INVESTIGATIONS ON HaNPV ISOLATES AND THEIR ASSESSMENT IN THE IPM OF *Helicoverpa armigera* (Hubner)

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CERTIFICATE

This is to certify that the thesis entitled "**INVESTIGATIONS ON HaNPV ISOLATES AND THEIR ASSESSMENT IN THE IPM OF *Helicoverpa armigera* (Hubner)**" *submitted by* **Mr. D. N. KAMBREKAR** *for the degree of* **DOCTOR OF PHILOSOPHY** *in* **AGRICULTURAL ENTOMOLOGY** *to the University of Agricultural Sciences, Dharwad is a record of research work done by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any other degree, diploma, associateship, fellowship or other similar titles.*

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September, 2004

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Dedicated

To

My Beloved Mother & Brothers

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Introduction

I. INTRODUCTION

Use of viral insecticides, particularly, baculoviruses have several advantages over chemical insecticides. Baculoviruses are naturally occurring pathogens that are specific to a single or a few related insect species. They are environmentally safe, produce no toxic residues and are harmless to non-target animals including beneficial insects and vertebrates. In addition, they can provide economic short-term and long-term control and are quite effective at low dosages under suitable environmental conditions.

Of the premier groups of insect viruses, the genus *Baculovirus* constitutes one of the largest and most diverse groups of insect pathogenic viruses. A wilt disease in the larvae of *Helicoverpa armigera* (Hubner) was reported by Steinhaus (1949) from North America. It was later identified as NPV as confirmed by electron micrographs of POB and virions.

Four viruses were reported from *Helicoverpa* species comprising three inclusion viruses and one non-inclusion virus (Parsons, 1936; Teakle and Bryne, 1989). Since the virions of HaNPV are rod shaped, it is a baculovirus and has been named as *Baculovirus heliothis* (Ignoffo and Allen, 1972). In India, occurrence of NPV in *H. armigera* was first reported during 1968 (Patel *et al.*, 1968) from Gujarat and later Rabindra and Subramanian (1974) described the symptoms of disease, susceptibility of different instars of *H. armigera* and host pathogen relationship. Of the several insect viruses tested against *H. armigera,* the nuclear polyhedrosis virus (NPV) holds great promise in the management of *H. armigera* on a number of crops due to its high efficacy and safety to the natural enemies (Rabindra and Jayaraj, 1990).

Though HaNPV has been found to be a promising microbial insecticide, certain factors have strained its practical utility and commercial success. The time lag between the ingestion of a lethal dose and death of the pest under field condition is long and varied (3 to 10 days). During this period, the feeding may continue even up to the last one or two days before the death and the efficacy of HaNPV may be reduced. Hence there is an urgent need to identify, develop and multiply a virulent isolate in large quantities on commercial scale that can result in early death of the targeted insect species.

Presuming that a refined multiplication method of HaNPV is available, commercial utilization of HaNPV has several limitations including its bio-efficacy under field conditions. The germicidal action of UV radiation of sunlight and substrate environment have greater influence in reducing the potency of viruses to kill the pest. Therefore, the identification of HaNPV isolate which can persist for a longer period on foliage and in soil is the need of the hour.

NPV is known for high epizootic levels and is naturally occurring, self-perpetuating, safe to natural enemies due to host specificity and environmental friendly.

Since, NPV can be produced only in living larvae, the cost of production can be quite high, as *H. armigera* requires isolated rearing. The yield of virus from a single caterpillar can therefore be an important factor in the cost of production. If a virus isolate is capable of producing a larger number of bigger polyhedra with an increased number of virions per POB per cell and thereby per larvae, which is possible expression of genotypic variations (McIntosh *et al.,* 1987), the total number of larvae required to produce a certain amount of virus would be fewer, enabling a reduction in production cost. An important study therefore be of the polyhedra yield in different viral isolates. Hence, a more intensive search may yield a viral isolate with more virulence, greater resistance to certain physical factors (UV fraction of sunlight) and biotic factors (plant surface environment and plant bio-chemicals) than the existing ones that would greatly enhance the capability and utility of NPV of *H. armigera* as a microbial insecticide.

In India, there has been a rapid growth in the production and use of NPV products in the last decade, but this was accompanied by reports of problems on the product quality. The causes of the problem of poor quality lay as much in deficiencies in production technologies and poor quality control procedures which are absent currently. Assessment of quality of the HaNPV products produced by the private enterprises is therefore an important study, which helps in building up the user confidence, ensure product safety and maximize the product performance.

Among the different species of *Helicoverpa* occurring in India, *H. armigera* is the most widely prevalent and devastating pest. It is known for its extensive host range and severe damage it causes to many food and fibre crops (Anon., 1977). Its economic importance as a pest is magnified due to its direct attack on fruiting structure, voracious feeding habit, high mobility, opportunist and multivoltine nature.

