

**SURVEY AND EVALUATION OF NUCLEAR POLYHEDROSIS VIRUS
(NPV) FOR THE CONTROL OF *Helicoverpa armigera* (Hübner)
(LEPIDOPTERA:NOCTUIDAE) ON SELECTED VEGETABLE CROPS
IN KENYA. //**

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**A THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE
DEGREE OF MASTER OF SCIENCE (AGRICULTURAL
ENTOMOLOGY) OF KENYATTA UNIVERSITY**

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*Survey and
evaluation of*



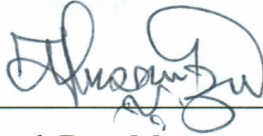
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DECLARATION BY THE CANDIDATE

This thesis is my original work and has not been presented for a degree in any other University.



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DEDICATION

To my wife Merceline, my daughter Amina and my parents for their continued support and encouragement

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ABSTRACT

Samples of *Helicoverpa armigera* larvae obtained from several sites and crops in Kenya in surveys carried out during June 1998 to October 1999 were examined at ICIPE, Nairobi. They revealed natural infection by nuclear polyhedrosis virus (NPV). Sixteen isolates were collected altogether. In laboratory bioassays on field-collected third instar larvae fed on contaminated diet with two local NPV isolates and the Indian (ICRISAT) isolate, the cumulative percent larval mortality did not differ significantly but the local isolates were apparently superior. Percentage larval mortality recorded 9 days after inoculation for the Kenyan isolates were $78.3 \pm 7.38\%$ and $68.3 \pm 5.68\%$ respectively, compared to $55.9 \pm 2.10\%$ for the Indian isolate. Larval mortality was found to be dose-dependent and increased with the larval instars. The Kenyan isolate 1 (Kibwezi isolate) had a higher slope of 0.685 ± 0.1480 compared to 0.0981 ± 0.14680 for the ICRISAT isolate while the LD_{50} values were 1.59976×10^{10} OBs/ml and 3.178×10^{13} OBs/ml respectively by probit analysis at 95% fiducial limits. In age-response studies with laboratory-reared larvae, mortality was found to decrease with later instars. Cumulative percent mortality of second instar larvae reached $78.3 \pm 2.2\%$ by the third day, $93.3 \pm 1.36\%$ by day 6 and 100% by day 8. In pot culture tests, when aqueous suspension sprays of native HaNPV were applied on pigeon pea and snowpea potted plants artificially infested with third instar *H. armigera* larvae, significant reduction in larval damage to pods and seeds were obtained. The grain yield per

plant was also at par with yields of plants sprayed with two chemical pesticides, Lambdacyhalothrin (Karate[®]) and Bifenthrin (Brigade[®]). Replicated small plots testing on vegetable pigeon pea also confirmed the potential of native HaNPV in providing satisfactory reduction in pest damage leading to grain yield benefits comparable to the chemical pesticide (Lambdacyhalothrin) spray. Molasses (1%) increased viral activity both in the laboratory, pot-culture and field tests compared to 1% charcoal powder. Further efforts should be made to test the biological potential, characterize, improve and demonstrate the potential of native HaNPV for augmentation biocontrol of *H. armigera* in Kenya.

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List of abbreviations**B. t.** – *Bacillus thuringiensis***CGIAR** – Consultative Group of International Agricultural Research**DNA** – Deoxyribo Nucleic Acid**ELISA** – Enzyme Linked Immuno Sorbent Assay**GV** – Granulosis Virus**HaNPV** – *Helicoverpa armigera* Nuclear Polyhedrosis Virus**HCDA** – Horticultural Crops Development Authority**I.C.I.P.E.** – International Centre of Insect Physiology and Ecology**ICRISAT** – International Crops Research Center for the Semi-Arid Tropics**IPM** – Integrated Pest Management**KARI** – Kenya Agricultural Research Centre**KISE** - Kenya Institute of Special Education**LD** – Lethal Dose**LT** – Lethal time**MNPV** – Multiple Nucleocapsid Virus**NPV** - Nuclear polyhedrosis virus**OB** – Occlusion Body**PCR** - Polymerase Chain Reaction**SNPV** – Single Nucleocapsid Virus**SP-IPM** – System wide Programme for Integrated Pest Management**USA** – United States of America

CHAPTER ONE

1.0 GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Vegetable production is emerging as an important income generating activity in Kenya as a result of the increased year round production by small-scale farmers who have access to supplementary irrigation. A recent estimate by Horticultural Crops Development Authority (HCDA) has shown that, about half a million small-scale farmers benefit from this enterprise in the country (HCDA, 1998). The volume of production in 1997 was estimated at 30.9 tonnes valued at 52.2 million US dollars (HCDA, 1998). Smallholder farmers earn much higher price per unit produce harvested than in other crops, thus resulting in increased profit margins, which permits them to invest more on the inputs like fertilizers and pesticides.

By 1994, reports from the Kenya Ministry of Agriculture, Livestock Development and Marketing (MOALDM) indicated that 46.8% of the land under horticultural crops was under vegetables. The major vegetable concentration areas include: around Nairobi, Machakos (especially along Athi River), Kiambu, Thika, Murang'a, Embu, Meru, Isiolo, Nakuru, Kirinyaga, Njoro, Naivasha, and Limuru (Ouko, 1997). Other areas growing export vegetables and others with potential, major vegetables produced and volumes of export vegetables are furnished in Appendices 1 and 2.

Kenya's climate and soils are not only excellent for horticulture production but also offer ideal environment for pests and diseases which often

reduce crop yield and quality (Anon., 1997). Yield losses due to insect pests have been reported in the East African region. They are known to vary with locations and seasons, often ranging between 20-50%. Fruitborers, especially, *H. armigera*, are recognized as an important constraint to vegetable production in Africa (Ikin *et al.*, 1993) causing direct and indirect damage to some malvaceous and solanaceous vegetables. In Kenya, *H. armigera* is a serious insect pest in the production of the income generating vegetables, especially tomatoes, chillies, okra, eggplant, peas and sometimes Frenchbeans (Farrel *et al.*, 1995; Wabule, 1997). This pest has also been listed among other pod borers as a major pest on pigeon pea (Okeyo-Owour, 1978; Sithanatham and Reddy, 1986; 1990). Research conducted by the International Centre of Insect Physiology and Ecology (ICIPE) scientists around Mbita Point (western Kenya) in 1997 reported an estimate of about 24% damage by *H. armigera* on tomatoes (ICIPE, 1997). Similarly, Sithanatham *et al.* (1998) reported the borer as a serious pest on capsicum and okra in Nguruman (among other fruit borers). Oduor *et al.* (1998) reported significant yield losses by *H. armigera* on tomato in Kiambu.

Production of vegetable crops in Kenya, as well as the quality, is not only being maintained but enhanced. Most vegetable produce are high value crops and even minor damage from arthropod pests can lead to significant reduction in market value (Anon., 1997). Vegetables grown for export market need special attention due to the demand for produce that is free from insect damage (even cosmetic damage) as well as harmful levels of pesticide residues. Use of safe and sustainable crop protection practices is essential in

maintaining the increase in production and high quality. Pest management is an integral part of production and post-harvest packages in Kenya's horticulture to produce a final product of premium quality without any blemishes or residues (Ouko, 1997). Ikin *et al.* (1993) reported that the sustainability of horticultural cultivation will be achieved through selective use of environmentally safe options and rational use of pesticides which are compatible with the stringent limits set for pesticide residue levels on fresh produce.

In the developing countries, major emphasis is placed on reducing pest populations through the indiscriminate use of chemical pesticides by both commercial and subsistence farmers although returns are minimum (Ikin *et al.*, 1993). Vegetable farmers tend to rely heavily on the use of chemical pesticides and this results in reduction of efficacy and increase control costs. At present, most farmers are getting concerned about the relatively greater costs in pest control that consequently reduces their profit margin. Thus, there is need for other alternatives that are cheaper and sustainable in order to arrive at profitable management of pests on the vegetables as well as other farm produce. Alternative pest management options such as host-plant resistance, cultural practices, crop rotation, field sanitation, botanicals and biological control should be evaluated with the aim of developing sustainable pest management systems for vegetables and other crops.

Small-scale vegetable farmers in Kenya consider insect pests as a major factor affecting the production and marketing of their produce. In a survey carried out in three districts by GTZ-IPM Project, farmers growing

Frenchbeans, tomatoes and brassicas ranked crop protection as first among different constraints affecting vegetable production (Gathui *et al.*, 1994). Ninety-three percent (93%) of the farmers reported the use of pesticides to protect their crops against pests and prevented loss of harvest which they believed would be as high as 90% (Gathui *et al.*, 1994). This clearly indicates the unilateral dependence on pesticides for producing acceptable crop produce for both local and export market. Since vegetables grown for export market fetch premium market prices if they are free from cosmetic damage, smallholder growers are largely motivated to intensively protect their crops. Quite often, these farmers use their own doses or those suggested by agrochemical retailers, largely due to lack of adequate or timely extension advice. The excessive (indiscriminate) use of pesticides is also attributed to the fact that, choice of insecticide is mainly determined by its availability and cost than specificity. They mostly resort to over reliance on chemical pesticides and this has drawbacks such as destruction of natural enemies, other beneficial insects, increased pest resistance to pesticides and the aggravation of the pest problems (Anon., 1997). In Kenya, the growers of export vegetables are also subjected to increasing pressure by consumers to minimize pesticide use on their crops, to avoid the problem of residues. Use of illegal chemicals and high concentrations of pesticide residues on the produce are therefore a serious threat to the farmers. The export markets, especially in Europe, are becoming increasingly aware of the environmental concerns and are making demands on the industry to move towards a more ecologically rational approach to pest management (Anonymous, 1997). Due to overdependence on chemical

pesticides, very little is being done about the use of other methods such as cultural practices, biological control or integrated pest management.

Pesticide resistance in insects is a common phenomenon. Its occurrence in *H. armigera*, and even in other vegetable pests [such as the diamondback moth (*Plutella xylostella*), whiteflies (*Bemisia tabaci*), leaf miners (*Liriomyza* spp), beanfly (*Ophiomyia* spp), aphids and spider mites] in Eastern Africa has been reported (Georghiou, 1981, 1986; Ikin *et al.*, 1993). Hence, the need for the development of rational and sustainable pest management systems is of great importance. Management strategies to keep pest infestations below economically damaging levels should be devised using the most appropriate combination of biological, mechanical and chemical methods, i.e. integrated pest management (IPM). They should be based on a thorough understanding of the situation, taking into account the previous experience of pest incidence and crop susceptibility, weather conditions, regular crop inspection to identify and assess pest levels and use of established thresh-holds. A number of IPM components are known and occasionally used with some positive effects such as seed-bed sterilization, crop sanitation, crop rotation, resistant varieties, planting trends/dates, mixed cropping and trap crops. Several biological control agents and activities to promote natural enemies have also been recommended (Wabule, 1997).

The International Centre of Insect Physiology and Ecology (ICIPE) is promoting biological pest control method as part of its TechnoPark project intended to commercialize its research results. This entails promoting the use of natural enemies such as predators, parasitoids and pathogens like viruses,

bacteria and fungi that cause insect diseases. It has over the years identified several natural enemies that are active against a wide range of arthropod pests as biocontrol agents including microbial pesticides. The production of the biopesticides is in conformity with the highest standards worldwide as the ISO 9000 series (Dr. Ochieng-Odero, pers. com.). This study is part of ICIPE's goal of developing biocides to be used in IPM measures. Biopesticides have been in use in Europe since 1900 though large-scale use of natural enemies of crop pests spans about 30 years (Anon., 1997).

Baculoviruses are desirable alternatives to chemical pesticides for the control of insect pests because of their compatibility with the environment and easy application by use of conventional spraying techniques (Stewart *et al.*, 1991; Cunningham, 1982; Huber, 1986). They have been seriously considered as biocontrol agents since they are virulent to insects but do not replicate in vertebrates. This provides the necessary safety insurance aspect (Dent, 1991; Adams, 1991). Evaluations carried out on nuclear polyhedrosis viruses (NPVs) in field crops, including vegetables, in the USA, Canada, Europe, Asia and South Africa show their potential for the field control of caterpillar insect pests. There is therefore, a scope to develop pest control systems using NPVs in Kenya.

1.2 Literature review

1.2.1 The African bollworm, *Helicoverpa armigera* (Hübner)

1.2.1.1 Occurrence and distribution

Helicoverpa (*Heliothis*) species are widely distributed throughout the tropics and subtropics (Manjunath *et al.*, 1985). *H. armigera* (Hübner) is distributed all over Africa, Southern Europe, the Middle East, India, Central and Southern Asia, Japan, the Philippines, Indonesia, New Guinea, Australia, New Zealand and some Pacific islands (Annecke and Moran, 1982). The polyphagous nature and wide geographical spread of some of *Helicoverpa* (*Heliothis*) species and economic importance merit their consideration at the international level (Hardwick, 1965; Reed and Pawar, 1982).

1.2.1.2 Host plants and pest status

Helicoverpa species are among the most dreaded agricultural pests and are considered to be the world's most destructive insects because they have defied human efforts to check their spread and consequent economic damage on several important crops (Manjunath *et al.*, 1985). By 1981, *H. armigera* was recorded damaging 60 cultivated plants across Africa, Asia and Australia, and these are likely to be only a fraction of the total number of plants which it feeds on (Reed and Pawar, 1982). Its host species belong to a broad spectrum of families including important agricultural crops such as cotton, maize, chickpea, pigeon pea, sorghum, sunflower, soyabean and groundnuts (Fitt, 1989). In Africa, *H. armigera* is one of the most important and highly

polyphagous as well as injurious pest of agricultural crops and home gardens (van den Berg and Cock, 1993; Kfir, unpublished). In Eastern Africa it is a sporadic serious pest of cotton, tobacco, maize, sorghum, millet, beans, pigeonpea and other legumes, vegetables (especially tomatoes), sunflower and citrus (Annecke and Moran, 1982; Karel, 1985; Greathead and Girling, 1985; Sithanantham and Reddy, 1986, 1990; Minja *et al.*, 1996). In Kenya, it is one of the most important pests and attacks a wide range of crops including many different weeds (van den Berg and Cock, 1993). Recent observations have shown that this pest also attacks export vegetables such as peas beans, capsicum, okra and export flower crops such as carnations (van den Berg and Cock, 1993; Farrel *et al.*, 1995; HCDA, 1996; Sithanantham *et al.*, 1997), besides tomatoes (Oduor *et al.*, 1998). The ability of the female to locate and utilize a wide range of hosts from a number of families as oviposition sites is one of the major factors contributing to its present pest status (Zalucki *et al.*, 1986; Fitt, 1989). It is also known to be resistant to many chemical insecticides commonly used in Eastern and Southern Africa (Hill and Waller, 1988).

1.2.1.3 Biology of *Helicoverpa armigera*

The biology of this pest in the tropics was described by Hill and Waller (1988) and in Eastern Africa by van den Berg and Cock (1993). Spherical eggs, about 0.5 mm in diameter, white-yellow turning brown (Plate 1) are laid singly. They are stuck onto the host plant (preferably on the fruiting and flowering parts or leaves) and hatch after 2-4 days. Older eggs are more difficult to locate against the dark plant surface. They have a distinct ridge

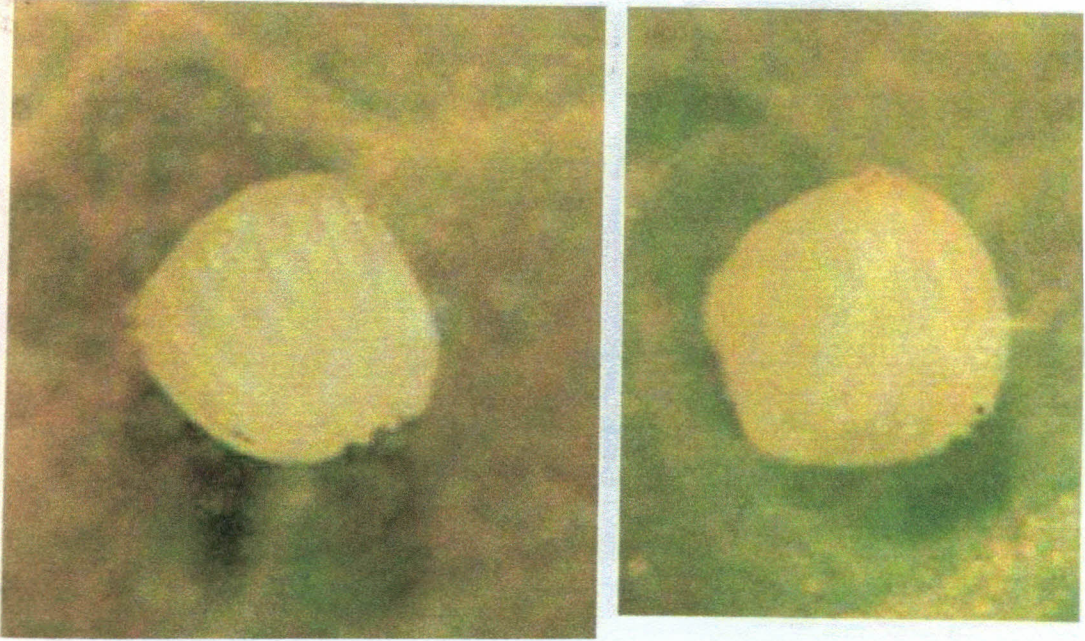
structure and a raised micropyle on top and can be identified in the field with a hand lens. Each female may lay 1000 eggs or more in her life span of about two weeks. On hatching, the larva consume part of the eggshell and then look for suitable food. There are six larval instars and larvae are extremely variable in colour and patterns, greenish to white-yellow or brown to entirely dark brown or blackish, with several dark and pale longitudinal bands and short hairs (Plate 2). Fully-grown larvae (5th-6th instar) may be about 40-45 mm long. The total larval period lasts between 14-24 days depending on temperature and type of food consumed. Pupation occurs in earthen cells in the soil and is completed in 10-14 days in the tropics but in cooler climates, they overwinter. The pupa (Plate 3) measures about 16-20 mm long. A complete life cycle can be as short as 28 days in the tropics with approximately 5-6 generations annually. The adult (Plate 4) is a stout-bodied dull-brown to buff nocturnal moth (about 2 cm long in a resting position) with a wingspan of about 40 mm-44 mm. The hind wing is pale and has dark-dotted border.

1.2.1.4 Damage and yield loss

Young larvae cause damage by feeding on soft plant parts rich in protein (especially buds and flowers) and on young pods but the main damage is caused by old larvae burrowing into green pods and eating developing seeds (Karel, 1997). Larvae or caterpillars also feed on leaves and buds in some host plants. Normally, they consume only a small portion of the flower or fruit and move to the next, leaving a trail of damaged flowers or fruits that produce no

harvest (van den Berg and Cock, 1993). Secondary rots may develop within buds or fruits of vegetables like tomatoes after the insect attack. Voracious larval feeding leads to substantial economic loss (Reed and Pawar, 1982). *H. armigera* has a wide range of alternative host plants and can cause substantial direct, indirect and cosmetic damage and consequently lead to losses in the quality and quantity within different crops. Research by ICIPE scientists in 1997 around Mbita revealed a damage estimate of about 24% by *H. armigera* on tomatoes (ICPE, 1997). Sithanatham *et al.* (1998) reported *H. armigera* (among other fruit borers) as a serious pest on capsicum and okra in Nguruman while Oduor *et al.* (1998) reported significant yield losses by the pest on tomato in Kiambu. Silvie and Goze (1991) estimated over 50% loss of cotton in Chad whereas Srinivasan (1985) reported a yield loss as high as 20-50% on tomatoes in India.

Plate 1: *Heicoverpa armigera* eggs X60



Heicoverpa armigera adults, male (left) and female (right)

Plate 2: *Heicoverpa armigera* larva



Plate 3: *Heicoverpa armigera* pupa



Plate 4 : *Heicoverpa armigera* adults, male (left) and female (right)



1.2.1.5 Management of *H. armigera*

1.2.1.5.1 Chemical control

In Eastern Africa, control of *H. armigera* using chemicals such as DDT, (Benzene hexachloride (BHC) or a mixture of DDT and BHC, carbaryl, cypermethrin and endosulfan was initially effective (Swaine, 1969; Karel *et al.*, 1981; Karel, 1985) but presently pyrethroids such as permethrin and cypermethrin are increasingly being used. DDT has been banned and BHC restricted. Prior to spraying, field scouting is required to monitor eggs and larvae in order to have timely spraying aimed at killing the first instar larvae. Widespread occurrence of resistance to chemical insecticides, including the widely used pyrethroids, in the late 1980s caused an increase in losses due to this pest and has made control by chemicals increasingly unreliable and expensive (Kibata, pers. com.). In recent years, the chemical resistance problem has worsened, resulting in further increases in estimated crop losses due to this pest. For instance, in India, in 1996-97 the losses were estimated to be 158 million US\$ (Russel, 1999), causing widespread hardship especially among poor farmers. This has prompted efforts to adopt alternative control methods which include the use of insect viruses (Cherry *et al.*, 1999).

There is evidence of development of resistance in *H. armigera* populations in the East African region (Georghiou, 1981, 1986; Hill and Waller, 1988). In Kenya, during the 1980s synthetic pyrethroids became popular pesticides for bollworm control on cotton as they were extremely effective and there was ample evidence that carbaryl and DDT no longer conferred the expected suppression of the bollworms (Kibata, pers. com.).

During the same period, the increased use of pyrethroids demonstrated that minor pests such as red spider mites on cotton immediately gained prominence and became a wide spread problem (Murega *et al.*, 1985). It was demonstrated that the continued sole dependence on pesticides for pest control in cotton would not only result in negative returns for the farmer but would eventually be deleterious to the environment (Nandwa, 1984).

1.2.1.5.2 Cultural control

Cultural practices such as destruction of residues, uniform and early planting together regular weeding have been recommended for the control of caterpillars but are seldom used to control *H. armigera* in Kenya. The use of resistant varieties has also been recommended and host plant resistance easily blends with other cultural practices such as regular weeding. It has been used elsewhere with considerable success against insect pests of some crops such as pigeonpea (Verulkar *et al.*, 1997) and sorghum (Azrag *et al.*, 1993; Sharma, 1993). In cotton, many resistant varieties have been developed and used against *H. armigera*. For instance, the highest mortality and lowest weight were recorded on young larvae that fed on cotton genotypes (upland cotton) with a high gossypol gland density on the ovary surface (Mohan *et al.*, 1996a) and early maturing cottons (Cook and Sosa, 1995). Intercrops, trap and companion crops have been tested using crops such as sorghum, maize, sunflower and beans planted in cotton fields and vice versa, (Abate, 1988, 1991; El-Heneidy and Sekemate, 1996; Karel, 1993). These methods coupled with regular weeding have also been tested (Abate, 1991). Other trap crops

tested with considerable success include use of *Xemenia americana* (Ajayi, 1991) and the African marigold (Srivastava *et al.*, 1994). Similarly, use of insecticides with mulching was successfully tested by Uvah *et al.* (1989). Botanicals have also been tested. For instance, neem equally gave good control (Renou *et al.*, 1987; Fagoone, 1987).

In Eastern Africa, use of sex pheromone traps has been recommended for scouting of eggs, larvae or adults for effective cotton spraying in Western Tanzania (Nyambo, 1988,1989a,b). This has been applied elsewhere with considerable success (USA and Asia). Pheromone trap network for *H. armigera* is well developed in India and constitutes an integral part in the management of this pest at many locations across the subcontinent (Srivastava and Srivastava, 1995).

1.2.1.5.3 Biological control

The role of biological control in the regulation of pests in a natural system is well known. Biological control involves the use of predators, parasitoids and insect pathogens (microbial pesticides). The rate of parasitism, predation and mortality due to pathogens has been observed to be high in the fields where no pesticide application is practised (Bhatnagar *et al.*, 1982). Several larval parasitoids of *H. armigera* have been recorded in Africa (Reed, 1965). A number of predators that attack *H. armigera* have also been reported *in the field* (van den Berg, 1993).

Successful records with parasitoids include the use of the egg parasitoids *Trichogramma* spp (Trichogrammatidae) (Bhatnagar *et al.*, 1982; Hassan,

1988; Huo *et al.*, 1988; Jong *et al.*, 1990; Mimouni, 1991; Silva *et al.*, 1991; Kumar *et al.*, 1994) in India. Success was also reported with *Brinckochrysa scelestes* in India at 50,000 individuals/ha 120 days after cotton sowing (Dhandapani *et al.*, 1992) and *Microplitis croceipes* (Cresson).

