from *Fusarium semitectum* (Syn. *F. pallidoroseum*) and reported that it showed toxicity against Colorado potato beetle in a foliar spray assay. *Fusarium semitectum* (Syn. *F. pallidoroseum*) is cytotoxic as reported by Grove and Pople (1980) and possesses some insecticidal properties against mosquito larvae and blowfly.

The results of this study show that strains of the genus *Aspergillus*, *Penicillium* and *Fusarium* have potentials as biological control agents against the DBM and should be further studied and mass produced in the sustainable management of DBM.

REFERENCES

Abro GH, Dybas RA, Green ASJ ,Wright DJ (1988). Toxicity of avermectin B1 against a susceptible laboratory strain and an insecticide-resistant strain of *Plutella xylostella* (Lepidoptera: Plutellidae). *J. Econ. Entomol*. 81:1575-1580.

Anderson TE, Roberts DW , Soper RS (1988). Use of *Beauveria bassiana* for suppression of Colorado potato beetle population (Coleoptera: Chrysomelidae) in New York State. *Environ. Entomol*. 17(1): 140-145.

Asonkan R, Mohan KS.,Gopalakrsihnan C.(1996). Effect of commercial formulations of *Bacillus thuringiensis* Berliner on yield of cabbage. *Insect Environ.* 2, 58-59.

Balogun SA, Fagade OE (2004). Entomopathogenic fungi in population of *Zonocerus variegatus* L. in Ibadan, Southwest, Niger. Afric. J. Biotech. 3 (8), 382-386.

Batra LR, Batra SWT ,Bohart GE (1973). The mycoflora of domesticated and wild bees (Apoidea). *Mycopath. et mycol. Appl.* 49, 14-44.

Carter DJ (1984). Pest Lepidoptera of Europe with special reference to the *British Isles. Series Entomol*. 31: 81–83.

Costa GL ,Oliveira PC (1998). *Penicillium* species in mosquitoes from two Brazilian regions. J. Basic Microbiol. 38, 343-347.

de Moraes AML, da Costa GL, de camargo-Barcellos MZ, de Oliveira RL , de Oliveira PC (2001). The entomopathogenic potential of Aspergillus spp. in mosquitoes vectors of tropical diseases. J. Econ. Entomol. 93, 963- 970.

Finney.D.J.(1971). Probit Analysis. Cambridge University, London.

Gillain M, Prest DB, Morton HL (1974). Fungi isolated from Honey Bees, *Apis mellifera*, fed 2,4-D and antibiotics. J. Invert. Pathol. 24, 213-217.

Gillian M ,Prest DB (1972). Fungi isolated from the intestinal contents of foraging worker honey bees, *Apis mellifera.* J*. Invert. Pathol.* 20, 101-103.

Gupta S, Krasnoff SB, Underwood NL, Renwick JAA ,Roberts DW (1991). Isolation of bauvericin as an insect toxo=in from Fusarium semitectum and Fusarium moniliforme var. subglutinans. Mycopathol. 115 185-189.

Hardy JE (1938). *Plutella maculipennis*, (Curt.), its natural and biological control in England. *Bull. Entomol. Res*. 29: 343-372.

Humber RA (1998). Entomopathogenic fungal identification. APS/ESA Workshop. November 7, 1998. APS/ESA joint annual meeting, Las Vegas, NV. 26 pp.

Ivey PW ,Johnson SJ (1998). Integrating control tactics for managing cabbage looper (Lepidoptera: Noctuidae) and the diamondback moth (Lepidoptera: Plutellidae) on cabbage. *Trop. Agric*. 73(3)., 369-374.

Kaya GP ,Okech MA (1990). Microorganisms associated with tsetse in nature; Preliminary results on isolation identification and pathogenicity. *Insect Sci. Appl.* 11, 443-448.

Kumar R (1986). Insect Pest Control with special reference to African Agriculture. ELBS/Edward Arnolds, 298pp.