NPV of *H. armigera* has a great potential for control of this pest and it has received a great deal of attention owing to several advantages. Considering the reliability and suitability of HaNPV in terms of economic and ecological reasons, its utilization in pest management has received a great deal of significance. However, vast differences exist in the pathogenecity and virulence of different geographic isolates of HaNPV against the local natural populations of the pest all over the world (Battu and Arora, 1996). The major causes for the variation in the genome level may be due to the change in the sequence coding for a particular protein, insertion of host DNA into viral genome (Shapiro *et al.,* 1991) and deletion of part of the viral genome (Heldens *et al.,* 1996). The present investigations are therefore, under taken to characterize the native isolates of HaNPV representing different areas of the country. Further their virulence, persistence and assessment in the Integrated Management of *H. armigera* in chickpea have been studied with following objectives.

1. To characterize the HaNPV isolates
2. To study the influence of cotton species on HaNPV isolates
3. To know the persistence of HaNPV isolates in different soils
4. To assess the quality of HaNPV produced by private firms, and
5. To assess the efficacy of promising HaNPV isolates in the IPM of *H. armigera* on chickpea.

Review of Literature

II. REVIEW OF LITERATURE

The literature pertaining to *Helicoverpa armigera* NPV isolates is scanty, hence, the information on the virus isolates of different insect species has been thoroughly reviewed and presented hereunder.

2. 1 Characterization of NPV isolates

Inheritable variations in the virulence were observed among the strains obtained from diverse geographic locations (Hukhara, 1968). According to the author, the field isolates collected from diverse geographic locations are often genetically heterogeneous. Viruses from members of the same host species from different locations show variability in virulence, biological characters and their DNA differs in restriction endonuclease and protein patterns.

The biological activity of 34 *Heliothis* nuclear polyhedrosis virus isolates was determined by Shapiro and Ignoffo way back during 1970. The LD_{50} (Lethal dose) of the 34 isolates of *Heliothis* NPV ranged from 0.7 to 39.0 polyhedral inclusion bodies (PIB) per mm^2, a difference of about 56 folds. At least two, and possibly three distinct activity groups were formed out of 34 isolates. Approximately 60 per cent of the isolates exhibited LD_{50} value ranging from 0.70 to 11.70 PIB per mm^2. The strainal variations in viruses of *Neodipram sertifer, Lymantria dispar* and *Hyphantria cunea* have also been reported (Smirnoff, 1972 and Hukhara, 1968). Vlak and Groner (1980) studied two *Mamestria brassicae* multi nucleo-capsid NPV (MbNPV) isolates from Germany and Netherlands. The LD_{50} values of the two isolates were similar.

Vail *et al.* (1982) demonstrated a total of eight isolates including variants and natural recombinants of *Autographa californica* multicapsid NPV (AcMNPV) for their *in-vivo* virulence and maintenance of the genetic characters after infection of *Trichoplusia ni* (Hubner) and *Heliothis virescens* (L.). Only one of the isolates was more virulent to *T. ni* than the wild type. However, it was less virulent to *H. virescens.* One recombinant exhibited low production of polyhedra.

McIntosh and Ignoffo (1983) studied the DNA of three baculoviruses propagated in larvae of a common host species *H. zea*, which were easily distinguished from each other by restriction endonuclease patterns. A molecular weight of 79.70, 119.00 and 86.6 $X10^6$ Da was estimated for the viral genome of HzNPV, HaNPV and HaGV, respectively. The fragments differed in size with fewer restriction sites for the viral genome of HzNPV and HaGV as compared to the viral DNA of HaNPV. The authors concluded that this variability might be due to genetic heterogenity or a high frequency of recombination between virus and host genome.

The existence of variation in the virulence among the *Heliothis* NPV isolates collected from different countries was investigated by Hughes *et al.* (1983) from New York. Fourteen isolates were collected representing both single nucleo-capsid NPV (SNPV) and multi nucleo-capsid NPV (MNPV). Based on the LT_{50} values, all the 14 isolates have been grouped into six activity classes. Among the 12 single enveloped viruses (SEV), the isolates collected from South Africa were similar in their LT_{50} value with two of the three isolates collected from United States. The two Australian isolates were grouped with the remaining isolate of United States and one isolate from Botswana, which together formed activity class II. The three New Zealand isolates were put together in activity class –III while the China isolate was separated in to class –IV. Of the two multi-enveloped viruses (MEV), the USSR isolate was put under class V where, the Indian isolate was also merged. Among the SEVs, the SEV collected from Southern India (Madurai) was found to be least virulent.

The author opined that the SEVs were generally more virulent than the MEVs except the SEV of South Indian origin.

Shapiro *et al.* (1984) studied the existence of variation in the virulence of *Lymantria dispar* NPV isolates collected from different continents. As high as 2950 fold variation in the LC_{50} values could be among the different isolates across the continents. The least LC_{50} value was observed in North America isolate while, the Asian (Japan) isolate recorded highest LC_{50} value with a difference of 2950 folds. The European isolates were moderately effective as compared to other isolates. The existence of variation in the biological activity among the different isolates was drastic when they were compared across the continents.