Predators include *Chrysoperla carnae* Steph. (Chrysopidae) tested in China and India (Glazer *et al.*, 1992) and the tachinid, *Goniophththalmus halli* Mesnil. Sex pheromones together with parasitoids, have also been used against this pest in Asia (Manjunath, 1993).

In Eastern Africa, several predators and parasitoids have been reported to cause significant mortality of *H. armigera* in the field (Nyambo, 1990; van den Berg and Cock, 1993, van den Berg *et al.*, 1995; 1997; Kabissa, 1996) but their large scale field use has not yet been tried.

1.2.1.5.4 Microbial control

Bacillus thuringiensis subsp. *kurstaki* (Berliner), *aizawai*, *israeliensis* and *kenyae* have been used in Asia and USA with variable success against *Helicoverpa* spp, especially *H. armigera* (Kulkarni and Amonkar, 1988; Khaliq *et al.*, 1989; Navon *et al.*, 1990; Broza and Sneh, 1994; Hou, 1997). Products based on this biopesticide are already available in the Kenyan market and are currently being evaluated. *B. thuringiensis* is known to be effective against many lepidopteran species and has a large market potential. Transgenic plants containing the cryIA(c) gene incorporated in them have been developed and inhibit the development of the *H. armigera* larvae (Sanchayita *et al.*, 1997). Protozoa in the order Microsporidia that have also

been tested with varied success include the following: *Nosema litura* sp. (Li and Wenn, 1987) in China, *Vairimorpha necatrix* Kramer (Wen *et al.*, 1992) in China and USA and *V. plodia* in England. Similarly, nematodes of the genera *Steinernema* (or *Neoaplectana*) and *Heterorhabditis* have shown considerable success in controlling *H. armigera* larvae (Glazer and Navon, 1989; Vela *et al.*, 1998). These include *Neoaplectana* (*Steinernema*) *carpocapsae* Weiser (Gupta *et al.*, 1987) in India, *Neoaplectana feltiae* (Glazer and Navon, 1989) in Israel and *S. glaseri* (Glazer, 1992). For all of them, the fastest invasion was recorded in small larvae. Fungal pathogens have shown potential in controlling *H. armigera* larvae. *Beauveria bassiana* (Balsamo) Vuillemin tested under field conditions was found to be very effective in India (Gopalakrishnan and Nayaran, 1990; Saxena and Ahmad, 1997).

Nuclear polyhedrosis virus (NPV) as a biocide (Elcar[®]) has been in use against *Helicoverpa* spp on cotton in California for many decades (Bell, 1982). Tests have been carried out on other crops including vegetables in other countries. In India, field efficacy of HaNPV against *H. armigera* affecting vegetable crops such as tomatoes (Narayanan and Gopalakrishnan, 1988; Mohan *et al.*, 1996b; Gopalakrishnan and Asokan, 1998) and pigeonpea (Dhandapani *et al.*, 1993a; Narayan and Gopalakrishnan, 1987) was proved. This biological control agent is desirable due to its compatibility with the environment and its amenability for application by spray techniques (Cunningham, 1982; Stewart *et al.*, 1991; Bijjur *et al.*, 1991b). Tests using NPV in combination with botanicals and chemical pesticides have been carried out with considerable

success on various crops (Santharam *et al.*, 1992; Abbas, 1987; Silvie *et al.*, 1993; Sarode *et al.*, 1995; Rajasekhar *et al.*, 1996; Zhang *et al.*, 1996). Tests in combination with larval parasitoids such as *Trichogramma* spp (Abbas, 1987; Balasubramanian *et al.*, 1989; Munir *et al.*, 1992; Kumar *et al.*, 1994) and *Chrysoperla carnea* Steph. (Rabindra *et al.*, 1994b) have also been reported.

1.2.2 Scope of microbial pesticides

Heiz *et al.* (1995) reported that an increasing awareness of environmental problems and widespread pest resistance (such as that displayed by *Helicoverpa armigera*) poses a severe threat to the management programmes based on conventional synthetic insecticides. One of the alternative pest control methods is the use of biological pesticides. Microbial control may be defined as the use of microbes to control pests (SP-IPM, 1999). It often involves the application of the microbe as a biological pesticide or biopesticide. The latter are generally termed as beneficial micro-organisms and include bacteria, fungi, protozoa, viruses and nematodes. They provide vital services to agriculture (such as nitrogen fixation) or help indirectly by causing diseases that attack various pests, crop diseases (such as *Trichoderma* spp, *Pseudomonas* spp) or weeds. Some pathogens, however, cause diseases to (or directly attack) man, livestock and crops (SP-IPM, 1999). Research on beneficial micro-organisms has been going on for decades and the vast majority of the microbes causing diseases in invertebrates are safe to vertebrates (SP-IPM, 1999).

Biological pesticides are required to have desirable properties that can match or at least show close flexibility to that of chemical pesticides in terms of different formulation products and diverse application equipment in order to be accepted by the end-users. They also must be registered before commercial use, and the registration process provides a check of their safety. Many microbes are already registered and are being used as safe and effective commercial products (Table 1.1). One of the main reasons for developing these products is that they offer safer pest control than chemical pesticides.

Bacillus thuringiensis is available as a commercial formulation in the Kenyan market. It is very useful as an alternative to chemical pesticides, especially to lepidopteran pests. For the last two decades, ICIPE has focused on developing and applying environmentally safe pest management technologies and successfully implemented farmer participatory strategies for the sustainable management of the tropical insect pests. Research is going on to evaluate the use of fungal pathogens (*Metarhizium anisopliae*) against thrips on vegetables and flowers and its potential has already been demonstrated (ICIPE, 1998).

Among the microbial pesticides, the viral-based pesticides are also gaining importance. Baculoviruses, which include the nuclear polyhedrosis virus (NPV) have been used in the control of a wide range of lepidopterous pests, including *H. armigera*. NPV has been used against these pests in the field in America for a long time and is an old local practice in Asia (Ignoffo, 1973). The use of the virus has reduced the hazards caused by chemical pesticides and more so, can easily be adopted by smallholder farmers.

The spectacular control of the potato tuber moth in Egypt and Tunisia by use of a baculovirus (granulosis virus -GV), with on-going local production by local farmers and NGOs is a case to reckon (SP-IPM, 1999). Research by the International Crops Research Institute for the Semi Arid Tropics (ICRISAT), International Potato research Centre (CIP) and ICARDA with Natural Resources Institute (NRI) has shown that NPVs are highly effective against some legume pod borers (including *H. armigera*) and local production is being encouraged (SP-IPM, 1999). Other studies in South Africa, Egypt, Tunisia, Botswana, Cameroon and Togo have shown the potential of NPV for the control of *H. armigera* in Africa.

Table 1.1 Commercial biopesticide products: Introductions and local production

<p><u>Successful introductions of biopesticides.</u></p> <ol style="list-style-type: none"> 1. <i>Oryctes</i> baculovirus against coconut palm rhinoceros beetle in India 2. Nuclear polyhedrosis virus (NPV) against gypsy moth in the USA 3. <i>Entomophaga maimaga</i> fungus against pine sawfly in the UK 4. <i>Entomophthora</i> fungus against aphids in Australia
<p><u>Local production successes</u></p> <ol style="list-style-type: none"> 1. <i>Beauveria bassiana</i> against sweet potato and banana weevil in Cuba. 2. Granulosis virus against potato tuber moth in Egypt. 3. Nuclear polyhedrosis virus against soybean looper in Brazil
<p><u>Some commercially available biopesticides</u></p> <ol style="list-style-type: none"> 1. <i>Bacillus thuringiensis</i> (<i>B.t</i>) against caterpillars, mosquitoes and beetles from Abbott, Mycogen and Thermo Trilogy (USA). 2. <i>Beauveria bassiana</i> (fungus) against caterpillars available in several formulations from Troy, Mycotech, Bioscience (USA), NPP (France) and Nitto Denko (Japan). 3. <i>Metarhizium anisopliae</i> (fungus) produced by Ecoscience (USA), BioCare (Australia), BCP (S. Africa) and several Brazilian companies for use against termites, locusts and sugarcane frogopper respectively.

Source: SP-IPM (CGIAR), 1999.

1.2.3 Insect viruses

There are in about 14 different families of viruses known to infect insects. However, biopesticide development is concerned almost exclusively with members of one family, the Baculoviridae (Grzywacz, 1997). The family Baculoviridae contains the most diverse and largest group of viruses (Wood and Granados, 1991). Fenner (1976) and Mathews (1982) classified those in the genus Baculovirus into 3 subgroups: Nuclear polyhedrosis virus (NPV), Granulosis virus (GV) and Oryctes virus (OBV). NPV and GV are the most important (Grzywacz, 1997). Baculoviruses infect over 600 insect species, mostly lepidopterans (Wood and Granados, 1991), including many important pest species but also insect species in other orders including Hymenoptera (31 species), Diptera (27 species) and Coleoptera (5 species) (Grzywacz, 1997). Neuroptera and Trichoptera are also known to be affected (Fenner, 1976). Baculoviruses are common and have been isolated from these orders (Fenner, 1976; Martignoni and Iwai, 1986). Ignoffo (1968) reported over 500 insect species infected by NPV, nearly 88 by GV and 184 by (cytoplasmic polyhedrosis virus (CPV) and other non-occluded viruses. Martignoni and Iwai (1986) reported 337 named isolates of baculoviruses from insects; 280 from the order Lepidoptera and the remainder from Hymenoptera, Coleoptera, Diptera, Neuroptera, Orthoptera and Tricoptera. Most baculoviruses were NPVs and GVs. NPVs account for 41% of the arthropods described viruses (Ignoffo, 1974). Baculoviruses are generally highly pathogenic to target hosts and therefore have received considerable attention as biopesticidal candidates

for biological control (Rothman and Myers, 1996). They are among the most promising alternatives to chemical insecticides, and have been used as insect control agent for a many years (Huber, 1986; Wood *et al.*, 1994; Bonning and Hammock, 1996). Many appear to be specific to the orders they have been isolated from (Adams and Bonami, 1991) though a few MNPVs have been reported to infect some members outside their specific orders. An elaborate review on the host range of some known NPVs has been reported by McIntosh and Grasela (1994). Baculoviruses do not infect any non-arthropod species. This specificity is one advantage of using them as biopesticides because they are known to be completely safe to man, animals and such non-target insects as bees, predatory insects and parasitoids (Gröner, 1986; Grzywacz, 1997). Over 20 species of baculoviruses have been developed or registered as commercially available insecticide. Out of these, over 30 different products have been registered as commercial insecticides based upon NPV or GV (Grzywacz, 1997). Burges and Hussey (1971) stated that for baculoviruses to be acceptable and usable as microbial pesticides, they have to meet the general requirements of entomopathogens: virulence, predictability of control, ease of production, low cost, good storage properties, aesthetically safe, acceptable and ability to reduce pest populations to sub-economic levels.

This study will focus upon NPVs, and particularly the *Helicoverpa (Heliothis) armigera* NPV (HaNPV). NPVs have received more attention as potential insect control agents because their occlusion bodies (OBs) are relatively easy to see and count using a light microscope. The others (GVs and

OBVs) are too small to be seen except with an Electron Microscope and as such are much more difficult to identify, isolate and work with (Grzywacz, 1997).

1.2.4 Nuclear polyhedrosis viruses (NPVs)

Nuclear polyhedrosis viruses are considered to have great potential in pest control (Kawase, 1983; Fuxa, 1987). They have attracted the attention of pest control scientists interested in looking for an alternative to pesticides because they cause a highly infectious disease that kills in 5-7 days; and attack some of the most important lepidopteran crop pests, including species of *Heliothis*, (*Helicoverpa*) and *Spodoptera* (Copping, 1993). The NPVs are seen in dying or dead larvae as bright irregular crystals called “occlusion bodies” (OBs) or “polyhedral inclusion bodies” (PIBs) (Adams and Bonami, 1991). These are protein crystals, 1-7 μm across that show up at X400 magnification as bright refractive crystals, especially under phase-contrast (Adams and McClintock, 1991). The crystals are many-sided (polyhedral) and are composed of a protein called polyhedrin (Granados and Federici, 1986; Adams, 1991). Individual virus particles, called *virions*, are embedded within each crystal (composed of a uniform lattice of polyhedrin), each containing about 20-200 virions depending on the species (Granados and Federici, 1986). In HaNPV, there are commonly upto 30 virions in each OB (Grzywacz, 1997). The virions are the actual infective virus particles and are composed of a rod-shaped (baculo) DNA/protein structure, called a nucleocapsid, inside a membranous envelope. A calyx or membrane of a protein surrounds the whole OB crystal (Granados and Federici, 1986). These OBs are the infective stage

of the virus designated to transmit the infection from insect to insect (Grzywacz, 1997). Two morphological sub-groups occur: the single-nucleocapsid NPV (SNPV) with only one nucleocapsid per envelope and the multi-nucleocapsid NPV (MNPV) with several nucleocapsids packed per envelope (Huger, 1966; Payne, 1974). The polyhedrin crystal helps to protect the vulnerable virions from inactivation by environmental factors. Baculoviruses in this form are extremely stable and can retain infectivity for many years, if not exposed to UV light or high temperatures ($\geq 50^{\circ}\text{C}$) (Jaques, 1977; Grzywacz, 1997).

1.2.4.1 Mode of virus infection and gross pathology

Once ingested by the insect, the proteinous occlusion bodies (OBs) are dissolved in the insect midgut lumen by the alkaline environment and digestive proteases, releasing the enveloped virions (Tanada and Hess, 1984). The virus enters the midgut cells especially the columnar epithelial cells by fusion with the membranes (Granados, 1978). The nucleocapsids are released, enter the cell and migrate to the nucleus through the nuclear pores (Harrap, 1970). Externally visible (morphological and behavioural) pathological changes in the host occur normally when infection is well advanced (2-5 days) and the insect becomes moribund (Aizawa, 1963; Poinar and Thomas, 1978a, 1985). NPV infected larvae may gather in a typical way at the top of the plants (Aizawa, 1963), they cease feeding, the integument changes in colour and luster (becomes opaque, milky and glossy) and the insect becomes flaccid and fragile. The normally clear haemolymph turns cloudy and milky (Tanada and Kaya, 1993). As the disease advances, larvae may hang suspended from the

tops by their abdominal and caudal prolegs, a phenomenon called the “wilt symptom” (Tanada and Hess, 1984) and they darken rapidly after death (Tanada, 1956).

1.2.4.2 Histopathology

Most NPVs cause systemic secondary infections in lepidopterous larvae, infecting major tissues such as the fat body, tracheae, hypodermis and blood cells (Tanada and Kaya, 1993), though some are tissue specific infecting a few larval tissues (Scheepens and Wysoki, 1989). Other organs and tissues infected are malpighian tubules, reproductive organs, salivary glands, midgut, pericardium and nervous tissue (Tanada and Kaya, 1993). OB production in *H. armigera* starts around 5 days after infection and peaks 7-8 days after infection. The massive destruction of body tissue that accompanies the production of OBs eventually kills the insect (Norris, 1978; Grzywacz, 1997). OBs can be seen in wet mounts of extracts of infected insects by phase-contrast or dark-field microscopy where because “shine” brightly in unstained preparations (Aizawa, 1963). The virus is also known to produce various protease enzymes that assist in the disintegration of the insect tissues to release the OBs that can infect other tissues. It has been reported that a dead insect may have about 10% of the body weight comprising of OBs. This astonishing high productivity of NPV in larvae is another reason for their attraction as biopesticides and is unmatched with any other types of viruses (Grzywacz, 1997). The successfully infected insects secrete NPV during the later stages of infection and move about the host plants spreading virus extensively before dying. In pupae, there may be no external observable changes during the

incubation period. However, at the end, the skin is easily ruptured while handling and the pupal body shows a homogenized appearance. Sometimes black markings are observed on the pupae surface around the time of death (Aizawa, 1963). OBs also remain active if eaten and passed out by predators in faecal material. Therefore, the activities of birds, mammals and other larval predators may be important in spreading NPV epidemics (Grzywacz, 1997). Healthy larvae cannibalise NPV-infected ones. Dhandapani *et al.* (1993b) observed cannibalism of NPV-infected and dead larvae by healthy ones, hence disseminating the virus. Pollen searching insects also disseminate the virus. Gross *et al.* (1994) outlined the importance of honey-bees in the autodissemination of NPV in a hive-mounted device.

1.2.4.3 Detection of baculoviruses

A number of methods have been used to detect, characterize and identify *Helicoverpa* NPVs. These include: agar gel diffusion, fluorescent antibody labelling, haemagglutination, viral neutralization, gel dissociation techniques, restriction enzyme analysis (REN) (Shapiro and Ignoffo, 1975; Young *et al.*, 1975; Kelly, 1977; Miller and Dawes, 1978; Bilimoria, 1983,1986), peptide mapping, (Smith and Summers, 1978), the enzyme-linked immunosorbent assay (ELISA) (Kelly *et al.*, 1978) and polymerase chain reaction techniques (PCR) (Rindich *et al.*, 1975; Wang and Kelly, 1983). Kelly *et al.* (1978) suggested that a rapid, sensitive and simple technique - the enzyme-linked immunosorbent assay (ELISA) should be used in detecting and identifying NPVs. Polymerase chain reaction techniques (PCR) was found to be more sensitive, it reduces sample preparation and no DNA purification is required.

Restriction enzyme analysis (REN) was found to be more effective than the other methods but is more reliable for a single late instar larva. Other characterization methods used include flow cytometry (Bouwer, 1997a; Khan *et al.*, 1997) and SDS polyacrylamide gel electrophoresis (Fielding *et al.*, 1997). A number of NPVs are pathogenic to *H. armigera* and based on morphology, biochemistry, physico-chemistry, microbiology, pathology and host specificity, *Heliothis* baculovirus found in different parts of the world are considered to be the same (Ignoffo and Couch, 1981).

1.2.4.4 Dose rate determination

Dosage of baculoviruses is expressed in terms of larval equivalents (LE) or in terms of the number of occlusion bodies (OBs). One LE is equivalent to the number of OBs produced by a fully infected fourth instar larva. In Thailand, the bioassay of *H. armigera* second instar larva using probit analysis indicated that the median lethal dose (LD₅₀) of the NPV of *H. armigera* was 1.9×10^3 OBs/larva and the sub-lethal dose was 5.187 OBs/larva (Sivilai-Sirimung, 1982). Other recommendations include LD₅₀ of 1.6×10^6 and 2.5×10^6 OBs/ larva for first and second instar larva respectively (Salama *et al.*, 1986), 450 LE/ha for second instar larvae (Dhamdhere and Khaire, 1986) and 250 LE/ha plus crude sugar adjuvant at 2.5 kg/ha at 10 days interval (Dhandapani and Babu, 1995) in field evaluations. The most recommended dose for field use in most crops is 250 LE/ha (1.5×10^{12} OBs/ha) while adjuvants are variable. Generally, mortality increases with increase in dose rate, and susceptibility varies with age of the insect. Young larvae are more susceptible than old larvae and need lower doses (Teakle and

Byrne, 1989). Lethal time, (LT_{50}), when 50 % mortality is achieved varies from 3 to 7 days (Smiths and Vlak, 1988).

1.2.4.5 Formulation of baculoviruses

Baculoviruses are formulated similarly as other pesticides. NPVs have been formulated as liquid and emulsifiable suspensions, wettable powders, bait formulations, freeze-dried formulations, ULV sprays and capsulated forms (Bell and Kanavel, 1977; Rabindra and Jayaraj, 1988, Rabindra *et al.*, 1989; Vail *et al.*, 1993). Because of their rather unusual physicochemical properties, polyhedra can be stored in the form of a dry powder or in flame-sealed tubes for as long as 20 years without significant changes in their solubility and without loss of infectivity of the enclosed virus particles (Aizawa, 1954, 1963; Steinhaus, 1960). They have also been formulated as sprays (ultra- and high volume) or air-dried and diluted with an inert carrier or freeze-dried with a carbohydrate (usually lactose). Liquid suspensions are preferred because they are usually cheaper and can kept for long or may be frozen (Jacques, 1977). Cherry *et al.* (1999) tested several formulations of NPV (including an emulsifiable concentrate, a ULV suspension and a microencapsulated preparation) but none were consistently more effective than a filtered unpurified aqueous suspension. Locally available adjuvants such as, stickers, U-V protectants and phagostimulants (e.g. clays, minerals, wetting agents, thickening agents, and plant extracts) have been added to increase larval feeding and viral activity (Rabindra and Jayaraj, 1988; Rabindra *et al.*, 1989; Jones and Grzywacz, 1990). Synergists which may be added include: oils, lecithin, alkaline compounds such as sodium carbonate that increases gut

pH, and pyrolytic activators (e.g. calcium chloride and mildly toxic boric acid) which enhance mortality (Rao *et al.*, 1987; Tuan and Houroger, 1988). Vegetable oils that have been used are cotton-seed oil, corn oil, sesame oil; together with their flours. Yeast, crude cane sugar (molasses or jaggery), sucrose and oilseed cakes have been used as phagostimulants. Insect growth regulators such as diflubenzurone and botanicals such as cotton seedkernel oil also enhance effectiveness. Whole milk (20%), whole egg homogenate (10%), Rapinal (0.5%) and Robinblue (0.5%) have been used as adjuvants (Rabindra *et al.*, 1989). In a laboratory study, 0.5% jaggery, 0.5% sucrose, 3% egg white and 1% chickpea flour (as feeding stimulants) were found to effectively reduce the LT_{50} (Sonalkar *et al.*, 1997). Enhancin (a protein) increases susceptibility and larval mortality of lepidoptera to NPV infection (Lepore *et al.*, 1994).

1.2.4.6 Field tests

The use of virus-diseased insects formulated into sprays and dusts for the field control is an old established practice (Ignoffo, 1973). Evaluation of NPV on field crops, including vegetables, has been carried out in the USA, Canada, Europe, Asia and some African countries. Research has shown that HaNPV can be used successfully on various crops, with varying degrees of success in controlling *H. armigera* under field conditions (Dhamdhare and Khaire, 1986; Narayanan and Gopalakrishnan, 1987, 1988; Ketunuti and Tantichodok, 1990; Zhang-X.H *et al.*, 1996, Rabindra *et al.*, 1992; Bell and Bhardee, 1993; Dhandapani *et al.*, 1993a; Mohan *et al.*, 1996b; Gopalakrishnan and Asokan, 1998). Cherry *et al.* (1999) reported that

treatment of chickpea with Ha NPV at 1.5×10^{12} OBs/ha was as effective, or better at controlling *H. armigera* larvae, and increasing yield relative to the control than either a standard chemical insecticide (endosulfan) or *Bacillus thuringiensis* in two successive years. Puri *et al.* (1996) reported a proliferation in the production of HaNPV in India, with 43 producers of the products based upon this virus. The first reported field use of *Helicoverpa* virus in East Africa was by Coaker (1958) against the African bollworm, *H. armigera*, on cotton in Uganda but little research was followed after this. No research work has been undertaken in Kenya on the *Helicoverpa* virus, although its natural occurrence was reported by van den Berg (1993).

Baculoviruses have been found to be more effective when combined with other pesticides such as cypermethrin and endosulfan (Bijjur *et al.*, 1994; Pawar *et al.*, 1990; Datkhile *et al.*, 1992; Reddy *et al.*, 1992), and an advantage of this is that low chemical concentrations are required and this reduces chemical hazards. Basavana *et al.* (1997) also proved that viruses were effective in mitigating the resistance to insecticide in *H. armigera*. *Bacillus thuringiensis* var. *kurstaki* has been effectively combined with baculoviruses (Roome, 1975). Natural enemies of *H. armigera* have also been found to work well with NPVs since they are specific (Kumar *et al.*, 1994).