Laakso JA ,Gloer JB (1993). A new penitrum analog with antiinsectan activity from the sclerotia of *Aspergillus sulphureus. J. Agric. Food Chem.* 41, 973-975.

Lomer C, Lomer C (1996). Lubilosa technical Bulletins 1 - 7.

Malthi S, Sriramulu M ,Babu TR (1999). Evaluation of certain eco-friendly insecticides against lepidopterous pest of cabbage. *Indian J. Entomol*. 61(2), 127-133.

Mohan M.,Gujar GT. (2003). Larval variation in susceptibility of the diamondback moth, *Plutella xylostella* (L.) to insecticides and detoxification enzymes. *Crop Prot*. 22: 495–504.

Morallo RB, Inocencio EL., Eusebio JE (1996). Comparative effectiveness of IPM-DBM technology versus farmers' control practice for diamondback moth, *P. xylostella* L. control. *Phil. Entomol.* 10, 57-65.

Ng BB, Ong KH, Teo LM ,tang MP (1997). Evaluation of integrated pest management strategies for the control of diamondback moth in leafy vegetables. *Singapore J. Pri. Industries*. 25, 33-42.

Nnakumusa ES (1985). Laboratory infection of mosquito larvae by entomopathogenic fungi with particular reference to *Aspergillus parasiticus* and its effects on fecundity and longevity of mosquitoes exposed to conidial infections in larval stages. *Curr. Science.* 54, 1221-1228.

Norberg AN, Queiroz NMC, Maure EAP, Toledo RF, Gazeta GS, Norberg CMBM , Guimaraes RR (1999). Vectocao de fungos por moscas sinantropicas coletadas em hospitais, sestaurantes e fieras da Baixada Fluminense , Rio de Janeiro, Brasil. In XIV Congreso Latinoamericano de Parasitologia, Acapulco Mexico pp 84.

Pell JK, Eilenberg J, Hajek AE, Steinkraus DS (2001). Biology, ecology and pest management potential of Entomophthorales. In: Fungi as biocontrol agents: progress, problems and potential (Butt, T.M., Jackson, C. and Magan, N. Eds). CABI International, pp. 71-154.

Pionar GO Jr, Thomas GM (1984). Laboratory guide to insect pathogens and parasites. Plenum Press. New York and London. pp 105-109.

Powel KA, Renwick A and Perbody JF (eds) (1994). The Genus Aspergillus: From Taxonomy and Genetics to Industrial Application. Plenum Press, New York.

Rachana RR (2007). Bio-ecology of red spider mite *Tetranychus neocaledonicus* (Andre) (Acari: Tetranichidae) and its management using fungal pathogens on okra. MSc. (Agri.) Thesis. Univ. Agric. Sci. Bangalore (India).

Schlein Y, Polachek I, Yuaval B (1985). Mycosis, bacterial infections and antibacterial activity in sandflies (Pyschodidae) and their possible role in the transmission of leischmaniasis. *Parasitol*. 90, 57-66.

Sunnaveerappanavr VT, Viraktamath CA (1997). Management of insecticides resistant diamondback moth, Plutella xylostella L. (Lepidoptera: Yponomeutidae) Mysore J. Agric. Sci. 31(3), 230-235.

Talekar NS ,Shelton AM (1993). Biol., Ecol. and management of the diamondback moth. *Ann. Rev. Entomol*. 38:275-301.

Thayib MH (1983). Investigations on the bionomics of cabbage insect pest *Crocidolomia binotalis* Zeller, Lepidoptera: Pyrallidae. Ph.D. Thesis, Univ. of Gajah Mada, Yogyakarta. 181 p. (in Indonesian with English Summary).

Vandenberg JD, Shelton AM, Wilsey WT , Ramos M (1998). Assessment of *Beauveria bassiana* sprays for control of diamondback moth (Lepidoptera: Plutellidae) on crucifers. *J. Econ. Entomol*. 91: 624-630.

Varma GC,Grill GS (1977). Laboratory studies on the comparative efficacy of the biotic insecticides for the control of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *J. Res.* Punjab Agriculture University. 14, 304-308