Odak *et al.* (1984) found considerable difference in the infectivity of HaNPV isolated from different locations. The culture isolated locally from Narasinghapur recorded higher mortality (93 per cent) when compared to the cultures from other centers. Five baculoviruses, three granulosis viruses and two nuclear polyhedrosis viruses that infect the armyworm, *Pseudaletia unipuncta,* were analyzed with four restriction enzymes. All viruses gave unique restriction fragment patterns eventhough each progeny virus was produced in larvae of the same insect species. The results revealed that there was no homology between the Oregonian GV and the other two GVs, nor between the Hawaiian GV and the GV isolated from *Scotogramma trifolia,* both of which produce similar but distinct synergistic factors that enhance baculovirus infections (Harvey and Tanada, 1985).

Genotypic variation among the wild isolates of two nuclear polyhedrosis viruses isolated from Egyptian cotton worm, *Spodoptera littoralis* was assessed by Cherry and Summers (1985). The diseased larvae were collected from 21 different regions of Israel from which NPV was isolated and the viral DNA were compared by restriction endonuclease analysis. Two distinct virus types represented with approximately equal frequency were found. Plaque purification of the wild isolates revealed the presence of additional genotypic variants. Restriction endonuclease analysis of DNA from eight geographic isolates of potato tuber moth granulosis virus (PTMGV) exhibited minor genotypic differences between some of the isolates, which revealed the existence of three distinct but closely related genotypes. (Vickers *et al.,* 1991).

Diversity in the virulence within the NPV isolates among the different samples of *Heliothis zea* was studied by Shapiro and Robertson (1991) from United States. The results indicated highly variable activity among the samples of the same isolate in terms of relative effectiveness at LC_{50} and LC_{90} values. The difference in the LC_{50} values among the samples collected within a isolate (Abington) was to the extent of 15 folds and the variation was highest (20 folds) among the samples of the isolate collected from Hamden.

Somasekar *et al.* (1993) collected different isolates of HaNPV from different areas of Tamil Nadu and compared with the isolates of Gujarat and Rajsthan. The diameter of the POB ranged between 1.734 to 2.006 μm. The least being recorded in Ooty isolate while, the other extreme was observed in Rajasthan isolate. However, there was no significant difference among the isolates with respect to the POB size. Similarly, the molecular weight of isolates also varied from 71.73 to 78.19X10^6 Da, but without any significant difference. The Ooty isolate was highly virulent with lowest LC_{50} value closely followed by Coimbatore isolate. The isolates collected from Rajasthan required five times higher concentration than Ooty isolate to cause 50 per cent mortality of the test insect population. Further, the time mortality response studies proved that the Ooty isolate recorded shorter LT_{50} values as compared to other isolates at all tested concentrations.

The variation in the virulence among the different isolates of *Bombyx mori* NPV (BmNPV) was reported by Shivaprakasam and Rabindra (1997). There was no statistical difference in the LD_{50} and LT_{50} values among the various isolates tested, which was mainly attributed to the poor selection pressure between the host and pathogen. Arora *et al.* (1997) screened HaNPV isolates obtained in Punjab for their virulence in comparison with the isolates collected from the commercial firms of Bangalore (Karnataka) and Godhra (Gujarat). The three local isolates were collected from PAU (isolate maintained at Punjab Agricultural University), Mudki and Karaiwala. The LC_{50} values of all the isolates ranged between 0.3593 to 1.1214 LE/l. The native isolates were comparatively virulent against the pest, as indicated by less LC_{50} values than the two commercial formulations obtained from Bangalore and Godhra. Among the five isolates, the PAU isolate was the most virulent whereas, the least virulence was recorded in Godhra isolate with 5-6 times more LC_{50} value. Similarly, the LT_{50} values also confirmed high virulence of the native isolates of Ludhiana, by recording shorter LT_{50} values at all the tested concentrations.

Gopali (1998) confirmed the existence of variation in the geographical isolates of HaNPV collected from different geographical regions. Among the five isolates collected, Gulbarga and Coimbatore isolates recorded maximum mortality of the larvae at all the intervals of observations. Further these isolates recorded lesser LC_{50} and LT_{50} values, confirming their high virulence as compared to other isolates.

Sudhakar and Mathavan (1999) made studies on the genetic variation among different isolates collected from different countries *viz.*, South Africa, Poland, China and Russia. The multi nucleo-capsid NPV of *Helicoverpa armigera* was compared with the Indian isolate collected from Madurai. The maximum genome size was observed in case of Poland isolate (174.40 kbp) followed by

China and Russian isolates (150.75 and 149.46kbp, respectively). The authors also studied the genome size of single nucleo-capsid HaNPV. The genome size of HaNPV was comparatively smaller than multi nucleo-capsid NPV, which ranged from 111 to 115 kbp.

Three strains of NPV isolated from infected *Spodoptera frugiperda* (J.) larvae in the United States, Nicaragua and Argentina were subjected to a structural, genetic and biological comparison to select a candidate isolate for use in bio-control. Restriction endonuclease analysis of viral DNA confirmed that these isolates were strains of a single virus species. Droplet feeding bioassays indicated that the isolates from Nicaragua and United States had the highest infectivity when treated against second instar larvae of *S. frugiperda* (Escribano *et al.,* 1999).