1.2.4.7 Factors affecting efficacy of formulated baculoviruses

The success in the use of viral diseases in insect pest control depends on many factors; for instance, pest biology and characteristics of pathogenic agent, and environment. The variable characteristics of the pathogenic agent will affect the results either positively or negatively. These factors could

generally be divided into crop, pest and virus-related factors. Crop-related factors include growth and architecture of the plant. Host plant foliage reduces insect susceptibility to virus due to the presence of inactivating allelochemicals. The high pH of dew on the leaf surface also reduces persistence of the virus (Carnedy and Arant, 1968; Andrews and Sikorowski, 1973; Young *et al.*, 1977; Ali and Sikorowski, 1987; Baskaran *et al.*, 1996). Mortality decreases steadily as larval age and weight increase (Ignoffo, 1966b; Daoust and Roome, 1974; Whitlock, 1978) and there is a protracted time to death (LT₅₀) (Washburn *et al.*, 1998). Host density and age structure also influence NPV effectiveness (Ignoffo, 1966a; Daoust, 1974). More crowded young larvae are more susceptible than old less crowded ones (Salama *et al.*, 1986; Shafique and Luttrell, 1992). Moulting hormone (β -ecdysone) has been reported to inhibit replication of *Heliothis zea* NPV (Keeley and Vinson, 1975). The virus also loses activity in the outside environment (Young and MacNew, 1994). The polyhedra in soil may be viable for years but if exposed to the sun they lose viability within hours due to UV irradiation (Evans, 1986). Time required for lethal infection in NPVs is inversely proportional to the inoculum density and environmental temperature (Canerday and Arant, 1968).

The use of some adjuvants (buffers and synergistic factors) (Bijjur *et al.*, 1991a; Pawar *et al.*, 1992) and genetic engineering by cell lines have improved the efficacy of baculoviruses (Tanada and Kaya, 1993; Shuler *et al.*, 1991; Miller, 1995). UV protectants have also been widely developed and include starch encapsulations, tryptophan, shade, stilbene fluorescent brighteners (Ignoffo and Batzer, 1971; Zou and Young, 1994, 1996; Ignoffo

and Gracia, 1995), stilbene-derived optical brighteners, charcoal powder, Indian ink, white carbon, molasses (or a mixture of white carbon and molasses), chelating agents and Raymix powder (Martignoni and Iwai, 1986). These greatly increased the efficacy of baculoviruses (Rabindra and Jayaraj, 1988).

1.2.4.8 Commercially available NPVs

Several baculoviruses have been developed as microbial insecticides and are presently being used for agricultural and forest pests (Granados and Lawler, 1981). They are also important in the integrated pest management approach (IPM) for short term control of a wide range of lepidopterous pests. The following NPVs have been registered by the US Environmental Protection Agency (EPA): The gypsy moth, *Lymantria dispar* (L) NPV; the cotton bollworm, *Helicoverpa zea* NPV; the European pine sawfly, *Neodiprion sertifer* (Goeffrey) NPV; the Dougloss-fir tussock moth, *Orgyia pseudotsugata* (McDunnough) NPV; the beet armyworm, *Spodoptera exigua* (Hübner) NPV; the alfalfa looper, *Autographa californica* (Speyer) NPV and the celery looper, *Anagrapha falcifera* (Kirby) (Goetell, 1996; Wood *et al.*, 1994). *Heliiothis* spp NPV (HZSNPV) was the first to be registered while *Anticarsia gemmatalis* NPV is extensively used in Brazil (Wood *et al.*, 1991). In Russia, *Pieris* spp NPV has also been isolated and commonly is used. Nuclear polyhedrosis viruses for many lepidopterans have been isolated in Canada, Russia, China, Indonesia, India, the Philippines, Malaysia, Thailand, Egypt and South Africa. These include NPVs for: *Prodenia* spp, *Neodprion*

sertifer, *Neodprion leconti*, *Spodoptera* spp, *Tricoplusia* spp, *Mamestra brassicae*, *Helicoverpa armigera*, among many others.

1.2.4.9 Advantages and disadvantages of using viruses for pest control

Insect viruses are known to be specific and pathogenic only to a limited range of host insects and are non-pathogenic to vertebrates. They have been proved to be remarkably safe to mammals (including man) and other non-target organisms (Aizawa, 1954; Ignoffo, 1965; Burges and Hussey, 1971; Norris, 1978; Burges, 1982; Gröner, 1986; Bijjur *et al.*, 1991b). Because of their specificity they have been considered ideal for use in integrated pest management (IPM) programmes which are based on the selective control of the pest species below the economic damage levels. This ensures balance of the ecosystem and maximizes the potential of beneficial arthropods. Since they are compatible with chemical pesticides, mixtures can be applied to control pest complexes in a crop, a method that reduces the amount of chemical pesticides and their adverse effects on the ecosystem and the natural enemy activity (Richter and Fuxa, 1984). NPVs are known to multiply in host insects and can be artificially disseminated. Natural epizootics can also be initiated by spread of the virus in the population thus keeping the pest levels low for several years (Huber, 1996). Resistance in target hosts has not been recorded except for induced resistance observed in the laboratory reared insects (Huber, 1986). Whitlock (1977) experimentally induced resistance of *H. armigera* to baculoviruses in the laboratory for 25 generations but it failed to establish. Cross-resistance has also not been recorded. Application by

conventional techniques gives them an upper hand than employing predators and parasitoids. Viruses are rather persistent in the soil but are quickly inactivated by UV light (Jaques, 1977) and therefore pose no residue problems.

Despite a number of desirable traits, several major drawbacks have hampered the efficacious use of baculoviruses for pest management. Some of these factors, as cited by Washburn (1998) include:

- (i) Limited host range which assures a high degree of safety to non-target organisms but restricts the use of each baculovirus species to only a few insect pests.
- (ii) Dose-mortality responses vary widely even among suitable hosts, and under field conditions certain resistant hosts do not ingest enough virus to induce mortality and effect control. For example, *Helicoverpa zea* (Boddie) larvae are highly resistant to AcMNPV (Vail *et al.*, 1978; Vail *et al.*, 1982), unlike *Tricoplusia ni* (Hubner) and *Heliothis virescens* (F) and require ingestion of more polyhedra to achieve mortality levels comparable to *H. virescens* cohorts of similar ages (Washburn and Volkman, unpublished).
- (iii) Protracted time to death - for instance, AcMNPV and other baculoviruses can kill and liquefy their hosts but are not highly virulent and infected larvae continue to feed and grow while their tissues become replete with virus. This problem has been addressed by engineering recombinants of AcMNPV with enhanced virulence (Bonning and Hammock, 1992; Miller, 1995) or by incorporating

genes that encode invertebrate neurotoxins (Stewart *et al.*, 1991; Cory *et al.*, 1994).

- (iv) Loss of infectivity in the environment – polyhedra in soil may remain viable for years, but typically lose viability within only a few hours when exposed to light (Evans, 1986). This has been addressed by incorporating UV protectants such as stilbene optical brighteners into inocula formulations which coat on the polyhedra and absorb long wavelength ultraviolet light (Shapiro, 1992; Shapiro *et al.*, 1992; Hamm and Shapiro, 1992). These also have enhancing effects by increasing susceptibility to fatal infection and reduce the time to death after ingestion of the virus (Adams *et al.*, 1994).

Currently, it is possible to alleviate these problems because they can be used as expression vectors for foreign genes such as insect-specific genes. For instance, insect-specific toxins, hormones or enzymes (hence genetic engineering) have been used to widen the host range or increase the rate of kill of NPVs (Martens *et al.*, 1990; Hammock *et al.*, 1990; Maeda, 1989; Wood and Granados, 1991; Shuler *et al.*, 1991; Miller, 1995).

At the farmers' level, precise timing is the major limitation. It is not easy for the uninitiated farmer to know when to apply the virus against the pest; whereas the chemical method is very simple. Farmers need minimum training and back up before they can reliably apply the biological pest control method. There is also no immediate noticeable result as pests die slowly. However, the possibility of local production and the long-term returns merit the use of these biopesticides.

1.3 JUSTIFICATION OF THE STUDY

Vegetable production will continue to expand since they are increasingly becoming an important part of the local diet. They also constitute a source of income for farmers, particularly women and children, and a source of foreign exchange especially for small-scale farmers. Cabbage, curcubits, capsicum spp, egg-plant, okra, onion, peas and tomato, including export Frenchbeans will continue to be important. Heavy insect pest outbreaks on these crops call for their control in order for reasonable yields to be realized. Consumers also prefer produce that is free from damage by either pests (or diseases) and does contain pesticide residues. Such might fetch better prices, especially in the export market. Hence, the use of pesticides, especially on export vegetables is an area of great concern for both producers and consumers. There is urgent need to develop alternative crop protection methods, to be used especially during reproductive and pre-harvest stages. The major drawbacks highlighted in the use of chemical pesticides include a wide spectrum of toxicity. Most pesticides are too broad in their action and prove to be toxic to other non-target insects (natural enemies) and have high persistence which leads to rapid development of resistance and pose risk to human health and the environmental pollution. Most of the chemical pesticides are also unaffordable, especially to the resource-poor vegetable farming communities. To ensure that farmers' efforts are not wasted due to pest damage, at present, there is emphasis is placed on researching on alternative pest management strategies that are selective, environmentally safe and for which resistance might not developed too quickly. One strategy

involves the use of naturally occurring organisms which include bacteria, fungi, viruses, protozoa and nematodes. Of these, insect viruses have gained considerable attention. They have been used in for the control of many insect pests. Evidence from USA and Asia shows that insect viruses generally, and in particular nuclear polyhedrosis virus, are neither toxic nor toxicogenic to vertebrates and can effectively control many insect pests. A trial on these is therefore called for in Kenya.

Generally, microbial control can provide new options for tackling long-standing pest problems. Classical biological control by introduction of natural enemies from the place of origin of a pest can sometimes provide a complete and timely solution to “new pests” (pests introduced into new areas). These biocontrol agents sometimes take a long time to establish, or are in some ways limited in their effectiveness. Microbial control can provide a stop-gap while a classical biocontrol agent takes hold, or can supplement the action of classical biological control where introduced natural enemies are not fully effective in the new situation. Host plant resistance, biological control and cultural techniques, may be unable to provide satisfactory control. Chemical control may be harmful to human health and the environment; safer chemicals may be too expensive, or incompatible with other integrated pest management (IPM) measures. In such cases, biopesticides can provide new control options which are both cost-effective and environmentally safe. In addition, replacing chemicals with biopesticides may contribute to biodiversity conservation. Once developed, this will reduce the pesticide residue problem in the Kenyan export vegetables, besides the above stated advantages.

1.4 HYPOTHESES

This study was based on the following hypotheses:

1. The locally isolated Baculoviruses (NPVs) is effective in controlling *Helicoverpa armigera*.
2. Field efficacy of *Helicoverpa armigera* NPV will be improved by the addition of adjuvants, UV protectants and phagostimulants.
3. Field efficacy of *H. armigera* NPV will depend on both host-plant and abiotic factors.

1.5 OBJECTIVES

1.5.1 General objective

The general objective of this study was to evaluate the effect of nuclear polyhedrosis virus (NPV) for the control of *Helicoverpa armigera* (Hübner) larvae affecting some vegetable crops in Kenya.

1.5.2 Specific objectives

1. To survey for the natural occurrence of nuclear polyhedrosis virus (NPV) affecting *Helicoverpa armigera* within Kenya.
2. To evaluate the effect of local and exotic strains of NPV in laboratory bioassays.
3. To test some local spray additives on the efficacy of the improved NPVs in the laboratory bioassays.
4. To carry out preliminary tests using potted plants and field plants on the efficacy of NPV against *H. armigera* on pigeon pea and snowpea vegetables.

CHAPTER TWO

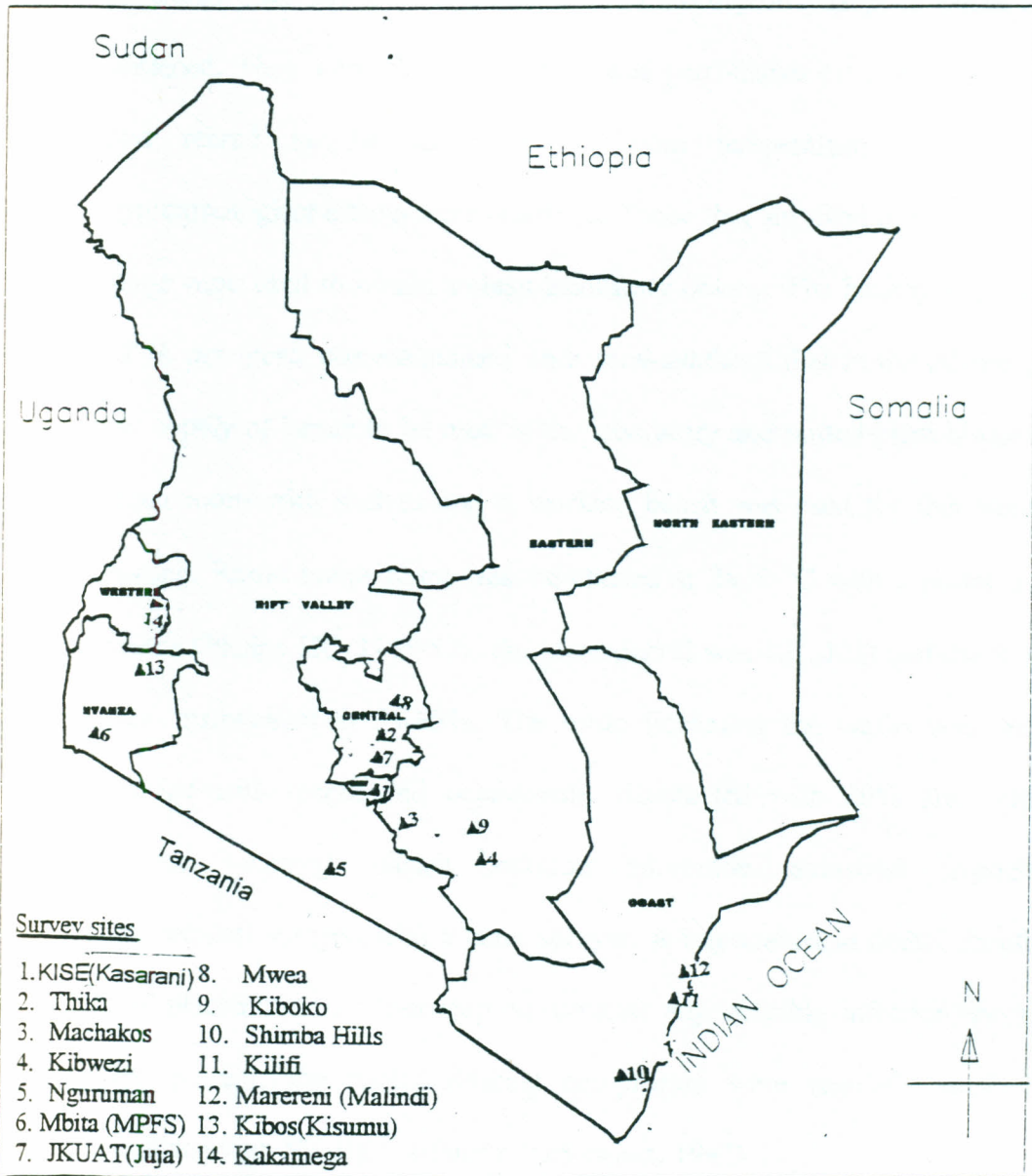
2.0 GENERAL MATERIALS AND METHODS

2.1 Study Sites

Vegetable farmers' fields around Nairobi (Kasarani), Thika, Machakos, Kibwezi, Mwea, Nguruman (Magadi), Mbita, JKUAT (Juja), Kwale, Kilifi, Malindi, Kisumu and Kakamega were periodically visited to collect *Helicoverpa armigera* (Hübner) larvae. The geographical positions of the surveyed areas are given in Figure 1.1. These sites were selected on the basis of the availability of vegetable crops prone to damage by this pest and their proximity to ICIPE or ICIPE's on-going projects and easy accessibility.

Laboratory rearing of the larvae and bioassays were carried out at Duduville (ICIPE headquarters, Kasarani-Nairobi). Potted pigeonpea and snowpea plants were also raised at Duduville while the preliminary field testing of NPV was carried out at Kenya Institute of Special Education (KISE), next to Duduville.

FIGURE 1.1: Map of Kenya showing survey sites



2.2 Laboratory rearing of host insects

Larvae collected alive in the field were reared on a natural diet (young pigeon pea pods) and on over-night soaked market grade chickpea seeds (changed after every 2 days) at times when pigeon pea pods could not be obtained. They were placed into individual petri-dishes on a moist filter paper and reared in the laboratory at room temperature and any NPV cytopathological effects were observed. Those that survived to pupal and adult stage were used to obtain a clean laboratory colony. The healthy stock colony of *H. armigera* was maintained on a semi-synthetic diet in the laboratory for the supply of larvae to be used in the laboratory and potted plant bioassays. A small room with shelves and a working bench was used for this laboratory rearing. Room temperature was maintained at 25 ± 2 °C with a portable room heater (Philips HD 3345-52), the photoperiod was 12L:12D and the humidity was maintained at 55-65%. The room (including the walls) was regularly washed with teepol and occasionally disinfected with 20% JIK[®] (Reckitt Colman, Kenya) bleach solution (domestic industrial hypochlorite disinfectant) sprayed with a hand sprayer. A rug soaked in deetol disinfectant was placed on the door-step to prevent any possible infection by people walking into the room. Rearing equipments were regularly washed and sterilized with 5% JIK[®] solution (Gryzwacz, 1997)

2.3 Rearing procedure

Adult *H. armigera* moths emerging from the field-collected larvae were used in the initial colony and sometimes infusions were made to stabilize

the population. Transparent plastic containers (Plate 5) were used for the adults. About 4-8 pairs were introduced into each container and they were allowed to mate freely. Black muslin cloth was used to cover the top of the containers which also acted as oviposition substrate for the females. They were fed on 10% (w/v) honey or sugar solution [Plate 6] or a modified Shorey and Hale (1965) adult diet (Appendix 3), soaked on cotton wool in a petri-dish and placed at the bottom of the container. The oviposition substrates were changed daily to remove the eggs laid.

The pieces of cloth with eggs were soaked in 0.2% sodium hypochlorite (van der Walt, 1988) or 2% formaldehyde for 10 minutes and then washed in tap water for 15 minutes (Kfir, unpublished). These were then hung to drip-dry and placed into small plastic containers (Plate 7) for hatching after which neonates were transferred into other small plastic containers pasted with semi-synthetic diet. Second instar larvae were reared individually (to prevent cannibalism) in glass vials containing about 10 ml of a semi-synthetic larval diet (Appendix 3) until pupation. Dead larvae were removed daily from the colony to prevent further entomopathogen contamination. Pupae were removed daily from the diet and transferred into petri-dishes, lined with filter paper.

Plate 5: Rearing containers for adult moths (covered with black cloth) and hatching containers (covered with white tissue paper)



Plate 6: Bottle containing adult diet



Plate 7: Small containers used for rearing neonates and first instar larvae



2.4 NPV occlusion body identification.

Apart from NPVs, there are other entomopathogens that have been reported to infect *H. armigera* in the field and cause mortality (van den Berg, 1993). In this study NPV polyhedra identification was done in order to ascertain that the dead insects collected were NPV-infected, and did not die from infection by any other pathogens. In this study NPV occlusion body (OB) identification was done by microscopic examination.

Wet mounts were made by extracting larval haemolymph using sterile Pasteur pipette and a drop was placed on a sterile microscope slide containing a drop of sterile distilled water and a sterile cover was slip placed. Observations were made under dark-field microscopy as detailed by Poinar and Thomas (1978b). A magnification of X400 was used to observe the OBs. Identified NPV-infected insects were preserved for further processing.

2.5 NPV multiplication in host insects.

Insect viruses can only replicate in a living cell (Grzywacz, 1997). In the laboratory, this can only be done in live insect tissues, either in whole insects (*in vivo*) or in isolated cell lines (*in vitro*) (Grzywacz, 1997). In this study multiplication was done in whole insects because it is much easier and cheaper, even for the smallholder farmer. This involved infecting healthy larvae, rearing and harvesting cadavers.

Dead insects collected from the field surveys were used to yield more viruses for bioassay and field spraying. The NPV was multiplied by feeding healthy insects on contaminated food. The infected insects were reared and

cadavers were harvested. This was done at the International Crops Research Institute for the Semi Arid Tropics (ICRISAT, Asia Center - India), under special arrangement in order to get facilities. Small quantities of two Kenyan virus isolates (NPV-K1 and NPV-K2 respectively) obtained from different sites in the initial surveys (KISE and Kibwezi) were used during multiplication of the virus. Second and third instar larvae were collected from an Indian weed (Lagata plants) at the ICRISAT fields and kept in semi-synthetic diet for three days to grow up to fourth instar. About 1 ml of HaNPV from these two Kenyan isolates were inoculated on chickpea seeds and fed to the larvae, each in a separate well of Linbro[®] Tissue culture multi-well plates, each with two seeds. Each culture plate consisted of 12 flat bottom wells measuring 2.4 cm x 1.7 cm, well capacity of 7.5 ml and area of approximately 4.5 cm². Seed inoculation was achieved by adding the virus onto the seeds in a small plastic container and then swirling it to ensure maximum contamination. A total of 500 fourth instar larvae were used for the first isolate and about 250 for the second isolate (due to shortage of larvae). Harvesting was done for 4 consecutive days after a period of 5 days post inoculation for NPV-K1 and three days for NPV-K2 when most larvae had picked up infection. Percent mortality was then calculated separately for the two virus isolates. The cadavers collected were stored in a refrigerator at 4 °C for later use in inoculum preparation.

2.6 NPV preparation and quality control

2.6.1 Homogenization and filtration

This was done by the method described by Grzywacz (1997). Cadavers stored in a refrigerator for 10 days were blended in a Philips Grinder with 1 % Sodium Dodecyle Sulphate (SDS) and minimum quantity of water (10 ml/1000 larvae) for two minutes. The homogenate was then passed through a double-folded white cotton muslin cloth and allowed to drip into a clean sterile glass beaker (Plate 8.). Stirring the homogenate with a glass rod enhanced the filtration process. The procedure was repeated to ensure that solid materials (mainly cuticular) were completely removed. The residue was washed with distilled water to carry through any NPV present. The muslin cloth was finally gathered together from the top and gently squeezed to extract the remaining liquid and thereafter immediately discarded to avoid any smell. The yellowish filtrate collected (Plate 9) was then taken for centrifugation.

2.6.2 Centrifugation

This was done by the method described by Grzywacz (1997). Equal volumes of the filtrate were poured into sterile 40 ml centrifuge tubes and the volumes balanced . A Sorvall RC-5B Refrigerated Superspeed centrifuge (Du Pont instruments) was used. It was set at a temperature of 15 °C (to provide cool conditions) and 5000 rpm for 15 minutes. Following centrifugation, the filtrate was separated into two layers. The top layer, (containing mostly lipids) and the supernatant (containing soluble wastes, bacteria and other insect wastes) were poured off and discarded. The yellowish sediment (containing

NPV pellets) was removed with a spatula, re-suspended in sterile distilled water and stored in a plastic jar at 4 °C. The concentration was determined by counting the OBs per millilitre in the stock suspension with an Improved Waerber Haemocytometer.

2.6.3 Dilutions for the counting of occlusion bodies

Two dilutions for each of the samples were made as below:

A : 1:100 (1 ml of stock suspension in 99 ml of sterile distilled water).

B : 1:1000 (1 ml of A in 999 ml of sterile distilled water).

Counting of occlusion bodies was done by use of an Improved Waerber Haemocytometer. One hundred (100) small squares were counted for each dilution.

The 1:100 dilution was used to determine the concentration of OBs/ml since it gave the best count.

2.6.4 Standardization of inoculum before storage (Quality control)

The stock suspension was diluted and the number of virus particles (a.i.) determined such that the suspension could be used even for field spraying in recommended doses for any crop and cause lethal effects. Since it is easy for the farmer to determine the dose in terms of larval equivalents (which can also be converted into OBs/ml), the concentration was set at 1 LE/ml (equivalent to 6.0×10^9 OBs/ml). The suspension was diluted by adding sterile distilled water such that 1 LE would be contained in 1 ml of NPV suspension and stored at 4 °C for further experiments.