Kariuki and McIntosh (1999) described a new baculovirus isolate from infected larvae of the diamondback moth, *Plutella xylostella.* It was identified as multi nucleo-capsid NPV and designated as MNPxNPV, which was compared with two broad spectrum NPVs *viz., Autographa californica* NPV and *Anagrapha falsifera* NPV. Bioassay studies on seven species of Lepidoptera were carried out by continuous feeding of the occlusion bodies through semi-synthetic diet. All the species of lepidopteran larvae were found susceptible to three baculoviruses exhibiting the typical symptoms of NPV infection including cessation of feeding, sluggishness and becoming flaccid. Significantly higher pathogenecity of PxMNPV against diamondback moth was observed as compared to other baculoviruses. The PxMNPV was equally effective against *H. virescens* and *Trichoplusia ni.* AcNPV and AfNPV followed similar pattern of infectivity against these two insect species. The AcNPV was the most pathogenic of the three baculoviruses against *H. zea* as compared to PxMNPV and AfNPV.

Rabindra (2000) made an effort to characterize the NPV of *H. armigera, Spodoptera litura* (F.), *Amsacta albistriga* (W.) and *Mythimna separata* (W.). The samples were collected from the same ecosystem. The DNA fragment profiles of Pst1 and Hind-III showed that the genome size ranged between 69.30 to 125.30 kbp. The size and number of fragments in each isolate varied drastically. Each isolate showed their unique fragments of varied size and the variation in the genome size was mainly due to the genetically controlled factors as well as the climatic factors prevailing in that region.

Homology and comparative activity of six isolates of granulosis viruses in sugarcane shoot and internode borer was reported by Easwaramoorthy *et al.* (2000) in an attempt to assess the possibility of obtaining virulent strains among the six GV isolates collected from different locations in Tamil Nadu. All the collections produced similar DNA fragment profiles. The biological activity of the GV isolates differed slightly indicating greater homology and highly conserved nature of genomes in case of shoot and internode borer granulosis virus.

Geetha and Rabindra (2000) characterized 11 HaNPV isolates collected across the country. They characterized the DNA of the isolates using double digestion with different restriction endonuclease enzymes. The genetic variability was studied with Pst1, Hind-III, BamH1 and EcoR1. There was variation in the restriction profiles and the molecular weight of the isolates ranged from 64.43 $X10^6$ Da to 81.50 $X10^6$ Da. Further, the virulence of these isolates was compared through LC_{50} and LT_{50} values. The isolate collected from Negamum was the most virulent with least LC_{50} value against both second and third instar larvae of *H. armigera*. On the other hand, the Rajasthan isolate was least virulent with greater LC_{50} values. The HaNPV isolates collected from Negamum and Ooty were 6.5 and 3.6 times more virulent over the Rajasthan isolate. However, there was no correlation between genetic similarity and virulence.

Manickvasagam (2003) studied the molecular characterization and pathogenecity of two different geographical isolates of *Spilosoma obliqua* (W.) NPV. The restriction endonuclease analysis of virions indicated that there was no much difference in DNA profiling and also the genome size was more or less similar. With regard to pathogenecity, the isolate collected from Punjab proved more virulent with a LC_{50} value of $1.758X10^4$ POBs/ml as compared to the Maharastra isolate, which recorded LC_{50} value at $5.05X10^6$ POBs/ml indicating a difference of 287 folds that exists among the two isolates.

2. 1. 1 Polyhedral occlusion body yield of NPV

Pawar and Ramakrishnan (1971) estimated the POB yield in both larvae and pupae of *Prodenia litura.* The number of POBs per larva on an average was 1.9644×10^9 with a minimum and maximum record of 0.8582 to 3.2340×10^9 POBs. The authors also concluded that there was no correlation between the larval weight and the number of POBs. Whereas, in case of pupae, the estimated number of POBs varied from 0.242×10^9 to 0.458×10^9 with an average of 0.333×10^9 POBs.

Battu (1991) observed the yield levels of occlusion bodies in *Hellula undalis* NPV obtained from various sources. The results showed that higher occlusion body production was observed in experimentally infected insects. The yield obtained from medium sized larvae was 0.86×10^9 POB per insect as compared to 0.97×10^9 POBs per insect in grownup larvae.

S. litura larvae bred through virus contaminated semisynthetic diet yielded maximum quantity of SlNPV (4.07×10^9 POBs/g larva), which was 1.95, 1.28, 1.25, 1.13, 1.11 and 1.08 folds greater than the virus yield obtained from the larvae bred on cotton, groundnut, sunflower, tobacco and castor, respectively (Murulibaskaran *et al.,* 1996).

The HaNPV production from different instars of *H. armigera* revealed that the fourth instar larvae not only produced higher quantity of virus per larvae (2.81×10^9 POBs) but also recorded higher larval mortality (95.20 %). Though the fifth instar larvae registered the highest viral yield per larva (3.49×10^9 POBs), exhibited lower larval mortality (24.60 %) thereby resulting in drastic decline in the total viral production. In contrast, the second and third instar larvae recorded cent per cent mortality, but with lowest virus production. The number of larvae required to yield one larval equivalent (6×10^9 POBs) from the second, third, fourth and fifth instar larva was 24.5, 3.39, 2.14 and 1.18, respectively (Gopali, 1998).

Hussain *et al.* (2002) investigated the polyhedral occlusion body counts in naturally diseased larvae of *S. litura* infected by SlNPV on cabbage. The old larvae (5th and 6th instar) yielded on an average 1.5×10^9 POB's per larva, while the young larvae (3rd and 4th instar) produced 7.95×10^9 POBs per larva suggesting that the dead cadavers had enough inoculum for further spread of disease.

Senthil Kumar and Rabindra (2003) studied the influence of dietary vegetable oils on the POB yield of tobacco cutworm, *S. litura* NPV. The results revealed that the yield of NPV could be significantly increased by the addition of mustard and coconut oils to the standard diet. When the early fifth instar larvae were inoculated with a dose of 1966.18 POB/mm^2 of diet surface, the diet incorporated with 0.6 per cent coconut oil yielded highest number of POBs/larva (8.7×10^9), which was significantly higher than that obtained from larvae fed on standard diet (1.90×10^9 /larva). At the higher inoculum level of 3932.96 POBs/ mm^2 diet surface, the mustard oil recorded highest yield of

$7.0X10^9$ POBs per larva. However the incorporation of mustard oil in the diet reduced the fecundity in *S. litura.*

2. 2 Influence of Cotton species on HaNPV isolates

Since the information on the aforesaid topic is negligible, the literature on the effect of different plants on the efficacy of NPV is reviewed and presented hereunder.

The rapid inactivation of viruses on leaf surface is a limiting factor in their development as biological control insecticides. The persistence of viruses on foliage is mainly influenced by exposure of virus particles to sunlight. Inactivation of viruses is so rapid under sunlight that their activity loss due to other factors such as temperature and moisture become insignificant.

David and Gardiner (1966) reported the activity of *P. brassicae* GV on cabbage leaves even after 16 weeks application under field conditions. However, when highly purified *P. brassicae* GV was sprayed on, it was inactivated within 12 to 19 hours due to direct exposure of virus to sunlight. As reported by many workers, the HaNPV could be slowly inactivated at a pH of 9.0 to 10.0 if the buffer had sufficient ionic strength and a lengthy time interval is allowed (Ignoffo and Garcia, 1966 and Gudauskas and Channerday, 1968).

The inhibitory effects of plant foliage could result from direct antagonism between leaf characteristics and microbes (Kushner and Harvey, 1962). The altered effectiveness of infection barriers such as the biochemical environment of the midgut lumen and the peritrophic membrane (Paschke and Summers, 1975) or possibly the physiological stress, which may inhibit resistance at the cellular level, are also reported (Steinhaus, 1958).

Laboratory experiments showed that the crude or partially purified preparations of *T. ni* GV were not significantly inactivated by exposure to simulated rain for 5 hours or by scrubbing with detergent and rinsing in water (David and Gardiner, 1966). Certain preliminary observations on the effect of light on the activity of a granulosis virus of the European cabbage worm, *Pieris brassicae* led to the general conclusion that when the purified virus was exposed to sunlight it rapidly lost its capacity to cause lethal infections in the larvae (David, 1965 and 1967). A crude virus preparation, on the other hand, retained some of its activity for 16 weeks on cabbage plants, which were kept in open. Another crude preparation containing solid matter from the larvae showed significant activity even after two years of storage as a dried film in glass tube under glass house conditions (David and Gardiner, 1967).

Jaques (1967) found that most of *T. ni* NPV activity on cabbage was lost after a month exposure to weathering. At lower rates of virus, major activity (79%) was lost within three to five days. However, a slight activity remained for weeks due to non-exposure of virus particles present in cracks and crevices of the leaves to direct sunlight. Field, glass house and laboratory studies confirmed that deposits of polyhedra of nuclear polyhedrosis virus of the cabbage looper, *T. ni* on foliage were practically non-infective after one month exposure to weathering. Inactivation by sunlight appeared to be more important than removal due to washing by rain. The virus was rapidly inactivated by exposure to ultraviolet light. The nuclear polyhedrosis virus of *T. ni* and the granulosis virus of *P. brassicae* (Linn) were found to be quite sensitive to ultraviolet (Jaques, 1968) and it was the protein that is largely responsible for inactivation under sunlight in the ultraviolet range of 290-380 nm.

When a highly purified preparation of the granulosis virus of *P. brassicae* was exposed to direct sunlight, the virus was rapidly inactivated (David *et al.,* 1968). After an exposure of 3h, the suspension applied to the leaves registered significantly less larval mortality as compared to a non-irradiated control suspension. Total inactivation of the virus under these circumstances was observed between 12 and 19 h exposure.

The thermal inactivation point was observed in the range of 60-95^{0}C for most of the viruses. However, the continuous exposure to lower temperature ranges also reduced the infectivity of the viruses (Stuermer and Bullock, 1968).