Plate 8: Filtering homogenized cadavers through double folded white muslin cloth to obtain NPV



Plate 9: Filtrate containing NPV (note the yellow colour)



CHAPTER THREE

3.0 SURVEY FOR NATURAL FIELD OCCURRENCE OF NPV PATHOGENIC TO *Helicoverpa armigera* (Hübner) LARVAE IN KENYA

3.1 Introduction

Natural incidence of NPV in the field has been reported by many workers. Some native isolates of NPV have been obtained and evaluated (in the laboratory and field) in many countries, including some African countries like Botswana (Roome, 1975), Chad (Cadou and Soubrier, 1974), Togo (Silvie *et al.*, 1993), Egypt (Jones *et al.*, 1994), South Africa (Bouwer, 1997b). These isolates might be more pathogenic than the commercial or imported ones. Arora *et al.* (1997) compared three native isolates of HaNPV from different areas in Punjab (India) with two commercially available isolates from Bangalore and found the native isolates significantly superior. The availability of pathogenic local isolates reduces the time taken in the developmental process as there would be no need of the rigorous quarantine procedure. The first report on the field use of *Helicoverpa* virus in East Africa was by Coaker (1958), mixed with *B.t.*, for preliminary trials on cotton in Uganda. Similar work was carried out in Tanzania by McKinley (1971) but there was no follow-up in both cases. In Kenya, the occurrence of HaNPV was reported by van den Berg and Cock (1993) at Kakamega (Western Province) and Msabaha (Coast Province) in surveys for natural enemies of *H. armigera* larvae carried

out between 1988-1990. This was in an attempt to promote biological control of this pest. There have been no surveys carried out thereafter and also there has been no follow-up studies on pathogenicity. In this study an extensive survey was carried out aimed at establishing the natural incidence of NPV in various parts of the country, determination of pathogenicity of isolates from different localities and possible utilization of these isolates as biological control agents.

Insect pathogens have not been known to occur naturally in large quantities to cause significant mortality in the field, although natural epizootic has been reported (Webb and Shelton, 1990; Caballero *et al.*, 1992; Sait *et al.*, 1994). Development and application of pathogens as biocontrol agents involves multiplication for pathogenicity tests and use in the field. Insect viruses can only replicate in a living cell (Grzywacz, 1997). Thus, this can only be done in live insect tissues, either in whole insects (*in vivo*) or in isolated cell lines (*in vitro*), unlike other biopesticides like *Bacillus thuringiensis* or fungi that can be mass produced by fermentation on simple media (Grzywacz, 1997). In this study multiplication of the virus obtained in the field was made in whole insects after the confirmation of infection because it is much easier and cheaper. This involved infecting healthy larvae, rearing them and harvesting of cadavers.

3.2. Materials and methods

In response to the record of NPV on *H. armigera* by the ICIPE scientists (Export Vegetables IPM Project) near KISE in 1997, surveys were

conducted in June to October, 1998 and February to September, 1999 around farmers' fields. The areas surveyed were Kasarani (Nairobi), Thika, Mwea, Machakos, Kibwezi/Kiboko, Nguruman (Magadi), Mbita (MPFS), JKUAT (Juja), Mtito Andei, Muhaka/Diani and Shimba Hills (Kwale), Kilifi, Malindi, Kibos (Kisumu) and Kakamega. These areas were chosen on the basis of being major vegetable production areas with major host crops of *Helicoverpa* sp. and even presence of other host plants. Larvae were collected by use of a camel hair brush directly from host plants, from plucked leaves or removed from harvested fruits or pods which showed damage symptoms. The larvae were then placed in petri dishes with their natural diet, sealed with masking tape and carried to the laboratory. Dead larvae were placed in homeopathic glass vials and stored in the refrigerator set at approximately 4 °C for later polyhedra identification by use of compound microscope in order to confirm NPV infection. Those confirmed dead due to NPV infection were later used in production and multiplication of OBs.

Larvae collected alive in the field were reared on natural diet (when available) or on over-night soaked market chickpea seeds (changed after every 2 days) at times when natural diet could not be obtained. They were placed into individual petri dishes lined with moist filter paper and reared in the laboratory at room temperature. Observations were made for any NPV cytopathological effects. Cadavers and dead pupae collected with NPV symptoms were stored in a refrigerator (at 4 °C) for later identification of viral polyhedra. The healthy adults emerging from such collections were used in the initial laboratory rearing stock.

Cadavers were used for multiplication of OBs which were used for inoculum preparation and quality control carried out as described in chapter 2 subsection 2.6.4 and 2.6.5.

Summary tables were drawn for survey data on sampled vegetable host plants in different sites and preferred parts/regions. Percent incidence of virus in each locality was also determined.

Minimum and maximum developmental periods (incubation period, larval, pupal periods and adult longevity) for each collection were recorded. Pupae were sorted out according to sex which is basis on colouration, where males are greenish and females are brownish (Paul *et al.*, 1979). Developmental periods for the laboratory culture were similarly recorded for two successive generations. Pupal weights were also determined by weighing them individually with a digital weighing balance. and results analysed by general linear models procedure (GLM) (SAS Institute, 1994).

3.3. RESULTS

3.3.1 Field collections

H. armigera larvae were obtained from a wide range of host plants where they feed on leaves, flower buds, pods and fruits (Table 3.1), leaving characteristic trail marks (such as shredded leaves, bored flower buds) and characteristic round holes on fruits and pods (Plates 14-22). The preference for feeding positions was found to vary with host plant (Table 3.1). No larvae were obtained from Mwea, Thika, Machakos, Kangundo, Kibos and Kakamega because it was off-season. The highest larval incidences were from farms where vegetables were grown for most of the year under supplementary irrigation, especially KISE (349 larvae), Kibwezi University of Nairobi (UoN) Irrigation Farm (287 larvae) and Marereni (Malindi) (168 larvae). In these farms (Plates 10-13) it was possible to sample out some larvae at every visit made, while under normal rain-fed crops there would be none. Most of the *H. armigera* larvae collected in 1999 long and short rain seasons were from rain-fed crops, particularly those from the Coast (Shimba Hills, Kilifi, and Malindi). In Nguruman (Kajiado), a strikingly high level of *H. armigera* larval infestation was detected (from observed damage symptoms) in okra fields under irrigation but only a few larvae (56) were obtained after sampling. High *H. armigera* larval infestation and damage were observed on pigeonpea and chickpea in KISE farm, chickpea in JKUAT and on lablab bean in Malindi. In Kibwezi, (UoN Irrigation Farm) high *H. armigera* larval infestation was observed in pigeon pea, tomato, sweet corn, sweet pepper, capsicum, okra and eggplant fields (Table 3.1). Among the fields visited, it was only in Kibwezi

(UoN Irrigation Farm) where eggplants were observed to be heavily infested with *H. armigera* larvae. In Nguruman, damage symptoms were observed on the eggplant fruits but no larvae were obtained.

The developmental periods varied according to laboratory room temperature which changed with seasons, ranging from 13-26°C (Table 3.2). The developmental period of the larvae ranged from 9-21 days, depending on the instar collected from the field. Pupal period averaged 11-13 days when temperatures were around 25°C and was increased to 16-22 days at temperatures below 23°C, with extreme variations in some instances (Table 3.2). Most of the adults survived for 7-14 days and sometimes for as long as 21 days.

In the laboratory where the colony was maintained on a semi-synthetic diet for 2 generations whilst the rearing temperature maintained at 25±2 °C, the mean larval period was 12.6±0.09 and 13.9±0.10 for the first generation and second generation while the pupal periods was 10.7±0.22 and 11.9±0.50 days respectively. The adult longevity was 12.2±1.67 and 13.4±0.95 12-13 days respectively (Table 3.3). Pupal weights were recorded for the laboratory colony and the mean was about 0.4±0.01 grams. Although there were significant differences between the two generations, these periods appear to be similar to field collection records where laboratory room temperatures varied between 23-25 °C (Table 3.2). Results could not be analysed together and therefore no clear comparison could be made.

Table 2.1: Seasonal occurrence, host plants and feeding positions of *H. armigera* larvae

Month	Site of collection	Number collected	Host plant	Position on host plant
June to Sept. 1998	KISE	25	Pigeon pea	Leaves, flower buds and pods
July 1998	Thika	-	Pigeon pea	*
Sept. 1998	Mwea	-	Tomato	*
	Machakos	-	Pigeon pea	*
	Kangundo	-	Pigeon pea	*
	Kibwezi	31	Tomato	Inside fruits
Oct. 1998	Kibwezi	43	Pigeon pea	Leaves, flower buds and pods
Feb. 1999	Kibwezi	48	Pigeon pea	Leaves, flower buds and pods
April 1999	Nguruman	56	Okra	Leaves, flower buds and fruits
April to May 1999	Mbita	29	Tridax and Kales	Leaves and flower buds
May 1999	JKUAT	48	Chick pea	Leaves, flower buds and pods
May to June 1999	KISE	140	Pigeon pea	Leaves, flower buds and pods
		60	Chick pea	Leaves, flower buds and pods
July 1999	KISE	124	Chick pea	Leaves, flower buds and pods
Sept. 1999	Shimba Hills	17	Tomato	Fruits
Sept. 1999	Kilifi	5	Pigeon pea	Leaves, flower buds and pods
Sept. 1999	Malindi	48	Lablab bean	Pods
Sept. 1999	Kiboko	21	Pigeon pea	Leaves, flower buds and pods
Sept. 1999	Kibwezi	84	Sweet corn	Ears, Silk and Cobs
		6	Sweet pepper	Fruits
		84	Egg plant	Fruits.
Nov. 1999	Mtito Andei	30	Tomato	Fruits.
Nov. 1999	Malindi	120	Tomato	Fruits.
Nov. 1999	Kibos	-	Cotton	*
Nov. 1999	Kakamega	-	Tomato	*

Note

* : no record could be made

Table 2.2: The developmental periods of field collected *H. armigera* larvae fed on natural diet

Month	Site of collection	No. (N)	Host plant	Rearing diet	Rearing temp.(°C)	Mean larval period (days)	Mean pupal period (days)	Mean adult longevity (days)
June to Sept. '98	KISE	48	Pigeonpea	Pigeonpea pods	13-25	16	22	10
July 1998	Thika	-	-	*	*	*	*	*
July 1998	Machakos/Kangundo	-	-	*	*	*	*	*
Sept. 1998	Kibwezi	31	Tomato	Soaked chickpea seeds	23-26	10	19	12
Oct. 1998	Kibwezi	43	Pigeon pea	Soaked chickpea seeds	23-26	12	16	13
Feb. 1999	Kibwezi	48	Pigeon pea	Soaked chickpea seeds	25±2	9	10	12
April 1999	Nguruman	56	Okra	Soaked chickpea seeds	23-25	13	13	13
April to May 1999	Mbita	29	Tridax spp and Kales	Soaked chickpea seeds	23-25	14	11	10
May 1999	JKUAT	48	Chick pea	Chickpea leaves	23-25	9	11	10
May to June 1999	KISE	140	Pigeon pea	Pigeonpea pods	23-25	9	11	11
		60	Chick pea	Chickpea leaves	23-25	9	11	11
July 1999	KISE	124	Chick pea	Soaked chickpea seeds	23-25	13	12	10
Sept. 1999	Shimba Hills	17	Tomato	Soaked chickpea seeds	24-26	10	12	10
Sept. 1999	Kilifi	5	Pigeon pea	Soaked chickpea seeds	24-26	11	11	10
Sept. 1999	Malindi	48	Lablab bean	Soaked chickpea seeds	23-26	10	11	12
Sept. 1999	Kiboko	21	Pigeon pea	Soaked chickpea seeds	23-26	21	11	15
Sept. 1999	Kibwezi	84 50 6	Sweet corn, Eggplant, Capsicum	Soaked chickpea seeds	23-26	14	12	12
Nov. 1999	Mtito Andei	30	Tomato	Soaked chickpea seeds	24-26	14	11	11
Nov. 1999	Malindi	120	Tomato	Soaked chickpea seeds	24-26	14	11	10

- No larvae collected

* No record made

Table 2.3: Aspects of growth and development of *H. armigera* reared on a semi-synthetic diet under laboratory conditions (Temperature-25±2 °C)

Generation	Mean larval period (days ± SE)	Mean pupal period (days ± SE)	Mean pupal weight (grams ± SE)	Mean adult longevity (days ± SE)
1	12.6±0.09 a	10.7±0.22 a	0.4±0.01 a	12.2±1.67 a
2	13.9±0.10 b	11.9±0.50 b	0.4±0.01 a	13.4±0.95 b

3.3.2 Natural incidence of NPV in the field

Only a few NPV-infected larvae were collected during the months of June to October 1998, and either showed symptoms of infection or died from virus infection while being reared in the laboratory (Table 2.4). A few virus-infected cadavers were collected from KISE farm near ICIPE but most them, including those from other study sites acquired infection while being reared in the laboratory. There were no *H. armigera* larvae collected from the surveys carried out in Thika and Machakos/Kangundo, hence no cadavers were obtained from these areas. In all the other sites visited later, varying numbers of larvae were collected. It was not easy to obtain NPV-infected cadavers in the field and only one was collected from a tomato field in Shimba Hills. However, most of the cadavers which were obtained showed the symptoms of infection while being reared in the laboratory (Table 2.5).

Larval parasitoids (mostly tachinid, hymenopterans and braconid wasps) also emerged from the larvae which were being reared in the laboratory. The presence of braconid hymenopterans was also evident in the field (presence of white cocoons attached to plants). It was also observed that some tachinid puparia emerged from larvae that had died with NPV

symptoms. This aspect was more common with the larvae collected from pigeon pea, lablab bean, tomato and sweet corn.

A summary of the results showing the percentage virus incidence at different localities are shown in figure 2.1 and Appendix 4-6. The virus incidences recorded in different cropping systems and sites were as follows: Malindi, 31.3% in lablab bean and 29.2% in tomatoes; Shimba Hills, 23.5% in tomato; Nguruman, 23.2% in okra; Kibwezi, 22.4% in pigeon pea, 20.2% in sweet corn, 20% in egg plant and 19.4% in tomato; KISE (Nairobi), 25.5% in pigeon pea and 12.5% in chickpea; Kiboko, 14.3% in pigeon pea; JKUAT (Juja), 12.5% in chickpea and in Mbita, 6.9% in *Tridax* spp. The highest total percent virus incidence was recorded in Malindi (29.8%) followed by Shimba Hills (23.5%), then Nguruman (23.2%) and Kibwezi UoN farm (20.9%). In the other sites, the total percent incidences in descending order were: KISE (18.6%), Kiboko (14.3%), JKUAT (12.5%) and 10% in Mtito Andei. The lowest percent virus incidence was recorded in Mbita (6.9%) which had low larval collections. A 40% virus incidence was recorded in Kilifi (Kilifi Institute) but only 5 larvae had been collected, out of which two died from NPV infection. Mortality by NPV in some sites was found to be higher than that by other causes (Table 2.4 and Appendix 4-6).

Table 2.4: Field occurrence of NPV of *H. armigera* larvae collected from different localities in Kenya during the months of June- October 1998.

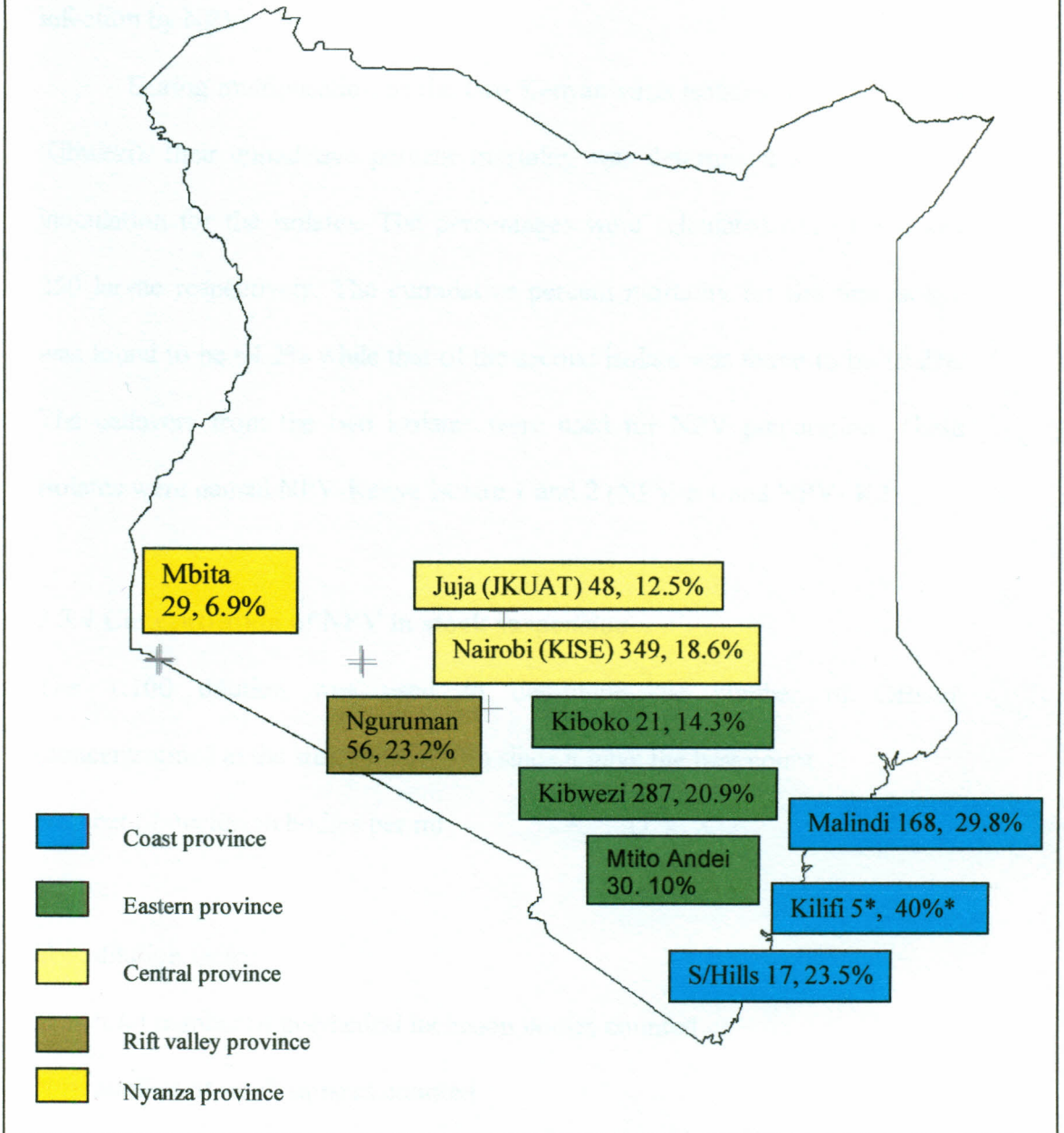
Month	Site	Host	No. collected	No. pupated	No. dead at larval stage	No. dead at pupal stage	Total % mortality	% mortality by virus
June to Sept.	KISE	Pigeon pea	25	11	10 Virus 2 others	1 Virus 1 Others	56	48
Sept.	Kibwezi	Tomato	31	16	5 Virus 6 Parasitoid 3 Others	1 Virus	48.4	19.4
Oct.	Kibwezi	Pigeon pea	44	30	5 Virus 4 Parasitoid 2 Others	1 Virus 2 Others	30.2	14

Table 2.5: Pilot survey results for natural occurrence of NPV of *H. armigera* from different localities in Kenya, June, 1998 –Nov., 1999.

Site	Crop	Number of <i>H. armigera</i>		Percent virus incidence	Total No. of larvae per site		Total % virus incidence per site
		Sampled	Infected by virus		Sampled	infected by virus	
KISE (Kasarani)	Pigeonpea	165	42	25.5	349	65	18.6
	Chickpea	184	23	12.5			
JKUAT (Juja)	Chickpea	48	6	12.5	48	6	12.5
MPFS (Mbita)	Tridax spp	29	2	6.9	29	2	6.9
KARI (Kiboko)	Pigeonpea	21	3	14.3	21	3	14.3
Kibwezi (UoN Irrigation Farm)	Pigeonpea	116	26	22.4	287	60	20.9
	Capsicum	6*	1	16.7*			
	Eggplant	50	10	20			
	Sweet corn	84	17	20.2			
	Tomato	31	6	19.4			
Nguruman	Okra	56	13	23.2	56	13	23.2
Mtito Andei	Tomato	30	3	10	30	3	10
Shimba Hills	Tomato	17	4	23.5	17	4	23.5
Kilifi (Insti.)	Pigeon pea	5*	2	40*	5*	2	40*
Marereni (Malindi)	Lablab	48	15	31.3	168	50	29.8
	Tomato	120	35	29.2			

* small sample size

Fig. 2.1: Map showing the natural occurrence and distribution of NPV on *H. armigera* in various sites in Kenya



Note: Figures after site name show number of *H. armigera* larvae collected followed percent virus incidence; * means limited sample size.

3.3.3 Identification and multiplication of NPV in host insects

Shiny spots of polyhedra (occlusion bodies) were observed in the wet mounts under X400 magnification. This characteristic was earlier described by Poinar and Thomas (1985). This was used to tentatively confirm *H. armigera* infection by NPV.

During multiplication of the two Kenyan virus isolates (from KISE and Kibwezi), their cumulative percent mortality was determined at 9 days post inoculation for the isolates. The percentages were calculated out of 500 and 250 larvae respectively. The cumulative percent mortality for the first isolate was found to be 64.2% while that of the second isolate was found to be 19.2%. The cadavers from the two isolates were used for NPV preparation. These isolates were named NPV-Kenya isolate 1 and 2 (NPV-K1 and NPV- K2).

3.3.4 Concentration of NPV in stock suspension

The 1:100 dilution was used to determine the number of OBs/ml (concentration) in the stock suspension since it gave the best count.

$$\text{Number of occlusion bodies per ml} = \frac{D \times X}{N \times K}$$

Where:

D = dilution factor

X = total number of polyhedral inclusion bodies counted

N = number of small squares counted

K = volume above one small square in cm³ = 2.5 x 10⁻⁷

Isolate one

$$\text{Dilution factor} = 100$$

$$\text{Number of small squares counted on haemocytometer} = 100$$

$$\text{Total number of polyhedra counted in 1:100 dilution} = 3406$$

$$\text{Therefore the number of OBs/ ml in stock suspension} = \frac{100 \times 3406}{100 \times 2.5 \times 10^{-7}}$$

$$= \frac{3406 \times 10^7}{2.5}$$

$$= 13.62 \times 10^9 \text{ OBs/ml.}$$

Isolate Two.

$$\text{Dilution factor} = 100$$

$$\text{Number of small squares counted on haemocytometer} = 100$$

$$\text{Total number of polyhedra counted in 1:100 dilution} = 2247$$

$$\text{Therefore the number of OBs/ ml in stock suspension} = \frac{100 \times 2247}{100 \times 2.5 \times 10^{-7}}$$

$$= \frac{2247 \times 10^7}{2.5}$$

$$= 898.8 \times 10^7$$

$$= 8.988 \times 10^9 \text{ OBs/ml.}$$

3.3.5 Standardization of the NPV (Quality Control)

This involved converting appropriate number of active OBs/ml into larval equivalents (LE) which is easily understood by farmers. This is the number of OBs that can be obtained from a fully infected fourth instar larva. Both the two isolates were standardized as explained below:

Isolate one

One larval equivalent (1 LE) contains 6.0×10^9 OBs

$$\begin{aligned} \text{For } 13.62 \times 10^9 \text{ OBs, number of LE} &= \frac{13.62 \times 10^9}{6.0 \times 10^9} \\ &= 2.27 \text{ LE per ml.} \end{aligned}$$

1ml of stock suspension is equivalent to 2.27 LE.

Commonly recommended dose of NPV for pigeon pea and chickpea (and many crops) is 250 LE/ha (Dhandapani and Babu, 1995), containing $250 \times (6.0 \times 10^9) = 1.5 \times 10^{12}$ OBs/ha

Therefore, 250 LE is contained in $\frac{250}{2.27} = 110.13$ ml of NPV stock suspension.

$$\begin{aligned} \text{For 100 LE, the stock suspension required} &= \frac{100 \times 110.13}{250} \\ &= 44.05 \text{ ml.} \end{aligned}$$

The suspension was diluted with distilled water such that 1 LE would be contained in 1ml of NPV suspension i.e. adding 44.05 ml of stock suspension in 55.95 ml of distilled water to make 100 ml that is equivalent to 100 LE.

Therefore 1000 LE would be obtained by adding $44.05 \times 10 = 440.5$ ml of stock suspension to 559.5 ml of distilled water to make 1000 ml.

This NPV suspension is a liquid formulation that can be used for the control of *H. armigera* in the field when mixed with the spray adjuvant.

Isolate two

One larval equivalent (1 LE) contains 6.0×10^9 OBs.

$$\begin{aligned} \text{For } 8.988 \times 10^9 \text{ OBs, number of LE} &= \frac{8.988 \times 10^9}{6.0 \times 10^9} \\ &= 1.498 \text{ LE per ml} \end{aligned}$$

1ml of stock suspension is equivalent to 1.498 LE.

Standard dose of NPV for pigeon pea and chick pea is 250 LE/ha, containing $250 \times (6 \times 10^9)$ OBs = 1.5×10^{12} OBs/ha.

Therefore, 250 LE is contained in $\frac{250}{2.27} = 166.9$ ml of NPV stock suspension.

$$\begin{aligned} \text{For 100 LE, the stock suspension required} &= \frac{100 \times 166.9}{250} \\ &= 66.76 \text{ ml.} \end{aligned}$$

The suspension was diluted with distilled water such that 1 LE would be contained in 1ml of suspension i.e. by adding 33.24 ml of distilled water to 66.76 ml of stock suspension to make 100 ml which is equivalent to 100 LE.

Therefore 1000 LE would be obtained by adding 667.6 ml of the stock suspension 332.4 ml of distilled water to make 1000 ml.

Plate 10: Sampling for *H. armigera* larvae in sweetcorn, Kibwezi Nairobi University Irrigation farm



Plate 10: Sampling for *H. armigera* larvae in sweetcorn, Nairobi (Kibwezi)

Plate 11: Sampling for *H. armigera* larvae in capsicum, Kibwezi Nairobi University Irrigation farm



Plate 12: Sampling for *H. armigera* larvae in pigeonpea field, KARI-Kiboko



Plate 13: Sampling for *H. armigera* larvae in tomato, Marereni (Malindi).



Plate 14: *H. armigera* damage on tomato



Plate 15: *H. armigera* damage on chilies



Entry/Damage
holes

Plate 16: *H. armigera* damage on sweetcorn

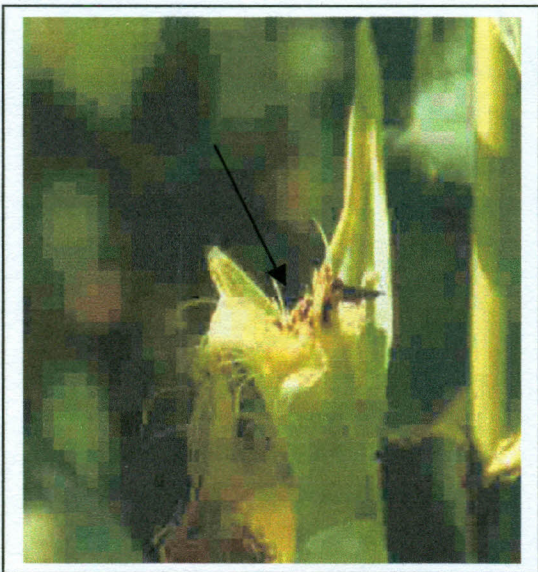
A: Larval feeding on silk



B: Larva inside silk fibres



C: Silk eaten by larva



D: Larval feeding on maize cob



Plate 17: *H. armigera* damage on okra

A: Young larva on mature fruit



B: Full grown larva on young fruit

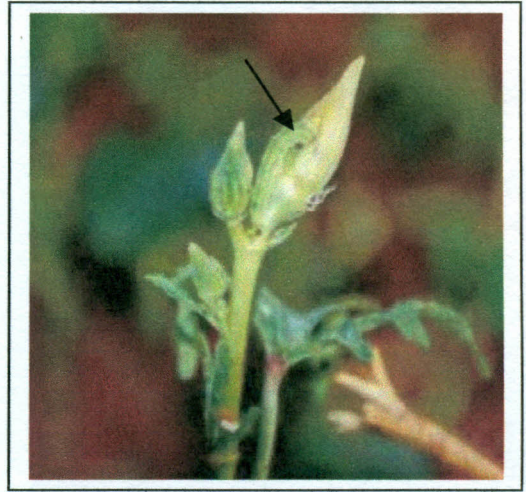
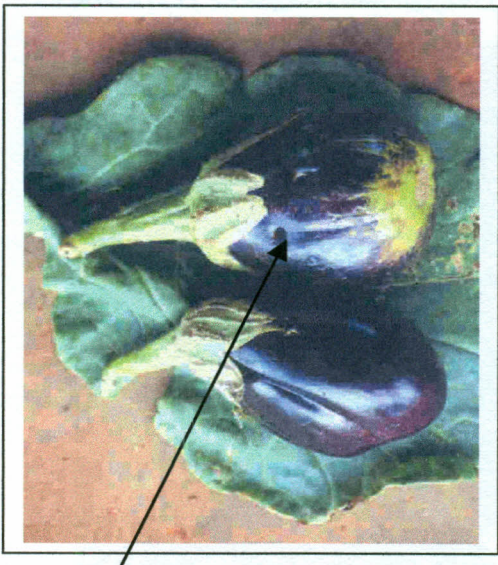
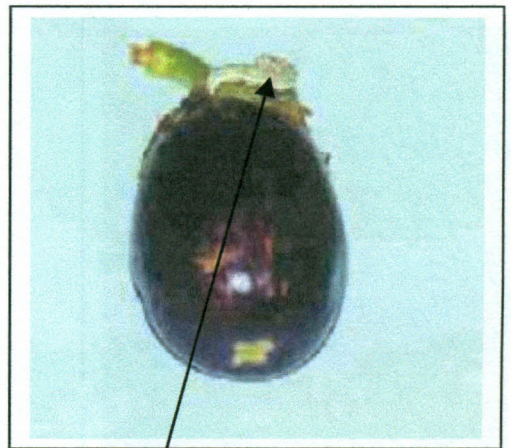


Plate 18: *H. armigera* damage on egg plant



A: Damage/entry hole



B: Larva on fruit

Plate 19: *H. armigera* damage capsicum

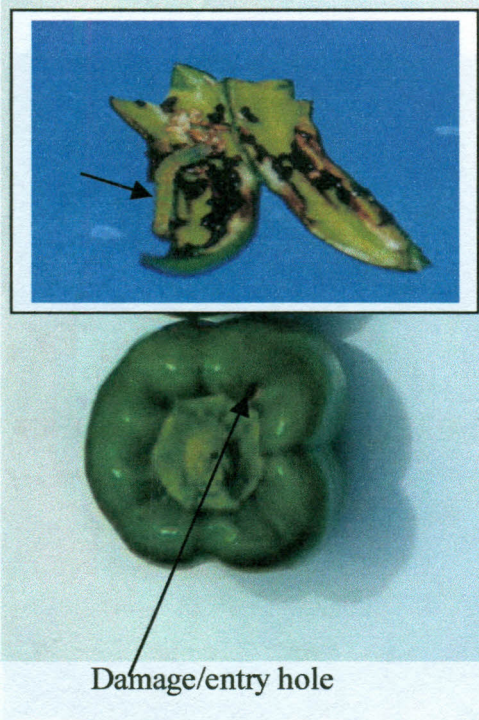


Plate 20: *H. armigera* damage on snowpeas

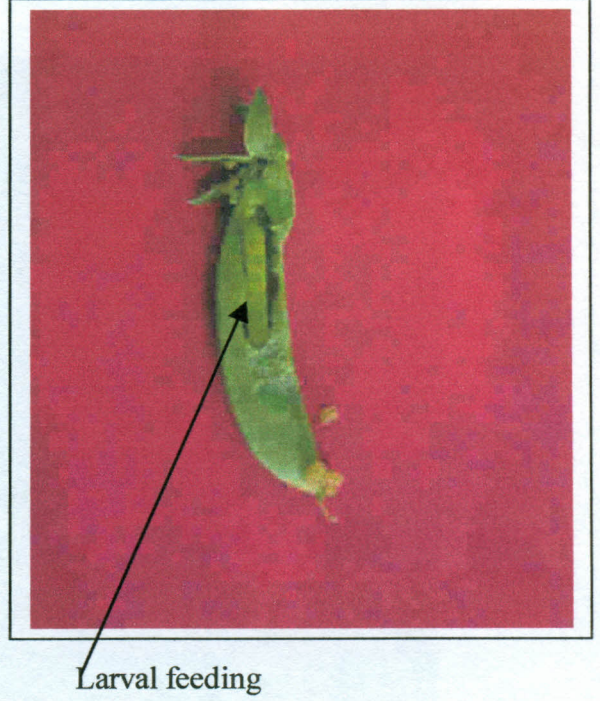


Plate 21: *H. armigera* damage on pigeon peas

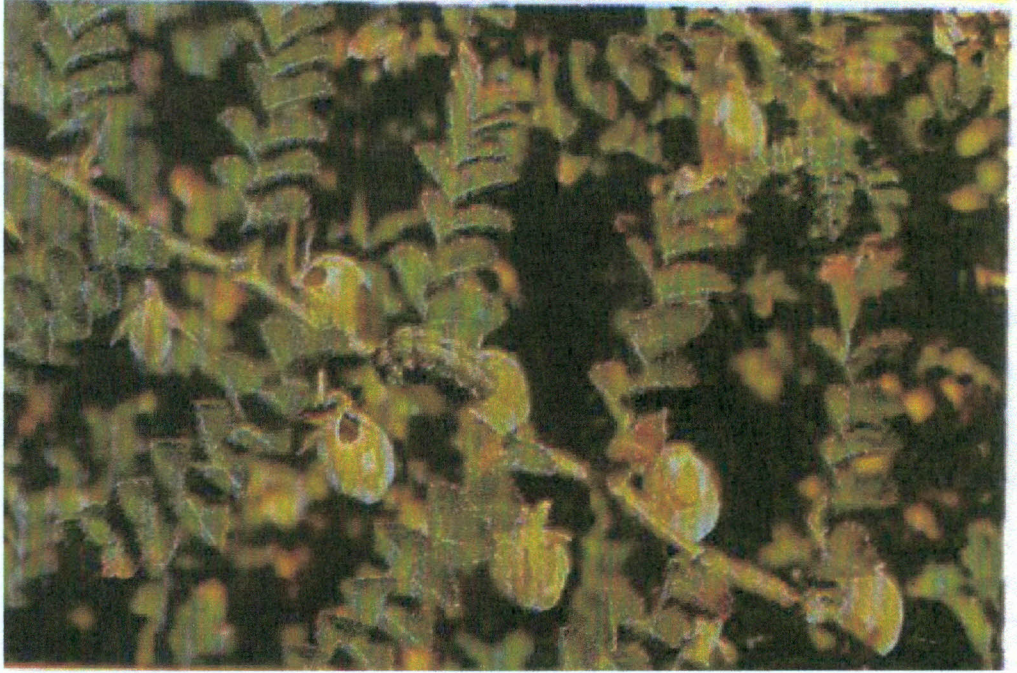


A: Young larva



B: Grown larva

Plate 22: *Helicoverpa* damage on Chickpea



3.4 DISCUSSION

The survey sites were selected based on the fact that *H. armigera* is a polyphagous pest and attacks most crops grown within available farms. However, in some study sites no larvae were observed on the available crops. For instance, when Thika, Machakos and Kangundo areas were visited, pod fly (*Melanagromyza chalcosoma* Spencer) and plume moths (*Exelastis atamosa* Walsingham) were the commonly observed insect pests on the pigeon pea plants. *H. armigera* probably may have completed their larval stage and had entered the pupal stage that normally burrow under the ground. As regards Mwea, Kibos and Kakamega, cotton fields were sampled but bugs and pink bollworms (*Pectinophora gossypiella*) were the only insect pests observed. It was more likely that *H. armigera* may already have completed their larval cycle. Occurrence of *H. armigera* in the field varied with seasons except for the irrigated farms. As would be expected, more larvae occurred during the rainy seasons than in the dry seasons and the abundance of host plants had effect on the occurrence of this insect pest. This insect pest has a wide host range and its occurrence in different seasons was certainly determined by the presence of alternative host plants (cultivated and wild) that were close to the farms visited. Most of the host plants targeted for sampling were tomatoes, pigeonpea and other peas (snow peas, snap peas). It has been reported that the occurrence of *H. armigera* in Eastern Province (the major pigeon pea growing region in Kenya) is in April to May and December to March when there are rains and thereafter pod flies take over, unless there are host plants under irrigation (Dr. Silim, pers. com.). Consequently, this could have been the

reason for the absence of *H. armigera* larvae when some fields were visited during the surveys. In Mbita, some larvae were collected from *Tridax* spp and Kales, which were the only available alternative host plants. As for the visited irrigated fields, host plants were present at least all the year round, including a wide range of weeds. The UoN Irrigation Farm (Kibwezi) is one of the sites where the pest was always present. This farm had at least one of the following vegetable crops grown all the year round: pigeonpea, tomatoes, egg plants, chilies and okra. Sweet corn was also frequently grown. Together with a wide variety of weeds (both monocots and dicots), these host plants supported the pest's population. These observations are in agreement with those by Zalucki *et al.* (1994) who observed that most of the native hosts of *H. armigera* are the agricultural crops, and more so those which are grown in dense patches. Maelzer and Zalucki (1999) stated that the abundance of *H. armigera* depends on the relative abundance of crops grown in the region as well as the abundance of the pest in each patch or field. Thus during control, there is need to consider possible pest source crops or carry out an area-wide pest management program. The pest has also been reported to exhibit learning behaviour and it has been predicted that more abundant hosts should receive proportionally more eggs than the less abundant ones and the more abundant species becomes the most preferred host by ovipositing moths (Cunningham *et al.*, 1999). This could have been the reason why females preferred tomatoes, sweet corn and pigeonpea (that were always abundant) and more than one field of these crops was present in the farm. Use of chemical pesticides in the UoN Irrigation Farm was also found to be prevalent and the Crop Protection

manager reported that the chemicals were being changed with time when found ineffective even after increasing the doses for marketable produce to be obtained. Of late, *H. armigera* is being considered as being one of the most difficult pests to control in the farm after the diamondback moth, *Plutella xylostella* (Linn.). It seemed to have developed resistance to most conventional chemical pesticides and this might have been due to their overuse or misuse. In Nguruman, the farmers reported that this pest was initially not a problem but due to misuse of chemical pesticides, the pest had become tolerant rendering them ineffective. It is now a major and serious pest of okra in the area. This was earlier reported by Sithanantham *et al.* (1998).

The variation in the incidence of *H. armigera* larvae in the sites visited could be explained by many factors. Pawar *et al.* (1986) reported that population levels of *H. armigera* at any location are the end result of complex interactions of many physical and biological factors: temperature and humidity (that directly affect reproductive rate and host plants indirectly), parasites, predators and diseases (including migration). Bouchard *et al.* (1999) observed a regularly distributed damage of tomato in the dry season which was significantly less (at the top of plants) during the rainy season. Madden *et al.* (1993) also observed that egg laying by *H. armigera* moths increased following many rainy days. The observations made in this study seem to agree with Hmimina's studies on food plants (Hmimina, 1988) in which he indicated that the reproductive potential of *H. arimgera* was dependent on seasonal succession of major food plants supporting successive generations. Srivastava *et al.* (1990) also reported obvious changes with latitude in patterns of

pheromone trap catches in India. Therefore, the understanding of these factors is important in the spatial and temporal distribution of this pest in relation to adopting the concept of integrated pest management, especially the use of insect pathogens. There is a need to monitor the population dynamics of the pest so as to understand the factors involved in its population build up and suppression. Such basic knowledge is essential for planning an effective pest management strategy that will help our farmers benefit financially without the risk of long term problems, including resurgence.

Incidence of NPV was recorded in quite a number of localities indicating natural occurrence of the virus in Kenya. However, hot humid conditions and low altitude seemed to favour the insect disease. It is suggested that the climatic conditions may be involved in determining the virus incidence. Rainfall and humidity might raise the pest incidence due to availability of food for the host and hence that of the virus. Crowding of larvae may occur and the level of disease incidence and contamination raised. The percentage virus incidence seemed to be higher in some cropping systems than in others, though within the same field and site of collection. The texture and morphology of the host plants could explain the differences noticed between plants. It is thought that the surfaces exposed to the environment (especially sunlight) and presence of allelochemicals (from exudates) in the host plants might have played a major role in the natural spread of the virus. It is thought that the greater the amount of surface exposed to oozing haemolymph, the greater the chance of contamination and feeding by larvae leading to enhanced probability of virus incidence. However, virus exposed to sunlight would be

deactivated by UV rays. Differences in allelochemical levels in host plants may have been the major factor. Differences in the pathogenicity of NPV from the effects of allelochemicals were reported by Rabindra *et al.* (1994a) where the most reduction in virulence was recorded in cotton, followed by chickpea, while pigeonpea, lablab bean and sunflower had no effect. The significant alteration of viral susceptibility of insects to pathogens is well underlined in the review by Benz (1987). Although there were variations in the percent incidence in the localities visited, this information can be used as a baseline data to exploit this baculovirus in biological pest control. The areas with high virulence could be used as multiplication/production sites and the multiplied virus can then be distributed to localities with low prevalence when required. However, the seasonality of the pest and varying virus prevalence are important factors in the production process.

The percent incidences obtained by the sampling method used in this study may also not have been a true representation of the situation in nature due to occurrence of low numbers of naturally dead larvae during the surveys. The reason could be that, most cadavers liquefy immediately after death. This observation was noted by Jorgen *et al.* (1995) who reported that quantitative sampling of dead or diseased insects, in many cases, will be useless due to difficulties in finding the quickly disintegrating cadavers. However, Steinhaus *et al.* (1995) reported that the counting of cadavers has been used as an estimation of the infection level of entomopathogenic fungi and this method can be borrowed for studying other entomopathogens. The method used in this study was based on the facts made by Fuxa (1987), that the number of hosts

afflicted with a disease at a given point in time and space (prevalence) is frequently measured by sampling and incubating living individuals and allowing infections to develop the typical macro- and microscopic features. Bearing this in mind, dead insects can be collected at different times and then prevalence can be determined. Therefore, this method was found to be of great usefulness in determining NPV prevalence.

CHAPTER FOUR

4.0. LABORATORY BIOASSAYS TO TEST PATHOGENECITY OF NPV TO *HELICOVERPA ARMIGERA* LARVAE

4.1. Introduction

Selection of viruses for use in pest management programmes requires determination of relative virulence of different isolates and preparations by testing the isolates on target species (Tompkins *et al.*, 1988). Various factors that have been cited to affect virulence include host plant sugar content, induction of metabolic changes in larvae or factors associated with the leaf surface and environmental factors. In this study virulence of two Kenyan and one Indian virus isolates were compared to determine their suitability for control of *H. armigera*. This enables one to establish the effectiveness of a local isolate and hence recommend, if there is need for importation of an exotic one, which always involves quarantine measures.

Dose-mortality data (i.e. quantity of pathogen required to kill the host), and time-mortality data (i.e. the length of time the pathogen requires to kill the host) are among the most basic information needed to characterize host-pathogen interactions (Farrar *et al.*, 1995). Properly analyzed and interpreted dose-mortality and time-mortality data on NPVs can aid in the understanding of the role of NPVs in the population dynamics of insect pests and the development of effective strategies for their application as pest control agents (Hughes, 1984; Hughes and Wood, 1986). Time-mortality relationships are

increasingly becoming important with the advent of genetically engineered NPVs that are able to kill insects faster (Farrar *et al.*, 1995). In this study, dose-mortality and time-mortality factors were investigated in order to determine the most effective dose that can be used to economically control *H. armigera* larvae in the field and also the expected effective period.

As stated earlier, potency and efficacy of insect pathogens depend on a number of factors. Whitlock (1978) stipulated that the combined effect of these factors can only be measured comprehensively through bioassay using the host itself. This is because its reaction to stimuli under different conditions is variable. Baugher and Yendol (1981) reported that insect larvae feeding on plant material treated with viruses require a higher concentration of virus to achieve the same level of control than when the insects ingest virus treated synthetic diet. In this study, a healthy colony of *H. armigera* larvae was therefore maintained on semi-synthetic diet for bioassays in order to reduce the influence of the above cited factors, although field collected larvae were also tested for comparison in one bioassay.

4.2. Materials and methods

4.2.1 The efficacy of two Kenyan isolates and one Indian NPV isolate against *H. armigera* larvae

Medium sized larvae (third and fourth instar) were collected from unsprayed pigeon pea fields at ICRISAT, Asia Center (India) in November 1998. These were placed into individual Tissue Culture multi-cell well plates with covers (12 flat-bottom wells, approximately 2.4 x 1.7 cm.; approximately

7.5 ml well capacity and approximate area 4.5 cm² per Well) (Falcon[®] - New Jersey). The larvae were starved for 24 hours to empty the gut contents and also to stimulate them to feed immediately after being supplied with food. Larvae were fed to overnight-soaked (in water) chickpea seeds contaminated with three NPV isolates, all replicated four times with thirty larvae per replicate and a control as follows:

Treatment 1 = NPV - Kenya isolate 1

Treatment 2 = NPV - Kenya isolate 2

Treatment 3 = NPV - ICRISAT (India) isolate

Treatment 4 = Control (uncontaminated seeds)

Three millilitres of each NPV isolate with a concentration of 6.0×10^9 OBs/ml were thoroughly mixed with 120 chickpea seeds in a small plastic bowl. They were then allowed to air-dry for about 5-10 minutes. Two NPV-treated chickpea seeds were placed into each cell-well for each treatment. Control larvae were placed in uncontaminated soaked chickpea seeds in a separate laboratory.

Daily mortality data was taken for each treatment for 9 consecutive days. The cumulative percentage daily mortality for each treatment was then adjusted using Abbott's Formula (Abbott, 1925) for calculating the dosage-mortality responses for pesticides:

$$\text{Adjusted percentage mortality} = \frac{(\% \text{ mortality} - \% \text{ control mortality}) \times 100}{100 - \% \text{ control mortality}}$$

Data on cumulative percent mortality on day nine was then subjected to ANOVA (SAS Institute, 1994) and means were separated by Student-Newman Keuls test. Time-mortality curves were also drawn to show the relationship between time of treatment and percent mortality of *H. armigera* larvae.

4.2.2 The effect of different NPV concentrations on the mortality of *H. armigera* larvae.

H. armigera eggs were collected from an unsprayed chickpea field at ICRISAT (India) and allowed to hatch in the laboratory at room temperature (about 20-25 °C). The hatched larvae (after three days) were placed in semi-artificial diet in small plastic containers and allowed to feed for 4 days. These were then transferred into individual wells of Tissue Culture multi-cell well plates with covers (12 flat-bottom wells, 2.4 x 1.7 cm approx.; Well capacity - 7.5 ml approx.; Area per Well = 4.5 approx.) from Falcon[®] (New Jersey). Chick pea leaves were picked from about 5 weeks old plants in the same field where the eggs were collected at about 1.30 p.m. to ensure that any naturally occurring viral spores were destroyed by the sun's UV rays. These were treated with 5 different concentrations of Kenya-NPV isolate 1 and ICRISAT (India) NPV isolate, made as shown below (Kenya NPV isolate 2 was not tested due to shortage of larvae). The initial concentration of all the isolates was 1LE/ml (1 LE is equivalent to 6.0×10^9 OBs/ml). All dilutions were made with distilled water as shown below:

1. 3 ml of virus suspension in 1 litre (equivalent to 750 LE/ha)
2. 2 ml of virus suspension in 1 litre (equivalent to 500 LE/ha)

3. 1 ml of virus suspension in 1 litre (equivalent to 250 LE/ha)
4. 0.5 ml of virus suspension in 1 litre (equivalent to 125.5 LE/ha)
5. 0.25 ml of virus suspension in 1 litre (equivalent to 62.5 LE/ha)

The chickpea leaves were soaked in each virus concentration for about 2 minutes and removed with clean forceps, placed onto absorbent paper and allowed to air-dry. 1-2 leaves were placed into each cell-well containing the larvae. Two replications of each treatment were used for each set with twelve larvae due to inadequacy of the test insects. A control was set using non-treated leaves in similar containers and placed in a separate laboratory.