The persistence studies with a HNPV on cotton leaves against *H. zea* and *H. virescens* indicated that the viral activity although evident after one day, only a slight activity remained on second day. Thus, the lack of persistence was an important consideration in utilization of virus for the management of *Heliothis* spp. (Bullock *et al.,* 1970).

Deposits of *T. ni.* and *P. rapae* NPV were largely inactivated within 10 days after field application. However, the deposits on plants retained for a long period in growth room or in green house due to non-exposure to direct sunlight. Further, the activity of virus deposits was extended substantially by addition of protectant materials to the spray suspension. The addition of charcoal mixed with skim milk powder, egg albumen or Brewer's yeast resulted in a 3-fold increase in the activity period (Jaques, 1972).

Tanada and Omi (1973) studied the persistence of virus from insect species in alfalfa fields. The insects included, *Autographa californica* (Sprayer), *Colias eurytheme* (Biosduval), *Pseudaletia unipuncta* (Haworth), *S. exigua* (Hubner) and *T. ni* (Hubner) and, the isolated viruses were the granulosis, CPV and NPV. The viruses persisted in the soil, on the foliage and on alternate hosts. In soil, the virus persisted even during winter months when no foliage remained on plants. Alfalfa sprout harbouring virus infected larvae of *C. eurytheme* and *S. exigua* could produce virus infections in treated larvae of these insects, while the larvae of *A. californica* and *P. unipuncta* did not cause any infections.

Plants such as cotton and some other Malvaceae family have a highly alkaline leaf surface. Andrews and Sikorowski (1973) reported that the pH of dew from cotton leaves was as high as 9.2 in the morning which rose up to 10.0 when the dew was about to dry. Brownian movement of virus particles was observed in suspended polyhedra stored in this high pH dew indicating polyhedral dissolution. The effect of temperature on persistence of viruses present on foliage has not been examined in the field. However, the laboratory studies discussed earlier, suggest that temperature has little effect on foliage. Many reports indicate the rapid inactivation of HaNPV on cotton leaves, to the tune of 95 per cent in three days (Bullock, 1967), more than 75 per cent in 12 hours and 90 per cent in 24 hours (Ignoffo and Batzer, 1971) and 75 per cent in three days (Ignoffo *et al.,* 1972). Leaf material or leaf extract from a wide range of host plants have inhibited the effects of NPV (Kunimi and Aruga, 1974).

Young and Yearian (1974) compared the activity of *H. zea* NPV for 96 hours on the upper surface of leaves and observed that the persistence was in the order of tomato>soybean>cotton. When plants were shaded against sunlight (dawn to dusk), the *H. zea* NPV activity on all three crops was retained even after 96 hour, indicating that the inactivation was mainly due to sunlight.

Rome and Daoust (1976) made studies on the survival of NPV of *H. armigera* on sorghum and cotton in Botswana and found that the virus activity lost rapidly on cotton but it remained at high level for upto 30 days on sorghum. The activity was detectable even on harvested sorghum grain i.e., after 80 days of spray.

Young *et al.* (1977) reported that cotton dew had a mean pH of 8.8 and was more alkaline than soybean dew (pH 7.8). When the dew in which the polyhedra were suspended was air-dried and resuspended daily in deionized water, the polyhedra in soybean dew remained active while in case of cotton the dew retained a little activity upto 7 days against *H. armigera.*

Among the environmental factors, the sunlight contributes to a large extent for the inactivation of the virus. Whereas, temperature, humidity, pH and foliage factors slightly affected the performance of virus (Pawar and Ramakrishnan, 1977). Many field and laboratory studies (Ignoffo and Hostetter, 1977; Young and Yearian, 1986) have proved that the microbial insecticides and more specifically NPV of *Helicoverpa* spp. are inactivated by exposure to either high temperature (Ignoffo, 1966; Bullock, 1967; Gudauskas and Channerday, 1968 and Witt and Hink, 1979) or sunlight (Broome *et al.,* 1974 and McLeond *et al.,* 1977).

A bioassay technique was used by Podgawaite *et al.* (1979) to estimate the concentration of infectious Gypsy moth, *Lymantria dispar* NPV. The results indicated that the NPV was a natural component of the host's habitat, persisting at high concentrations in soil, litter and bark atleast one year after natural epizootics. Activity of NPV was measurable for 3.15 days after application on foliage and bark. The application of NPV at 2.5 X 10^{12} polyhedral occlusion bodies (POB) per ha to an are already containing high concentrations of naturally occurring NPV did not cause an increase in the environmental NPV load.

When HaNPV was applied to flowers and pods of certain host plants, the mortality of fourth instar larvae was significantly higher on pods of chickpea and pigeonpea as compared to flowers and fruiting parts (Vijaykumar, 1980).