The treatments were as follows:

- Treatment 1 : 3 ml/litre of Kenya virus isolate 1
- Treatment 2 : 2 ml/litre of Kenya virus isolate 1
- Treatment 3 : 1 ml/litre of Kenya virus isolate 1
- Treatment 4 : 0.5 ml/litre of Kenya virus isolate 1
- Treatment 5 : 0.25 ml/litre of Kenya virus isolate 1
- Treatment 6 : 3 ml/litre of ICRISAT (India) virus
- Treatment 7 : 2 ml/litre of ICRISAT (India) virus
- Treatment 8 : 1 ml/litre of ICRISAT (India) virus
- Treatment 9 : 0.5 ml/litre of ICRISAT(India) virus
- Treatment 10 : 0.25 ml/litre of ICRISAT(India) virus
- Treatment 11 : Control (uncontaminated leaves)

The number of dead larvae was recorded for each treatment on daily basis for fifteen days. The cumulative percent mortality for each virus isolate

and concentrations was not corrected by Abbott's Formula because there was no mortality in the controls. Probit analysis (SAS Institute, 1994) was used on the percent mortality, taking the base ten logarithm of the concentration to determine the dose-mortality and time-mortality responses. Time-mortality curves were drawn by regression to determine the mortality effect with time for the different strains and doses in time.

4.2.3 The influence of age on larval mortality.

Larvae used in this bioassay were obtained from a laboratory colony maintained on a semi-synthetic diet. Second, third fourth and fifth instar larvae were tested for their response to a Kenyan HaNPV isolate at a standard formulation dose of 250 LE/ha. About 10 ml of semi-synthetic diet was placed into glass vials (7.62 x 2.54 cm). Five microlitres (5 μ l) of NPV suspension (at a concentration of 6.0×10^9 OBs/ml) were pipetted into each vial, spread with a smooth brush and allowed to air-dry. Larvae of the different ages which had been starved overnight were placed in each glass vial and allowed to feed until death or pupation. Each larval age had twenty test insects replicated four times. Daily mortality data was recorded for each larval age until all larvae pupated. The pupae were then collected and kept at the appropriate temperature and humidity to observe the adult percent emergence. Mortality data was corrected by Abbott's formula (Abbott, 1925) and then analyzed by ANOVA. Means were separated by Students New-Man Keuls test (SAS Institute, 1994). Age-mortality curves were then drawn for each instar.

4.2.4 The effect of some local adjuvants on the efficacy of HaNPV on *H. armigera* larvae.

The adjuvants tested here (1% molasses and 1% charcoal powder) are those which have been reported to have both phagostimulation and U-V protection effect and are locally available. Larvae used in this bioassay were obtained from the laboratory culture reared on a semi-synthetic diet. About 10 ml of semi-synthetic diet was placed into 7.62 x 2.54 cm glass vials and the surface was contaminated with virus. Surface contamination was achieved by pipetting 10 microlitres of each sample into the glass vials, spreading with a smooth brush and allowing for air-drying. The treatments were set as below, each with ten larvae and replicated four times.

- | | | |
|-----------|----|---------------------------------------|
| Treatment | 1: | NPV alone at 1ml/litre |
| Treatment | 2: | NPV at 1ml/litre + 1 % Molasses |
| Treatment | 3: | NPV at 1ml/litre + 1% Charcoal powder |
| Treatment | 4: | Distilled water (control). |

Six-day-old *H. armigera* larvae were placed individually into the glass vials and allowed to feed until death or pupation. Daily mortality data was taken for each treatment for nine consecutive days. Cumulative percent mortality was determined and then adjusted for mortality in the controls by Abbots Formula (Abbott, 1925). Data was analyzed by ANOVA and the means were separated by Student-Newman Keuls test (SAS Institute, 1994).

4.3 Results

4.3.1 The efficacy of two Kenyan isolates and one Indian NPV isolate against *H. armigera* larvae.

The Kenyan NPV isolates 1 and 2 caused of $78.3 \pm 7.38\%$ and $68.3 \pm 5.68\%$ *H. armigera* larval mortality respectively and were apparently superior to the Indian (ICRISAT) isolate which caused $55.9 \pm 2.10\%$ mortality. Kenyan NPV isolate 1 was also superior to the Kenyan NPV isolate 2 (Table 3.1; Figures 3.1 and 3.2). However, the differences were not statistically significant (d.f = 3,15; $F = 14.76$; $p = 0.05$). A relatively low mortality of $25.0 \pm 4.41\%$ that was recorded in the control was significantly different from the NPV treatments.

Daily cumulative percent mortality increased gradually with time but a steep increase was noticed as from day 4 and 5 and the highest on the eighth and ninth day in all the virus isolates (Fig. 3.2). The cumulative percent mortality was 67.5% on day 8 and 78.3% on day 9 for the Kenyan isolate 1, 65.8% on day 8 and 68.3% on day 9 for the Kenyan isolate 2, and 50% on day 8 and 55.8% on day 9 for the Indian isolate. However, after day 8 there was a high decline in the rate of rise in mortality for the Kenyan isolate 2 and a slight decline for the Indian isolate (an increase of 3% and 5.8% respectively).

Table 3.1: Percentage mortality of *H. armigera* larvae caused by two Kenyan and one Indian (ICRISAT) NPV isolates

Treatment.	% mortality at 9 days \pm SE
1. Kenya NPV isolate - 1	78.3 \pm 7.38 a
2. Kenya NPV isolate - 2	68.3 \pm 5.68 a
3. Indian (ICRISAT) Strain	55.9 \pm 2.10 a
4. No NPV (Control).	25.0 \pm 4.41 b
Mean	56.87
C.V.(%)	21.21

Means with the same letter are not significantly different at $p \geq 0.05$

Fig. 3.1: Percentage relative mortality of *H. armigera* larvae due to two Kenyan and an Indian NPV isolates

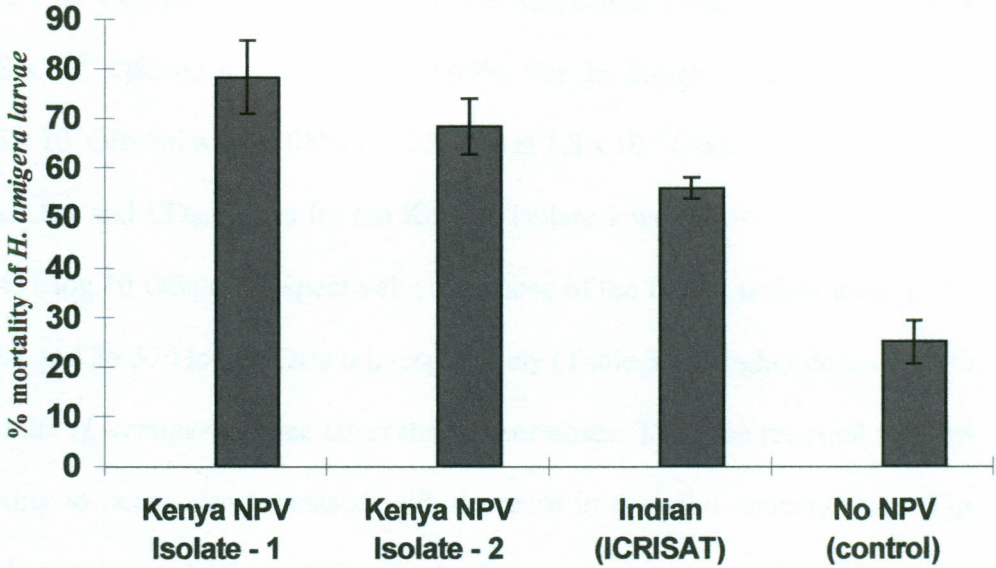
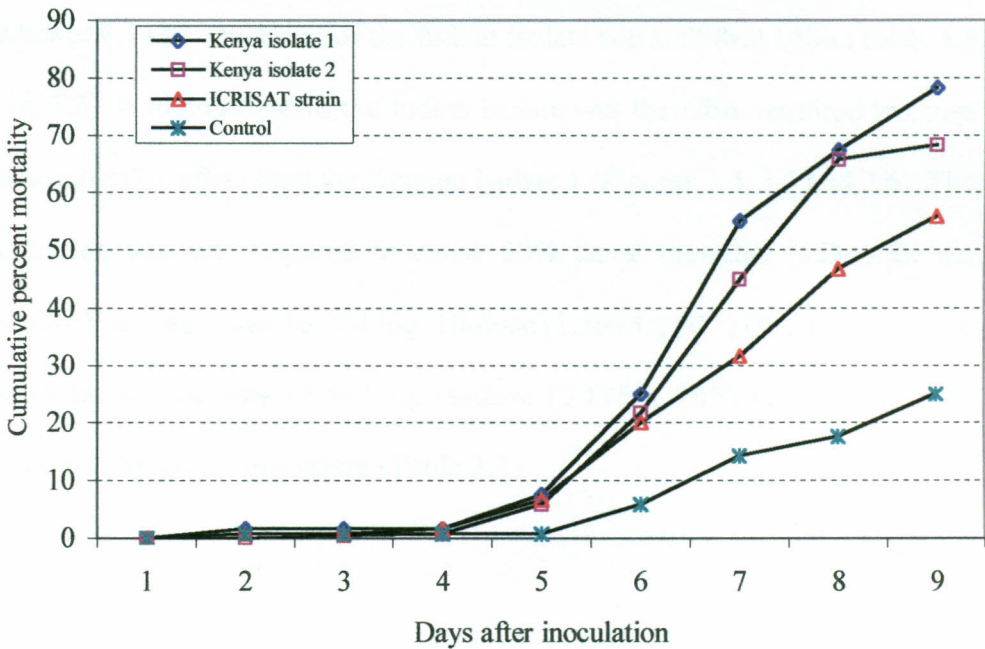


Fig. 3.2: Cumulative percentage relative mortality of *H. armigera* larvae due to two Kenyan and an Indian (ICRISAT) NPV isolates.



4.3.2 The effect of different concentrations of NPV on the mortality of *H. armigera* larvae

At 1.5×10^9 OBs/ml the response for the Kenyan isolate 1 was only 28.75% while at 1.8×10^{10} OBs/ml it increased to 52.09%. For the Indian isolate, the mortality at 1.5×10^9 OBs/ml was 32.08% and 38.33% at 1.8×10^{10} OBs/ml (Table 3.2). The LD₃₀, LD₅₀ and LD₉₀ values for the Kenyan isolate 1 were 9.3697, 10.2041 and 12.2431 log₁₀ OBs/ml, respectively; and those of the Indian isolate were 8.155, 13.502 and 26.570 log₁₀ OBs/ml, respectively (Table 3.3). Higher doses of NPV killed the *H. armigera* larvae faster than lower doses. The time required for 50% mortality to occur also increased with decrease in the viral concentration (Fig. 3.4). For instance, 50% mortality for the Kenyan isolate 1 at 1.8×10^{10} OBs/ml was achieved after about 4.8 days and about 6.5 days for the Indian isolate. At 1.5×10^9 OBs/ml, the periods increased to 8 and 9 days for the Kenyan Isolate 1 and Indian isolate, respectively (Fig. 3.4). The slope of the Kenyan isolate 1 at LD₅₀ was 0.6285 ± 0.1480 while that of the Indian isolate was 0.098 ± 0.1486 (Table 3.3 and Fig. 3.3). A higher dose of the Indian isolate was therefore required to cause the same mortality effect than the Kenyan Isolate 1 (Figures 3.4, 3.5 and 3.6). The concentration that was required to cause 50% larval mortality (LD₅₀) for the Kenyan NPV isolate 1 was 10.204 log₁₀-dose (1.5998×10^{10}) OBs/ml while that of the Indian isolate was 13.502-log₁₀-dose (3.178×10^{13}) OBs/ml at 95 % fiducial limits by probit procedure (Table 3.3).

Table 3.2: Dose-response analysis of a Kenyan and an Indian NPV isolates at ICRISAT, 1998.

Virus isolate	Dose (OBs/ml)	Response	Probit	SE	Xbeta
KENYA-1	1.5 x 10 ⁹	28.7500	0.25930	0.17605	-1.04963
KENYA-1	3.0 x 10 ⁹	30.8330	0.32273	0.12035	-0.74125
KENYA-1	6.0 x 10 ⁹	34.1655	0.39344	0.09337	-0.43288
KENYA-1	1.2 x 10 ¹⁰	50.0010	0.46891	0.11702	-0.12451
KENYA-1	1.8 x 10 ¹⁰	52.0850	0.51397	0.14691	0.05588
INDIAN	1.5 x 10 ⁹	32.0835	0.33573	0.16785	-0.68236
INDIAN	3.0 x 10 ⁹	37.4995	0.34651	0.11529	-0.63441
INDIAN	6.0 x 10 ⁹	35.0005	0.35745	0.09350	-0.58645
INDIAN	1.2 x 10 ¹⁰	35.4155	0.36854	0.12051	-0.53849
INDIAN	1.8 x 10 ¹⁰	38.3335	0.37509	0.15064	-0.5104

Table 3.3: Probit-dose response of a Kenyan and an Indian NPV isolates.

Virus isolate	Probit	LD	Log10 Dose	95% Fiducial limits	Slope±SE
KENYA-1	4.48	30	9.3697	8.9493-9.5716	0.6285±0.1480
	5.00	50	10.2041	9.9971-10.6443	0.6285±0.1480
	5.52	70	11.0384	10.6118-12.1502	0.6285±0.1480
	6.28	90	12.2431	11.4490-14.3747	0.6285±0.1480
INDIAN	4.48	30	8.155	-	0.0981±0.1468
	5.00	50	13.502	-	0.0981±0.1468
	5.52	70	18.849	-	0.0981±0.1468
	6.28	90	26.570	-	0.0981±0.1468

Fig.3.3 Dose-mortality responses of *H. armigera* larvae in a Kenyan and an Indian NPV isolates, Lab. study, ICRISAT, 1998.

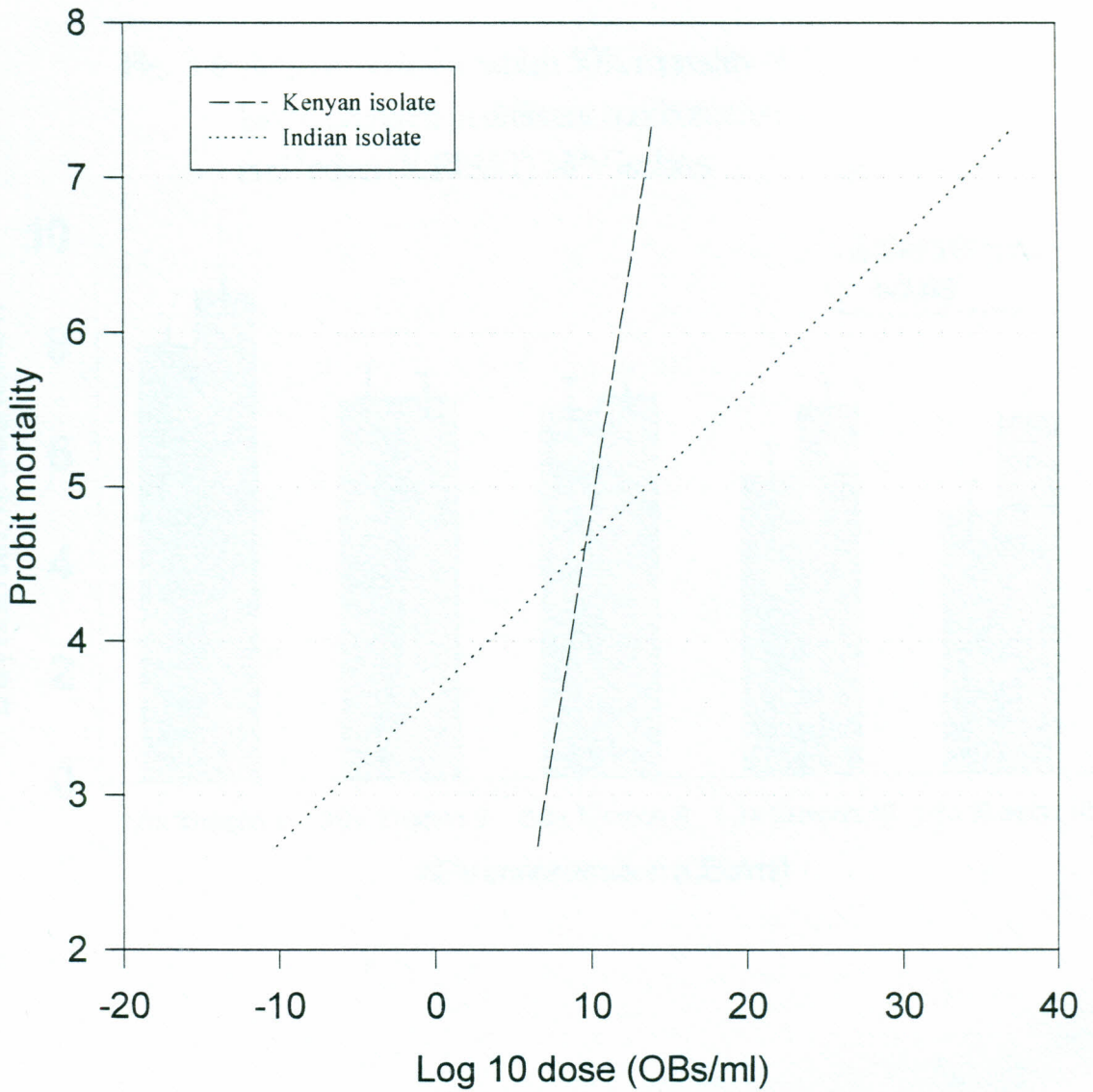


Fig. 3.4: Relative period at which 50% mortality of *H. armigera* larvae occurred in different concentrations of a Kenyan and Indian (ICRISAT) NPV isolates

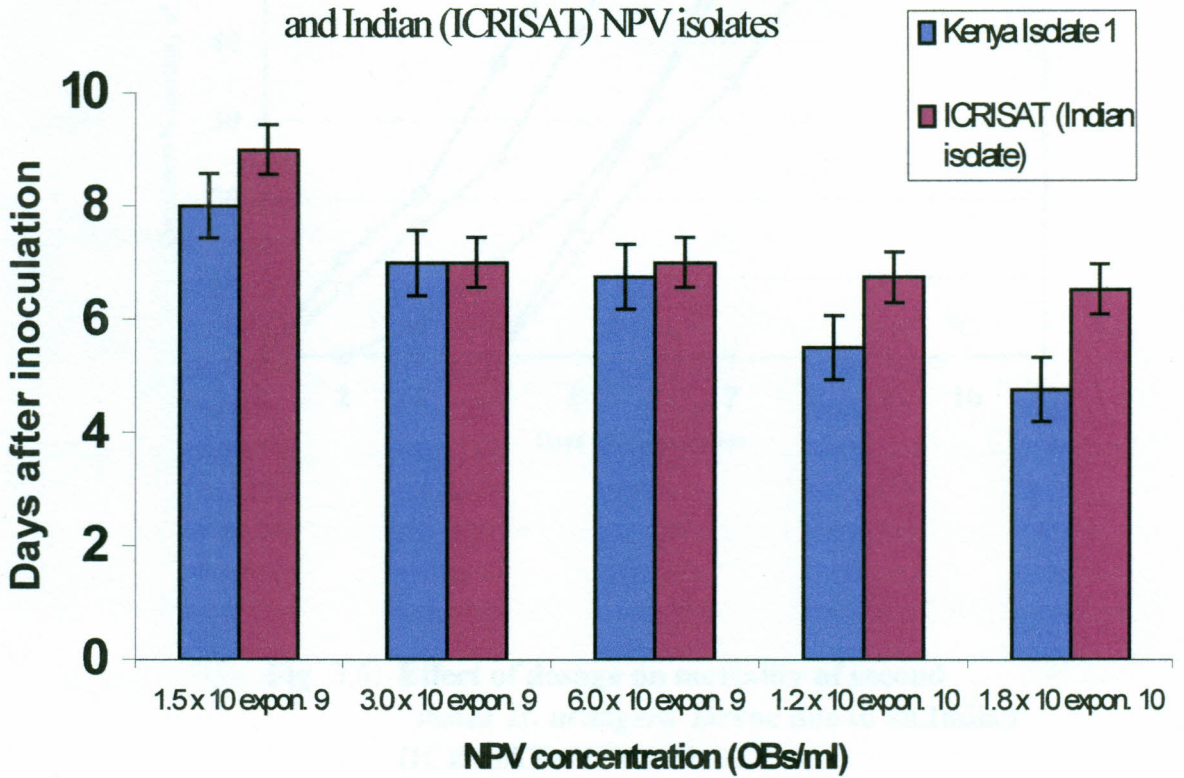


Fig. 3.5: Effect of dosage on mortality of second instar *H. armigera* larvae due to a Kenyan NPV isolate 1.

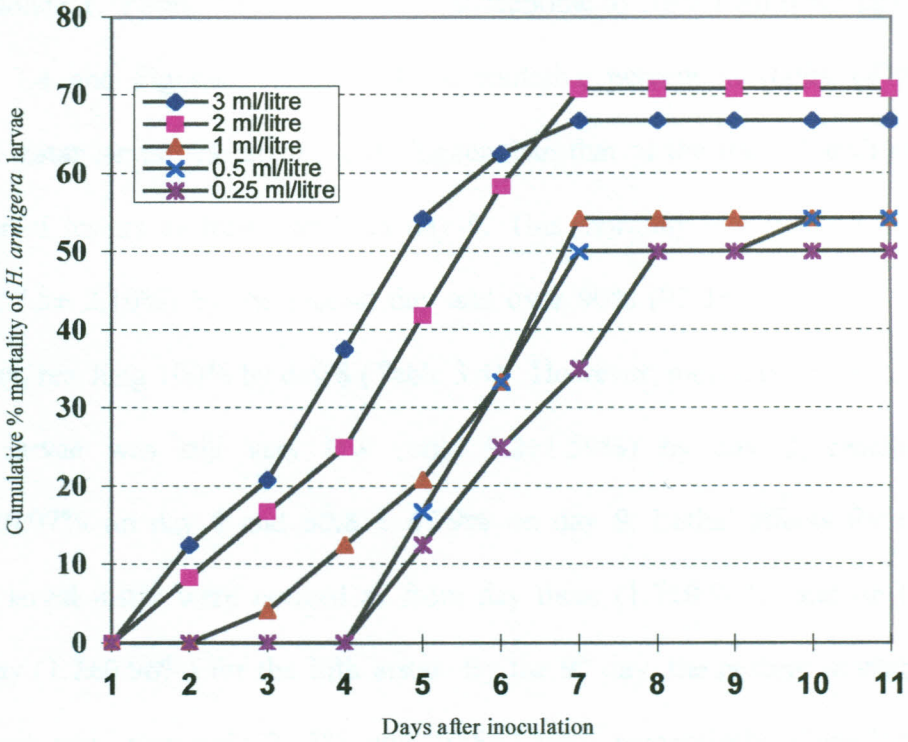
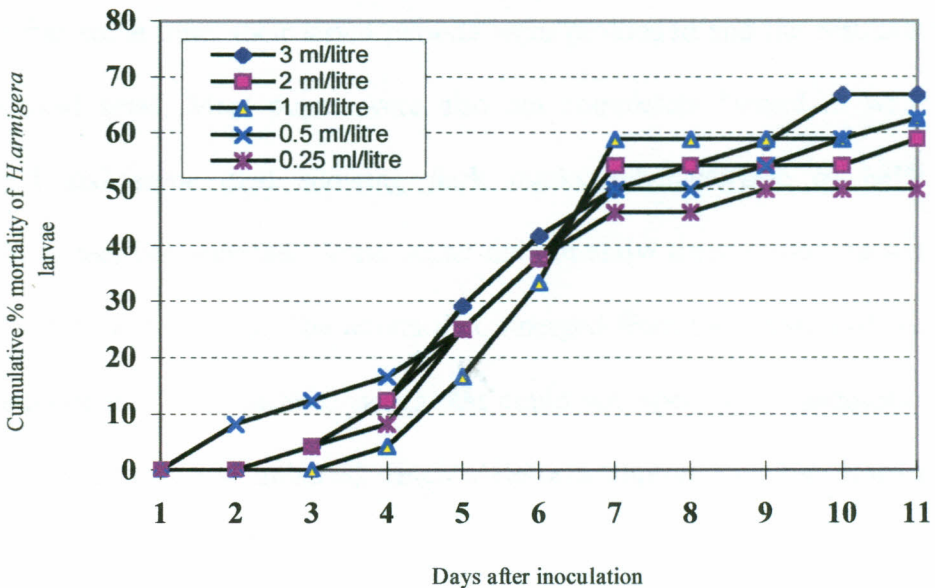


Fig. 3.6: Effect of dosage on mortality of second instar *H. armigera* larvae due to an Indian (ICRISAT) NPV isolate.



4.3.3 The influence of age on larval mortality.

Results given in this study are based on tests carried out on the Kenyan NPV isolate 1. Young larvae were more susceptible to NPV than older larvae (Table 3.4 and Figures 3.7 and 3.8). Cumulative percent mortality of the second instar larvae was significantly higher than that of the third, fourth and fifth larval instars as from day 2 to day 9. This mortality had reached over 50% ($57.5 \pm 2.10\%$) by the second day and over 90% ($93.3 \pm 1.36\%$) by the fifth day, reaching 100% by day 8 (Table 3.4). However, mortality of the third instar larvae was still very low (only $9.2 \pm 1.59\%$) by day 2, reaching $45.4 \pm 10.07\%$ on day 8 and $60.8 \pm 4.79\%$ on day 9. Lethal effects for the fourth larval instar were noticed as from day three ($1.7 \pm 0.96\%$) and on the fifth day ($1.7 \pm 0.96\%$) for the fifth instar. By the 9th day, the percent mortality for these ages was only 23.3% and $10.8 \pm 2.49\%$, respectively. Cumulative percent mortality recorded for the fourth larval instar was not above 40% while that of the fifth larval instar remained below 20% even after nine days post inoculation (Figures 3.7 and 3.8).

The surviving larvae, mainly the late instars (fourth and fifth) stopped feeding after some time, their larval periods were prolonged and the resultant pupae looked small. Most pupae were also not completely formed or were malformed and some died showing dark marks (characteristic of NPV infection) or had decomposed. Some pupae did not show these symptoms but did not transform to adults. The adults that emerged from the pupae had the wings reduced into wing pads or wings that could not open. Their abdomens were soft, elongated and inflamed. Such abdomens ruptured on the first or

second day releasing a brown-yellowish fluid leading to death of the insect.

The few adults that emerged either did not lay or laid very few eggs, most of which did not hatch (shrunk).

Table 3.4: Percentage mortality of different larval stages of *H. armigera* larvae caused by the Kenyan NPV isolate 1.

Instar	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
II	0	57.5± 2.10 a	78.3± 2.2 a	86.7± 1.36 a	93.3± 1.36 a	95.8± 2.50 a	98.3± 0.96 a	100 ± 0 a	100 ± 0 a
III	0	9.2± 1.59 b	15.8± 0.84 b	18.3± 0.96 b	23.3± 0 b	34.7± 0.84 b	42.2± 30.3 b	45.4± 10.07 b	60.8± 4.79 b
IV	0	0 c	1.7± 0.96 c	4.2± 0.84 c	9.17± 0.84 c	15.8± 0.84 c	20.8± 0.83 c	23.3± 0 c	23.3 ± 0 c
V	0	0 c	0 c	0 d	0 d	1.7± 0.96 d	6.7± 2.36 d	9.7± 2.37 c	10.8± 2.49 c
Control	0	0 c	0 c	0 d	0 d	0 d	0 e	1.7± 0.96 d	4.2± 4.17 d
Mean	0	13.3	19.2	21.8	26.0	31.1	35.1	37.9	41.2
C.V.	—	18.3	12.1	8.4	15.3	15.5	15.2	28.9	16.2

Means within the same column with the same letter are not significantly different by Student-Newman Keuls test (SNK) at $p \geq 0.05$.

Fig. 3.7: *H. armigera* mortality due to the Kenyan NPV isolate 1 at different larval stages.

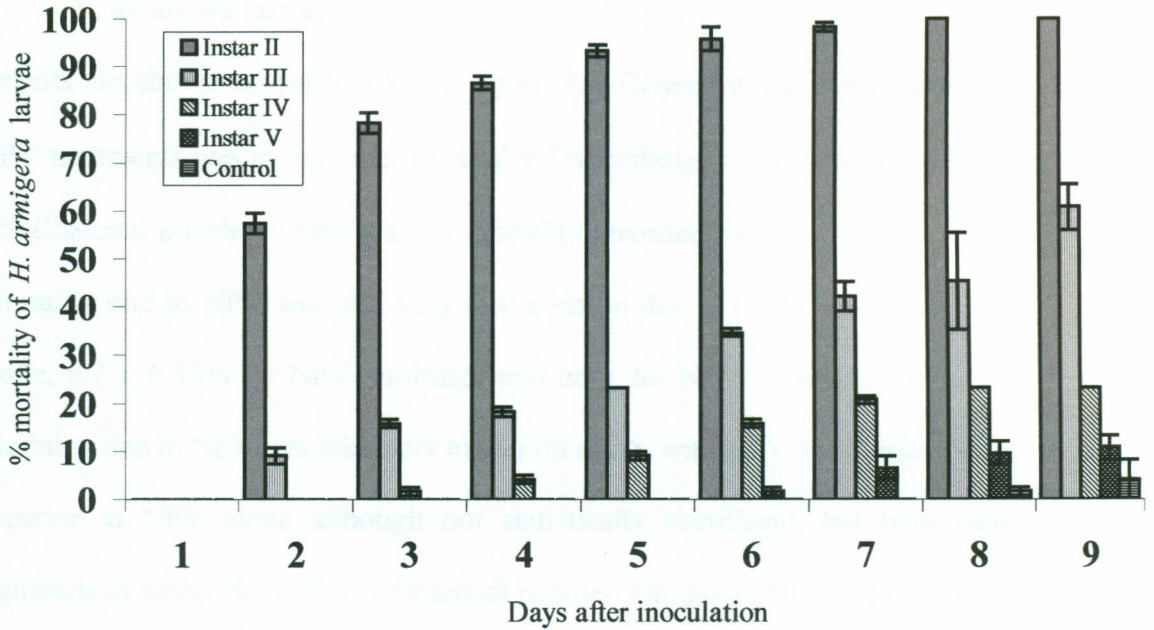
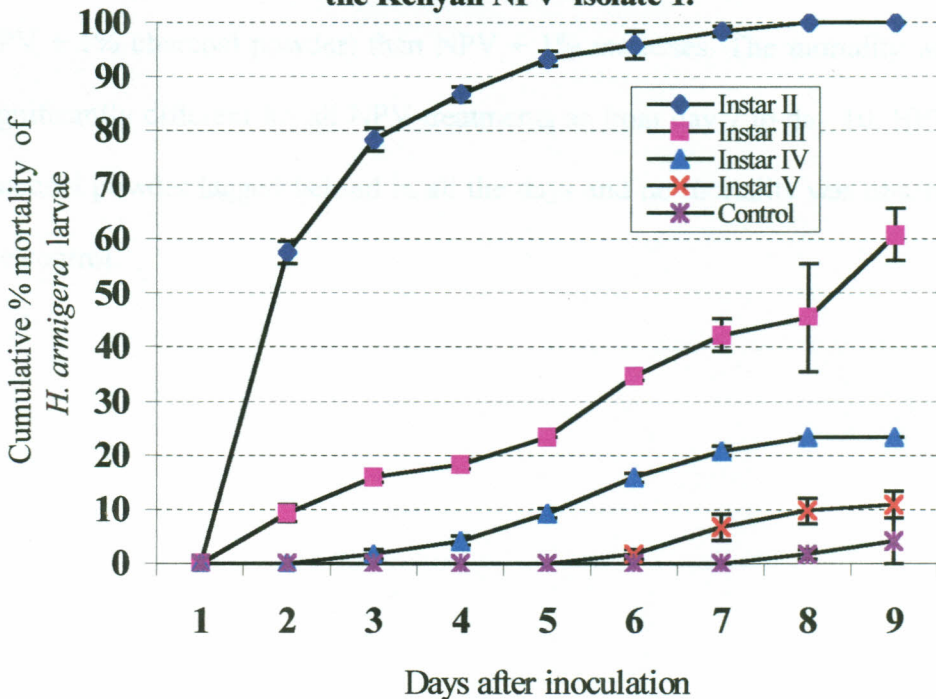


Fig. 3.8: Effect of larval age on the cumulative % mortality of *H. armigera* larvae treated with the Kenyan NPV isolate 1.



4.3.4: The effect of some local adjuvants on the efficacy of HaNPV on

H. armigera larvae

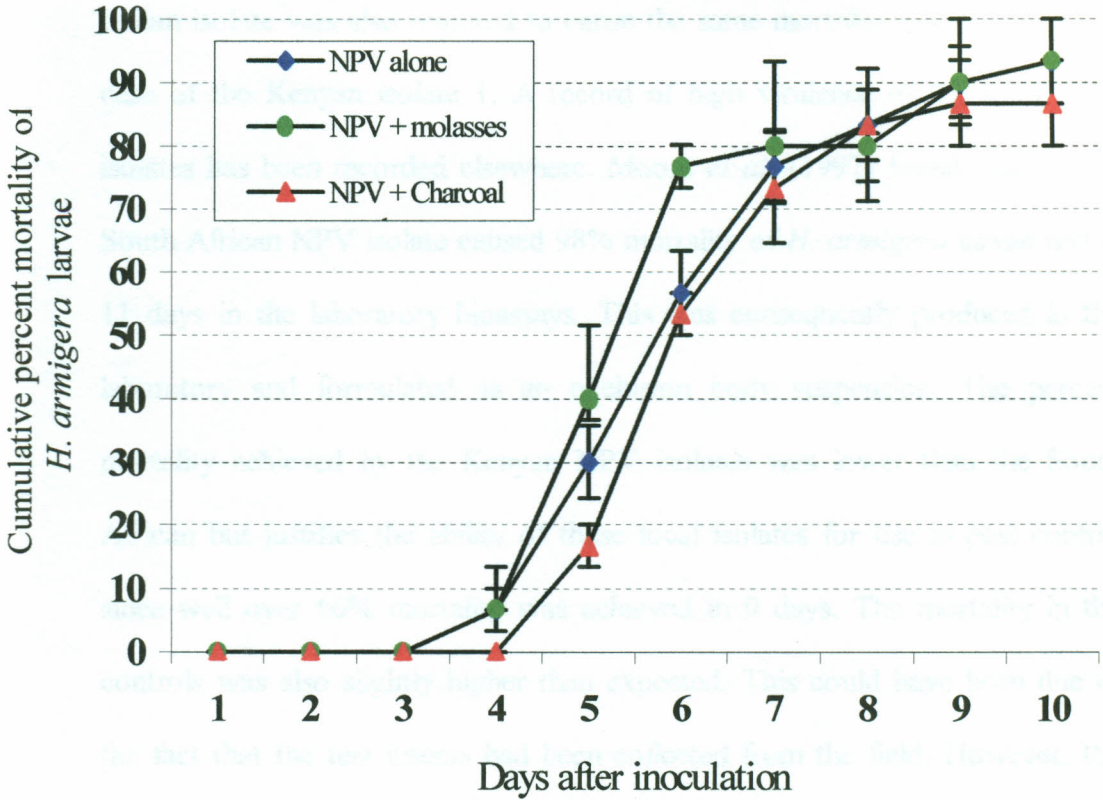
Results are shown in Table 3.5 and figure 3.9. Generally, the effectiveness of NPV treatments was in the order of NPV + 1% molasses > NPV alone > NPV + 1% Charcoal powder. There was no mortality recorded for the first three days. Mortality due to NPV was still very low even on day 4 ($6.7 \pm 6.67\%$ for NPV alone, $6.7 \pm 6.33\%$ for NPV+molasses and none for NPV + charcoal powder). Mortality due to NPV was relatively higher on day 5, with NPV + molasses being superior to NPV alone although not statistically significant, but both were significantly better than NPV + Charcoal powder. On day 6, NPV+1% molasses was significantly superior to NPV + 1% charcoal powder and NPV alone. Thereafter, there were no significant differences in all the NPV treatments. NPV alone however caused a slightly higher mortality on day 8 (which was at par with NPV + 1% charcoal powder) than NPV + 1% molasses. The mortality was not significantly different for all NPV treatments as from day 7 to day 10. NPV+1% charcoal powder lagged behind in all the days and no mortality was observed in the control.

Table 3.5: Relative percentage mortality of 6 day old *H. armigera* larvae caused by NPV with and without adjuvant.

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
NPV alone	0	0	0	6.7± 6.67 a	30±11.54 ab	56.7±3.33 b	76.7±13.3 a	83.3±2.02 a	90.0±10 a	93.3±6.67a
NPV + 1 % Molasses	0	0	0	6.7± 3.33 a	40±5.77 a	76.7±6.67 a	80.0±5.77 a	80.0±5.57 a	90.0±5.77 a	93.3±6.67 a
NPV+ 1% Charcoal powder	0	0	0	0 b	16.7± 3.33 b	53.3± 3.33 b	73.3±8.82 a	83.3± 8.82 a	86.7±6.7 a	86.7±6.7 a
Control	0	0	0	0 b	0 c	0 c	0 b	0 b	0 b	0 b
Mean	0	0	0	3.33	21.7	46.7	57.5	61.7	66.7	68.3
C.V. (%)	-	-	-	217.9	54.9	14.7	23.4	17.7	13.0	10.9

Means within the same column with the same letter are not significantly different by Student-Newman Keuls (SNK) test at $p > 0.05$.

Fig. 3.9: Influence of molasses and charcoal powder on the efficacy of HaNPV, Laboratory study.



3.4 DISCUSSION

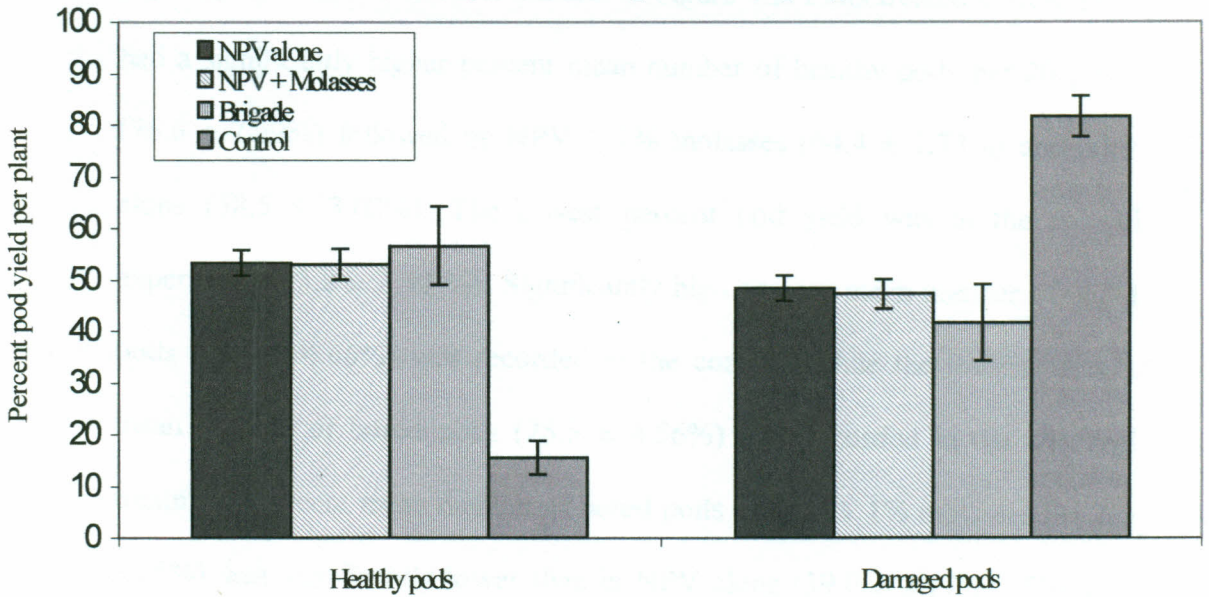
Pathogenicity tests showed that the local (Kenyan) isolates performed slightly better than the exotic (Indian) isolate. The most virulent (Kenya isolate 1) recorded a $78.3 \pm 7.83\%$ mortality of *H. armigera* larvae within 9 days, compared to $55.9 \pm 2.10\%$ of the Indian isolate. A higher dose of the Indian isolate was also required to cause the same mortality effect than in the case of the Kenyan isolate 1. A record of high virulence of the local NPV isolates has been recorded elsewhere. Moore *et al.* (1997) found that a local South African NPV isolate caused 98% mortality of *H. armigera* larvae within 11 days in the laboratory bioassays. This was consequently produced in the laboratory and formulated as an occlusion body suspension. The percent mortality achieved by the Kenyan NPV isolates was lower than the South African but justifies the ability of these local isolates for use in pest control since well over 60% mortality was achieved in 9 days. The mortality in the controls was also slightly higher than expected. This could have been due to the fact that the test insects had been collected from the field. However, the mortality due to the NPV treatments was significantly different higher than the controls. Better results probably would have been obtained with the laboratory reared test insects.

The effect of dosage on larval mortality was also been investigated and was found to be dose dependent. Higher doses killed faster, with lower lethal time (LT_{50}) values recorded than with lower doses. Similar results were reported by Whitlock (1978), Sivilai-Sirimung (1982), Dhamdhare and Khaire (1986), Dhandapani and Babu (1995) and Galande and Ajri (1997). All studies

Table 4.1: Relative efficacy of NPV to control artificially infested *H. armigera* on potted snow pea plants

Treatment	% healthy pods/plant	% damaged pods/plant
NPV alone	53.5±8.10 a	48.3±8.63 b
NPV +1% molasses	53.1±11.14 a	47.2±10.61 b
Brigade®	56.6±10.66 a	41.7±10.47 b
Tap water (Control)	15.5±3.23 b	81.7±4.00 a
Mean	39.63	59.65
C.V (%)	44.42	30.31

Fig. 4.1: Relative efficacy of NPV for the control of *H. armigera* larvae artificially infested on potted snowpea plants



5.3.2: Preliminary field testing for the bioefficacy of a local NPV isolate on pigeon pea.

Some larvae that had died with NPV symptoms were observed in the pigeon pea NPV-treated plots in the field, mostly noticed by the appearance of small scabs on the leaves. All the larvae sampled alive from these plots were observed dying in the laboratory with NPV symptoms 5-7 days after the treatment (i.e. 2-3 days after collection). Larvae on the Karate[®] plots rare and the few which were sampled from these plots developed into pupal and adult stages with minor malformation. In the controls, no dead larvae could be observed and among most those sampled a few died due to parasitism by tachinid flies or braconid wasps while being reared in the laboratory.

Results of the yield data in the preliminary field efficacy test with pigeon pea are given in Table 4.2 and in Figure 4.2. Plots treated with Karate[®] had a significantly higher percent mean number of healthy pods per 20 plants ($76.6 \pm 7.29\%$) followed by NPV + 1% molasses ($64.4 \pm 1.73\%$) and NPV alone ($58.5 \pm 3.02\%$). The lowest percent pod yield was in the control experiment ($43.8 \pm 2.40\%$). Significantly high percent mean number of bored pods ($62.5 \pm 4.66\%$) was recorded in the controls while the lowest percent mean number of bored pods ($25.5 \pm 4.56\%$) was recorded in the chemical treatment. Percent mean number of bored pods in NPV + 1% molasses ($31.1 \pm 2.05\%$) was significantly lower than in NPV alone ($39.0 \pm 2.17\%$). The seed weight from the healthy pods also followed a similar trend, with those from Karate plots weighing significantly more ($84.3 \pm 7.75\%$) than the NPV + 1% molasses ($77.4 \pm 2.40\%$) and then NPV alone ($73.7 \pm 0.59\%$). The latter two

were also weighed significantly more than the untreated control plots which weighed the lowest ($57.1 \pm 1.73\%$). There were no significant differences between the percent mean weight of healthy seeds from the NPV treated plots but the NPV+ 1% molasses was apparently superior, hence provided better control. However, the weight of these seeds was highest in the Karate treatment, followed by the NPV + 1% molasses and least in the control.

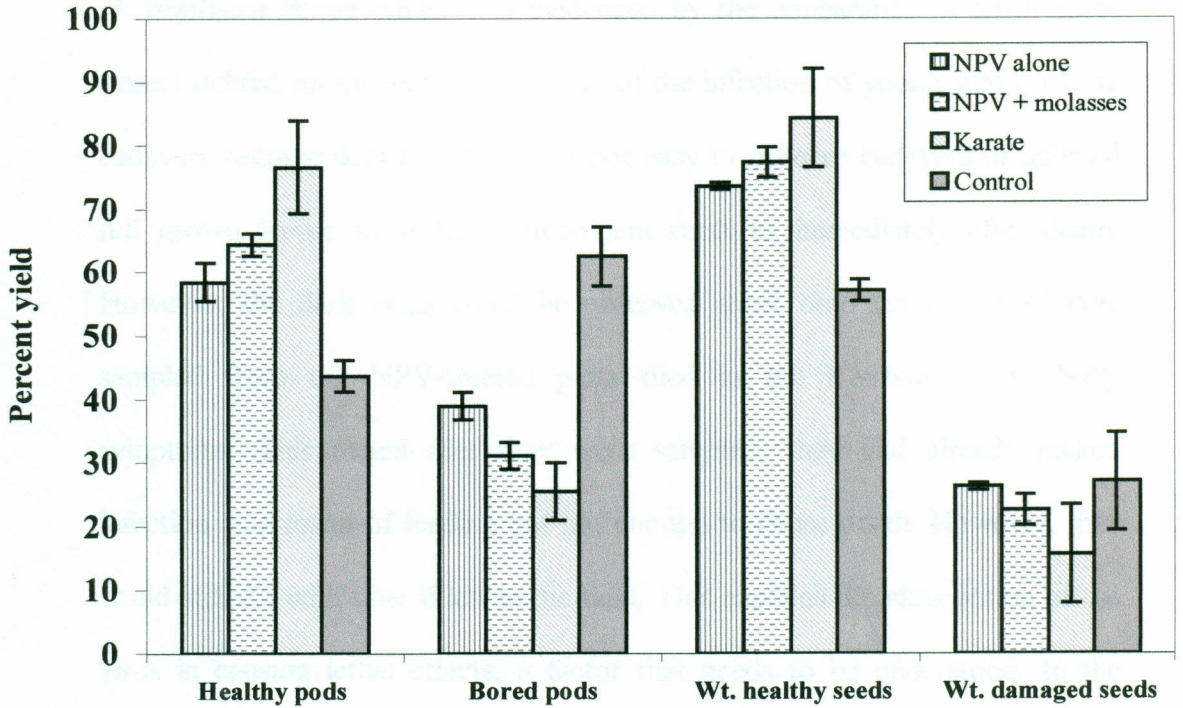
Table 4.2: Percentage pod and seed yield per 20 plants during a preliminary field efficacy testing of HaNPV against *H. armigera* on vegetable pigeon pea.

Treatment	Percent healthy pods	Percent bored pods	Percent weight of healthy seeds (g)	Percent weight of damaged seeds (g)
NPV alone	58.5 ± 3.02 c	39.0 ± 2.17 b	73.7 ± 0.59 b	26.3 ± 0.59 c
NPV + 1% molasses	64.4 ± 1.73 b	31.1 ± 2.05 c	77.4 ± 2.40 b	22.6 ± 2.40 b
Karate [®]	76.6 ± 7.29 a	25.5 ± 4.56 d	84.3 ± 7.75 a	15.7 ± 7.75 a
Tap water (control)	43.8 ± 2.40 d	62.5 ± 4.66 a	57.1 ± 1.73 c	27.1 ± 7.69 d
Mean	62.2	39.5	73.19	22.9
C.V. (%)	17.9	25.4	11.8	39.8

Means within the same column with the same alphabetical letter are not significantly different by Student-Newman Keuls test (SNK) at $p > 0.05$.