The persistence of NPV of *Neodiprion sertifer* was reported by Mohammed *et al.* (1982). The authors could recover the POB from soil and foliage in all plots sampled at fixed time intervals within 21 months of treatment imposition. The concentration of POB in soil and foliage samples during second year was generally lower in plots treated once than those treated twice. NPV induced cent per cent larval mortality in plots sprayed once in the first season whereas, its efficacy was as low as 8 per cent in the second season. The laboratory bioassay of soil samples at different intervals upto 21 months period indicated 11 to 80 per cent NPV induced larval mortality. The study suggested that the NPV of *N. sertifer* could persist and retain some activity for atleast 21 months under field conditions.

Though NPVs are considered to be relatively stable in the environment as the POB shield protect the virus to some extent, UV rays from the germicidal lamp or sunlight are known to inactivate the virus considerably (Chaudhari and Ramakrishnan, 1988).

Rabindra *et al.* (1988) evaluated controlled droplet application formulation of NPV against *H. armigera*. The treatments were applied to the shoots of chickpea and larvae were then allowed to feed on shoots for 24 h. The larval mortality was assessed at 90, 114, 126 and 132 h. after exposure to virus. It was found that the mean percentage mortalities were significantly high at 90 and 110 h after treatment application.

The persistent *Anticarsia gemmatalis* NPV in remains of soybean plants effectively killed larvae than that of applied NPV. The virus was thought to be shielded by debries from the dead insects and by plants canopy against sunlight (Young and Yearian, 1989). Rabindra *et al.* (1989) found that the Ranipal 0.5 per cent and Robin blue 0.5 per cent could protect HaNPV when applied as Controlled Droplet Application (CDA) on chickpea.

The observations on the natural mortality of larvae due to HaNPV when reared on chickpea, sunflower, bean and pea revealed higher percentage of larval mortality when reared on pea and sunflower as compared to beans and chickpea (Pawar *et al.,* 1989).

Dhandapani (1990) conducted pot culture studies to know the influence of cotton varieties on the efficacy of NPV. A dose of 2.16x 10^8 POB's per ml along with crude sugar (17.5%) +cotton seed kernel powder (2.5%) as ULV spray resulted in cent per cent larval mortality with shorter LT_{50} in DCH-32 than on LRA-5166 and K-10 cultivars.

The NPV of *Panolis flammea* persisted better on lower than higher branches of lodge pine due to reduced UV light as measured by radiometer and polysulphone film (Killick and Warden, 1991). In an experiment with NPV of *H. zea,* exposed to simulated sunlight, there was about 90 per cent loss of its original viral activity (Ignoffo and Garcia, 1992).

Mortality of second instar larvae of noctuid *H. armigera* on cotton due to nuclear polyhedrosis virus was significantly higher on *Gossypium barbadense* cv. DCH-32 than on *G. hirsutum* cv. LRA-5266 and *G. arboreum* (cv. K-10) under laboratory condition (Dhandapani *et al.,* 1993).

The influence of chickpea, pigeonpea, lablab, sunflower and cotton on the pathogenecity of nuclear polyhedrosis virus (NPV) against *H. armigera* and the effect of different plant parts of cotton and two species of cotton on the pathogenecity was studied by Rabindra *et al.* (1994a). The median lethal concentration and median lethal time (LT_{50}) in second instar larvae of *H. armigera* indicated that the cotton leaf surface was the most detrimental to the virus followed by chickpea. Pigeonpea, lablab and sunflower leaves did not affect the virulence of NPV. The LT_{50} was higher for pigeonpea, chickpea and cotton and lowest on lablab followed by sunflower. Among the different plant parts of cotton, virus applied to leaves was most affected while flowers, squares and bolls did not affect the virus significantly. The mortality of *H. armigera* NPV was higher on *Gossypium barbadense*.

Gopali (1998) evaluated HaNPV on different host plants like pigeonpea, chickpea, tomato, cotton, sunflower, safflower and chilli. Among different plants, pigeonpea and chickpea leaves caused least change in the virulence of HaNPV. Whereas, the efficacy was significantly reduced on tomato and chilli followed by sunflower and safflower. Further, the influence of different parts of pigeonpea on the efficacy of virus indicated that viral activity was least affected on pods ($2.4x10^3$ POB/m) followed by buds, flower and leaves ($7.47x10^4$ POB/m).

Nuclear polyhedrosis virus (NPV) solution was sprayed at 250-300 larval equivalents (LE)/ha to 45-day-old cabbage, castor, beans, cotton, groundnut, mulberry, potato, soybean, strawberry, sunflower and tomato plants in a field experiment conducted in Dharwad, India during 1996-97. The leaves from randomly selected plants were taken at 0, 1, 2, 3, 4, 5, 6 and 7 days after spraying and fed to third instar larvae of *S. litura*. Highest larval mortality was observed on the day of spraying with soybean leaves (96.33%) while, the lowest mortality (68.33%) was observed on cotton. More than 50 per cent larval mortality was recorded on leaves of soybean and groundnut at 3 days of spraying indicating higher persistence of NPV on these crops, which could be attributed to dense canopy and microclimate of these crops as compared to other host plants. Probit analysis of time mortality (LT_{50}) response of *S. litura* was highest in cotton and least in soybeans after 0, 24, 48 and 72 h (Kulkarni and Hugar, 1999).