Plot size - 3.5 m x 3.5 m

**Fig. 4.2: Percentage pod and seed yield per 20 plants, pigeon pea-
NPV preliminary field efficacy test.**



5.4 DISCUSSION

In the field experiment, it was evident that the NPV applications killed *H. armigera* larvae which was evidenced by the appearance of small scabs (insect debris) on the leaves as a result of the infection of young larvae whose cadavers become dehydrated. It was not easy to observe cadavers of infected full grown larvae since their integument ruptures immediately after death. However, the dark skins could be observed stuck onto leaves. All larvae sampled from the NPV-treated plots died in the laboratory with NPV symptoms. This meant that they were sampled, they had already picked infection as a result of feeding but not enough to cause death. However, they could still die with time if left in the field. This explains the slow action of the virus in causing lethal effects, a factor that needs to be understood. In the preliminary pigeon pea yield data, better control of *H. armigera* larvae was obtained with Karate[®] (Lambda-cyhalothrin) (i.e. better results on the mean number of healthy pods, least number of bored pods and highest mean seed weights). However, the results achieved with the NPV treatments were better than in the untreated plots, especially where 1% molasses was added to NPV. These results indicate the potential of local HaNPV isolates for the field control of *H. armigera*. The potential of NPV for the field control of *H. armigera* has been demonstrated elsewhere. Moore *et al.* (1997) reported good control of *H. armigera* larvae with a local South African NPV isolate when sprayed on tomato plants artificially infested with the larvae in a nursery pilot trial. When this NPV isolate was sprayed on navel orange plants in the field, 100% bollworm mortality was achieved in 14 days or slightly longer. Cherry

et al. (1999) reported that treatment of chickpea with HarNPV at 1.5×10^{12} OBs/ha was as effective, or better at controlling *H. armigera* larvae, and increased yield relative to the control than either a standard chemical insecticide (Endosulfan) or *Bacillus thuringiensis* in two successive years.

The yield data shown in this study does not reflect a true representation of the actual yield because pod setting was below average due to water stress. The infestation by *H. armigera* larvae was equally not high as shown in the control treatment results. NPV applied alone achieved lower mean number of healthy pods, higher mean number of bored pods and mean weight of damaged seeds compared to the NPV+ molasses treatment. Thus, the latter treatment increased the potency of the NPV isolate. Molasses is known to be a phagostimulant and a UV-protectant. It increases larval feeding, hence making larvae to take in more virus particles while feeding. It also reduces loss of viral activity by sunlight irradiation (Sonalkar *et al.*, 1997; Rabindra *et al.*, 1989). This material can be easily obtained locally and when added to the crude extracts of NPV, it may raise its potency and hence bring effective control of *H. armigera* on vegetable crops amongst the resource poor small scale vegetable farmers. The problem of slow action of the virus recorded in this study hence lower level of control is not a new phenomenon. It may be tackled by incorporating other pest control methods in an integrated pest management programme (IPM).

CHAPTER SIX

6.0 GENERAL DISCUSSION, CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FUTURE STUDIES.

6.1 General discussion

6.1.1 Natural occurrence of NPV pathogenic to *H. armigera* larvae

Mortality of *H. armigera* larvae by naturally occurring NPV was observed from different localities. There was a variation in the percent incidence with hot humid areas having a higher incidence. Hot humid conditions may favour NPV incidence in field populations than cool conditions. The survey covered most parts of the country and some sites were visited once while others were visited more than once. Thus, this may explain the variation in the occurrence and percentage incidence of this pathogen. The results obtained in this study indicate that NPV is fairly common and widespread. However, there is need to carry out thorough surveys aimed at recording NPV incidences throughout the growing seasons in all agro-ecological zones of Kenya. Results obtained from such survey will provide information on the potential use of NPVs as biocontrol agents. The survey carried out in this study provides baseline information. Other baculoviruses (GVs) affecting different lepidopterans (such as diamondback moth) have also been recovered from field populations by other researchers (Oduor *et al.*, 1998).

Serious research should therefore be carried out geared towards developing and packaging these baculoviruses for the control of lepidopteran pests in Kenya.

6.1.2 Production of NPV and quality control

A simple and cheap method of producing the NPV polyhedral suspension using host insects (*in vivo* production) described by Grzywacz (1997) was carried out in this study. *In vivo* production has been recommended to be the most cost-effective and practical means for viral pesticides as opposed to *in vitro* method which utilises host insect cell line culture systems (Kariuki and McIntosh, 1999). Infected larvae were easy to recognize as they became sluggish, stopped feeding and eventually died. Larvae were harvested 5-9 days after feeding on an NPV-contaminated diet of soaked chickpea seeds but it normally takes 5-15 days after infection before cessation of feeding and death of the insect pest (Grzywacz, 1997). Homogenizing or crushing the larvae with a limited amount of water and soap (or SDS), centrifugation and mixing with water made a mixture that was used as a cheap insecticide. This affected the larvae that fed upon foliage or pods contaminated by the virus particles and thus controlled the pest. The method may be adopted by small-scale entrepreneurs who may be required to purchase a few quality control facilities (medium sized refrigerated centrifuge, a compound microscope, a refrigerator, a haemocytometer, a homogenizer and rearing facility for host insects) to produce high quality inoculum with higher potency. The rest of the materials are cheap and easily available for the day-to-day running of the production system. NGOs may be encouraged to take up the initiative of running

production systems. Small-holder farmers can also organise themselves into groups and run a production unit. This method is being applied for the production of baculovirus (granulosis virus–GV) against the potato tuber moth in Northern and Western Africa (SP-IPM, 1999). A much simpler method involves the use of field larvae that can be homogenised with a pestle and mortar and the virus harvested by slow settling and decantation. The cadavers are often found in the field dead larvae with the head hanging downward from twigs, and full of a brown liquid which contains the virus particles (Tanada and Hess, 1984). If such larvae are crushed and mixed with water, the mixture can be used as a cheap biocide, though it will be of poor quality.

The NPV suspension used in this study had been stored at 4⁰C for six months when it was used for the field experiment. Some of the laboratory bioassays were conducted with a suspension that had been stored for eight months. It is therefore concluded that with the ordinary domestic refrigeration facility, one is able to prepare and store the virus at home and use it without much loss of activity within one year.

It is important to note that commercialization of viral insecticides has suffered some obstacles, amongst which have been high production costs, the lack of efficacious formulation and application technologies and a slow speed of action. Biotechnology has contributed several advances towards overcoming these problems and the new technology developed recently greatly improved the cost/benefit ratio for the production of viral pesticides. The high cost of *in vivo*

production can be reduced significantly using the high-density rearing system termed as "high efficacy rearing device" (Hughes, 1984) where many different species of lepidopterous larvae can be reared for the production of viral insecticides.

6.1.3 Comparison of local and exotic NPV isolates

Two local isolates were compared with one Indian (ICRISAT) isolate in this study. Higher percent mortalities were recorded in the Kenyan isolates ($78.3 \pm 7.38\%$ and $68.3 \pm 5.68\%$) 9 days post inoculation, compared to $55.9 \pm 2.10\%$ in the Indian isolate. Although there were no significant differences, the local isolates were slightly more virulent and might suit the local environmental factors than the exotic ones. Their local availability from farmers' fields may easily attract utilization by any interested farmer. Since it may be cheaper to produce a crude suspension that may effectively control the pest, the local isolates may be more economical. Furthermore, their use avoids the delays that are involved in quarantine procedures during importation and registration of exotic strains.

6.1.4 Dose-mortality

The dose-mortality studies showed that higher doses killed *H. armigera* larvae faster than lower doses, thus larval mortality was dose-dependent. For the two isolates studied, the LD_{50} of the Kenyan isolate by probit analysis was 1.59976×10^{10} OBs/ml while that of the Indian isolate was 3.178×10^{13} OBs/ml at 95% fiducial limits. It may be concluded that 1.60×10^{10} OBs/ml of the local isolate could be used for effective control of *H. armigera* in Kenya. These results

are important for effective and economic use of NPV. Farrar and Ridgway (1998) emphasized the importance of dose-response studies in characterizing the host-pathogen interaction. The dose mortality and time-mortality data enables the understanding of the role of NPVs in the population dynamics of insect pests and development of effective strategies for their application as pest control agents (Hughes and Wood, 1986). Baculoviruses are typically applied with aerosol applicators at the rates of $2.5 \times 10^{11-12}$ OBs/ha and with a total leaf index of 3 square metres of leaf area per square metre of ground area; the virus application rate is estimated to be 800-8000 OBs/sq. cm. Thus the results obtained in the current study were within the acceptable range. Laboratory studies on the application rate, even with a low targeting efficacy, it would be adequate to infect most target-pest larvae, though it is the amount of virus that would be present immediately after application.

6.1.5 Effect of local adjuvants on the pathogenicity of HaNPV to

***H. armigera* larvae.**

Two local adjuvants (1% molasses and 1% charcoal powder) were tested in this study. Molasses was found to increase viral activity both in the laboratory bioassay and in the field. It is known to have a phagostimulant and U-V protection effect and makes the larvae to ingest more virus and hence receives a higher infection. Many workers have recommended the use of cheap and locally available materials in tank mixes of NPV. Shapiro (1995) reviewed the high susceptibility of viral pesticides to inactivation by UV in sunlight. On plants and

plant tissues, the virus is inactivated within 1-2 days post inoculation but the development of cost-effective environmentally acceptable UV protectants is one of the most important requirements for commercial production. The use of optical brighteners is the most promising option (Shapiro, 1992) because it protects the virus from inactivation and also reduces the LD₅₀ of some virus-host interactions (Dougherty *et al.*, 1996).

6.1.6 Potential of NPV in integrated pest management

The control results achieved with NPV in the preliminary (pilot) field test is a pointer to the potential for use of NPV in the field control of *H. armigera*. The protection results conformed with those achieved by using Karate[®] (Lambdacyhalothrin), though the chemical gave better control. Effective control of *H. armigera* larvae with NPV has been reported in other parts of Africa (Cadou and Soubrier, 1974; AbouBakr *et al.*, 1986; El-Nagar and El-Sheik, 1990; Hunter-Fujita *et al.*, 1990; Silvie *et al.*, 1993; Jones *et al.*, 1994; Moore *et al.*, 1997; Bouwer, 1997). Tests have also been carried out on vegetable crops such as tomato (Lutwama and Matamni, 1988) with good results. This virus has been used even against resistant *H. armigera* strains (Basavana *et al.*, 1997), in combination with neem (Rabindra *et al.*, 1997) and even with *Bacillus thuringiensis* (Roome, 1975; Lutwama and Matamni, 1988; Montaldo, 1991; Silvie *et al.*, 1993; Moore *et al.*, 1997). NPV has also been used in combination with low doses of chemicals and gave good control (Silvie *et al.*, 1993). Basavana *et al.* (1997) reported that the resistance problem in *H. armigera* may be mitigated by the use of NPV and

Rabindra and Jajaraj (1985) stipulated that entomopathogens can break insecticide resistance when properly introduced into the field population.

The field efficacy of viral pesticides and other biocontrol agents requires cost effective, biologically based formulation and application technologies. Based on the current field evaluations of baculovirus pesticides, formulation/application technologies must be improved for viral pesticides to compete effectively and consistently in most pesticide markets.

6.2 Conclusions

1. Sixteen isolates of NPV obtained from *H. armigera* samples obtained from different localities in Kenya.
2. Larval mortality was found to be dose-dependent
3. Second instar larvae were highly susceptible compared to third, fourth and fifth instars
4. Molasses (1%) increased viral activity both in the laboratory, pot-culture studies and in the field.
5. Control of *H. armigera* with NPV compared favourably with that by two chemical pesticides, Lambdacyhalothrin (Karate[®]) and Bifenthrin (Brigade[®]).

6.3 Recommendations

Nuclear polyhedrosis virus (NPV) pathogenic to *H. armigera* occurs naturally in the country and has high potential for field use to control this pest on vegetable crops. The results obtained in this study may be used as good baseline data for further evaluation and use for insect pest control. However, its full potential and economic control will be realized if used with other IPM options for pesticide-free produce to be obtained. Farmers need to understand the need and advantages of using natural products in their farming systems. They need to be educated to understand the reduced cost of application as a result of bulimic symptoms of NPV and development of other controlling insects that reduce the populations of the destructive ones.

Biopesticides and especially NPVs have a market potential in East Africa for the control of lepidopteran pests, especially in high value crops like vegetables and flowers where there are stringent limits on residue levels. The demand will however be determined by population and economic trends, farming practices, technological development in the agricultural sector, product development by potential pesticide companies and competition from other pesticides, industry structure, pricing and mode of action.

Success will however come if the following issues are addressed:

1. Information flow on the advantages of baculoviruses in pest control, which is poor.

2. Understanding of the comparatively low crop yield from NPV than is usually expected in the chemical control.
3. Farmers must understand that baculoviruses have slow action and training is required to make them understand the long-term benefits.
4. Farmers and extension workers need to understand the simple technology of local production of the biopesticide.
6. Basic knowledge should be provided to research staff and the extension sector.
7. Farmers need to be involved in field trials and demonstration farms could be planned to make farmers understand the role of natural enemies in biopesticide sprayed plots.
8. The issue of the cost of local biopesticides and availability should be addressed.

6.4 Suggestions for future studies

1. A thorough survey on the natural occurrence of NPV should be carried out in all high potential agro-ecological zones to supplement this study. A detailed study of natural disease dynamics is important since naturally occurring epizootics cause several waves of larval mortality within a season that may result in tremendous decline in pest population.
2. Further bioassays need to be carried out on other standard NPVs from other parts of the world, especially the US and UK, in comparison with the local isolates to be able to gauge their potency in laboratory susceptible cultures.

3. Molecular characterization to confirm differences in geographic isolates to supplement the results of biological activity and electron microscope investigations for classification is required.
4. Experiments on the potency of local isolates need to be conducted for longer periods in order to select locally adapted strains.
5. More local additives (adjuvants) should be evaluated, including optical brighteners that could not be tested in this study.
6. More field tests should be carried out on other vegetable crops in different growing seasons to demonstrate field efficacy and impact.
7. Simple pilot local mass production units should be developed and efficacy demonstrated.
8. Farmers and extension staff should be trained in the simple production and utilization of baculoviruses.

Appendix 1: Horticultural production zones and examples of suitable growing areas

ZONE	DESCRIPTION		AREAS	SUITABLE VEGETABLE CROPS
	ALTITUDE (M)	MEAN TEMP (°C)		
9	> 3,000	<10		
8	2,700 – 3,000	10-12	Mao Narok, Kericho Kinangop	Brassicas, Snowpeas, Snappeas, Carrots
7	2,400 – 2,700	12-14	Kinangop	Brassicas, Snowpeas, Snappeas, Carrots
6	2,100 – 2,400	14-16	Limuru	Brassicas, Snowpeas, Snappeas, Green beans, Runner beans, Carrots
5	1,800 – 2,100	16-18	Kericho	Asparagus, Green beans, Snowpeas, Runner- Beans, Gherkins
4	1,500 – 1,800	18-20	Limuru, Kericho Eldoret, Nairobi	Asparagus, Baby corn Green beans, Snowpeas, Runner beans, Gherkins
3	1,200 – 1,500	20 -22	Embu, Thika, Athi River, Meru, Nairobi, Loitoktok, Yatta, Naivasha, Kisumu, Kakamega	Asian vegetables, Snowpeas, Green beans, Runner beans, Gherkins
2	900 – 1,200	22-24	Mitunguu, Isiolo Embu, Kibwezi, Meru, Marigat, Tana River, Taita Taveta, Mombasa	Asian vegetables, Onions, Gherkins
1	<900	24-30	Tana River, Mombasa, Taita Taveta, Garissa	Asian vegetables

Appendix 2:**Volume of individual vegetables exported from Kenya during 1995**

VEGETABLE	WEIGHT (Kg)
French Beans	14,693,734
Snow Peas	2,074,317
Chilies/Capsicums	1,580,319
Okra	1,898,065
Karella	1,384,094
Runner Beans	1,818,613
Bobby Beans	686,314
Aurbegines	786,845
Valore	319,287
Snap Peas	555,567
Dudhi	421,618
Cabbage	176,956
Onion	340,019
Spinach	110,001
Carrots	26,802
Mushrooms	20,672
Cucumber	19,296
Lettuce	9,378
Courgettes	15,045
Ginger	5,517
Cauliflower	5,042

Appendix 3: Semi-Artificial Diet used for *Heliothis armigera* rearing:

Larval diet	100 vials	Single batch	Double batch: % comp.	
Chickpea flour	180.6 gm	300 gm	600 gm	18.06
Ascorbic acid	2.8 gm	4.7 gm	9.4 gm	0.28
Methyl-4-hydroxybenzoate	3.0 gm	5.0 gm	10 gm	0.3
Sorbic acid	1.8 gm	3.0 gm	6.0 gm	0.18
Aureomycin powder	6.9 gm	11.5 gm	23 gm	0.69
Linseed oil (sunflower oil)	7.2 ml	12 ml	24 ml	0.72
Vitamin stock solution	6.0 ml	10 ml	20 ml	0.6
Water	270.8 ml	450 ml	900 ml	27.08
Yeast	28.9 gm	48 gm	96 gm	2.89
Agar	10.4 gm	17.3 gm	34.6 gm	1.04
Water (for yeast and agar)	481.6 ml	800 ml	1600 ml	48.15

Source: ICRISAT NPV project, 1997.

Making up larval diet

Weigh out all dry ingredients and have all wet ingredients in appropriate measuring cylinders.

Use a large white bowl. Add ingredients (a-g) in bowl. Add water (h) and mix thoroughly using hand held mixer. Meanwhile heat water for yeast and agar in saucepan on hot plate. When boiled, add yeast and mix thoroughly with mixer. Sprinkle in agar while stirring and mixing thoroughly all the time. Remove from heat and pour the ingredients into plastic bowl. Mix continuously until an even consistency is obtained. Pour hot diet into plastic home dispenser and serve about 10 ml volumes into glass vials or sterilized plastic trays on a level surface of 5mm depth of diet. Leave to cool and set completely. When cool, place vials or tray in plastic bag, exclude air and place in refrigerator.

Larvae are fed by placing them individually into glass vials and allowed to feed until pupation. Diet in trays was cut into one inch squares when required and place into vials for larvae.

Adult diet:Ingredients

Sucrose acid	50 gm
Methyl -4- hydrobenzoate	1 gm
Vitamin stock solution	10 ml
Water	500 ml

VITAMIN STOCK SOLUTION :Ingredients:

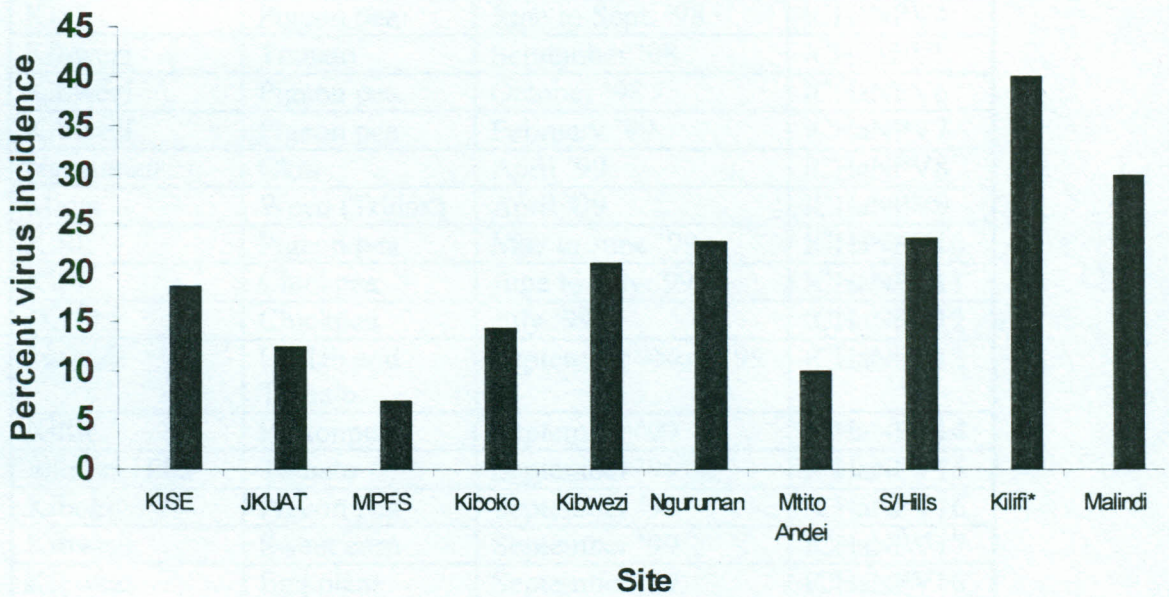
Nicotinic acid	1.528 gm
Calcium pantothenate	1.528 gm
Riboflavin	0.764 gm
Aniline hydrochloride	0.382 gm
Pyridoxine hydrochloride	0.382 gm
Folic acid	0.382 gm
D-biotin	0.305 gm
Cyanocobalamine	0.003 gm
Water	500.000 ml

Appendix 4: Survey results for the natural occurrence of NPV on *H. armigera* from different localities in Kenya, February-November 1999.

Month	Site	Host plant	No. collected	No. dead at larval stage	No. dead at pupal stage	Total % mortality	% Mortality by virus
Feb. 1999	Kibwezi	Pigeon pea	48	4 Virus 8 Parasitoid 3 Others	5 virus 2 Others	45.8	18.8
April 1999	Nguruman	Okra	56	6 Virus	7 Virus	46.4	23.2
April-May 1999	Mbita	Tridax Kales Pigeonpea	Tridax- 17 Kales- 2 P/pea- 10	2 Virus 1 Parasitoid 1 Others	4 others	27.6	6.9
May 1999	JKUAT	Chick pea	48	5 Virus 2 others	1 Virus 5 others	31.3	12.5
May to June 1999	KISE	Pigeon pea	140	25 Virus 10 Parasitoid	6 Virus 4 Parasitoid 5 Others	35.7	22.1
		Chick pea	60	5 Virus 2 Others	2 Virus 4 Parasitoid 1 Others	23.5	11.7
July 1999	KISE	Chick pea	124	11 Virus 5 Parasitoid 4 Others	5 NPV 10 Others	43.4	12.1
Sept. 1999	Shimba Hills	Tomato	17	4 NPV 1 Parasitoid 3 Others	1 Others	52.9	23.5
Sept. 1999	Kilifi	Pigeon pea	5	2 Virus	1 Others	60	40
Sept. 1999	Malindi	Lablab beans	48	13 Virus 8 Parasitoid 13 Others	2 NPV 1 Others	77.1	31.3
Sept. 1999	Kiboko	Pigeonpea	21	3 NPV 5 Parasitoid 1 Others	-	42.9	14.3
Sept. 1999	Kibwezi	Sweet corn.	84	17 Virus 8 Parasitoid 23 others	-	57.1	20.2
Sept. 1999	Kibwezi	Eggplant	50	10 Virus 6 Parasitoid 23 others	-	38	20
Sept. 1999	Kibwezi	Capsicum (sweet pepper)	6	1 Virus	-	16.7	16.7
Nov.'99	Mtito Andei	Tomato	30	3 Virus	5 Others	26.7	10.0
Nov. 1999	Malindi	Tomato	120	29 Virus 6 Others	9 Virus 6 Parasitoid 5 Others	45.8	31.7

Appendix 5

**Pilot survey results for natural occurrence of NPV of
H. armigera in Kenya, June 1998 - Oct. 1999**

**Note**

The percent virus incidence for Kilifi shown is high but there was limited sample size (see Table 3.5).

Appendix 6**HaNPV samples collected during the Field Survey, 1998-99.**

Origin	Host plant	Period of collection	Accession number
KISE	Pigeonpea	Jan. to March '98	ICHaNPV1
KISE	Cleome	Jan. to March '98	ICHaNPV2
KISE	Cabbage	Jan. to March '98	ICHaNPV3
KISE	Pigeon pea	June to Sept. '98	ICHaNPV4
Kibwezi	Tomato	September '98	ICHaNPV5
Kibwezi	Pigeon pea	October '98	ICHaNPV6
Kibwezi	Pigeon pea	February '99	ICHaNPV7
Nguruman	Okra	April '99	ICHaNPV8
Mbita	Weed (Tridax)	April '99	ICHaNPV9
KISE	Pigeon pea	May to June '99	ICHaNPV10
KISE	Chickpea	June to July, '99	ICHaNPV11
JKUAT	Chickpea	July '99	ICHaNPV12
Malindi	Lablab and Tomato	September –Nov. '99	ICHaNPV13
Kilifi	Pegionpea	September '99	ICHaNPV14
Shimba Hills	Tomato	September '99	ICHaNPV15
Kiboko	Pigeon pea	September '99	ICHaNPV16
Kibwezi	Sweet corn	September '99	ICHaNPV17
Kibwezi	Egg plant	September '99	ICHaNPV18
Kibwezi	Sweet pepper	September '99	ICHaNPV19
DUDUVILLE	Lab. Gen. 2	April '99	ICHaNPV20
DUDUVILLE	Lab. Gen. 3	May '99	ICHaNPV21

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