The activity of the *Anagrapha falsifera* nuclear polyhedrosis virus (AfMNPV) against the beet armyworm (*Spodoptera esculentum*) was least on cotton (*Gossypium hirsutum*) and was intermediate on kale (*Brassica oleracea*). Whereas, the activity of AfMNPV against maize earworm, *H. zea*, was in the order of maize >bean>cotton. Effects of host plants on viral activity were not closely related to larval feeding rates and host plants. Cotton and kale affected AfMNPV and homologous NPV of the beet armyworm to similar degrees. Similar results were found for AfMNPV and the homologous NPV of the maize earworm on cotton and bean. Levels of control provided by these viruses may thus be higher on tomato or maize than on cotton and intermediate on kale or bean (Farrar and Ridgway, 2000).

2. 2. 1 Effect of Bt cotton varieties on *Helicoverpa armigera*

Since the effect of Bt cotton varieties on the efficacy of HaNPV isolates is not available, the related reviews on the effect of Bt cotton varieties on *Helicoverpa armigera* is presented hereunder.

The effects of transgenic Bt [*Bacillus thuringiensis*] cotton on the development and reproduction of cotton bollworm (*H. armigera*] was studied using transgenic Bt cotton line R93-4 in comparison with the control variety CCRI-12. Transgenic Bt cotton exhibited an obvious effect on development and reproduction. All the first to fourth instar larvae fed with transgenic Bt cotton succumb to death. The rate of pupation and adult emergence decreased by 48.20-87.50 per cent and 66.70-100.00 per cent, respectively. Subsequently, the fecundity as well as rate of hatchability were reduced to the extent of 50.10-69.70 and 80.60-87.80 per cent, respectively (Jie *et al.*, 1999).

Studies were conducted to determine the effects of transgenic Bt [*Bacillus thuringiensis*] cotton on three larval instars of beet armyworm, *Spodoptera exigua*. The first, third and fifth instar larvae were fed with field collected Bt-cotton leaves for 1, 2, 3, 4 and 7 days or until pupation and then transferred to artificial diet. The larval mortality at pupation was low for all tested instars. The larval period enhanced with the increase of feeding time on Bt-cotton in 1st and 3rd instars, but not in 5th instar. Significant difference among three instars was noticed when fed continuously on Bt-cotton leaves. Similarly, the pupal weight also reduced with increased feeding time on Bt-cotton and, a greater reduction was recorded in later instars. Furhter, the feeding time on Bt-cotton also affected the survivability in 1st and 3rd instars but not in 5th instars (Ashfaq *et al.,* 1999)

A transgenic cotton [*Gossypium*] line expressing a modified Cry1A gene of *B. thuringiensis* (Bt) developed in China was used to assess the variation of insecticidal activity of Bt cotton and the response of the cotton bollworm (*H. armigera*) to the selection by Bt cotton. The insecticidal effect of Bt cotton flowers was least when compared to leaf, square and boll. The survivors of third and fourth generation *H. armigera* after feeding on flowers of Bt cotton produced offsprings, the neonate of which could feed on boll until pupation owing to selection of field populations of *H. armigera* against Bt cotton (Zhou *et al.,* 1999).

Zhou *et al.* (1998) evaluated the effects of Bt cotton on the growth and survival of different instar larvae of cotton bollworm, *H. armigera*. Significant insecticidal activity of Bt cotton leaves was observed only on the first instar larvae, which fed successively for 3 days. No larva could survive to pupation when fed on Bt cotton leaves continuously from the beginning of either 1st, 2nd, 3rd or 4th instar. However, if the larvae were provided with Bt cotton leaves only at the fifth instar, they could normally develop in to pupae. Thus, the studies indicated that the inhibitory effect of Bt cotton on the growth of larvae was more obvious than the lethal effect.

The survival and development of fall armyworm (*Spodoptera frugiperda*) on leaves and bolls of normal (variety DP 5415) and transgenic cotton plants with *B. thuringiensis* (Bt) CryIA (c) delta-endotoxin, variety NuCOTN 33B were examined in field studies conducted in Louisiana during 1996 by Adamczyk *et al.* (1998). No significant differences were observed in larval survival, per cent pupation and adult emergence between the two varieties at 2, 4, 6, 8, 10, and 12 days after exposure. However, larval weights of *S. frugiperda* differed significantly, being higher at 6 and 12 days after exposure on DP 5415 leaves than NuCOTN 33B leaves, and also time to pupation and adult eclosion were significantly shorter on DP 5415 leaves as compared NuCOTN 33B leaves.

Clear differences in bollworm (*H. zea*) and fall armyworm (*S. frugiperda*) survival and development were observed when the larvae were fed with leaves from 17 commercially available cotton (*Gossypium*) varieties having transgenic Bt (*B. thuringiensis*) delta endotoxin. A quantification assay (ELISA) method was used to quantify the levels of delta-endotoxin in two of these varieties (DP451B/RR and NuCOTN 33B) throughout the growing season. Differences in the amount of delta-endotoxin present in various plant parts including leaves of these two Bt varieties were correlated with larval survival and development data. The larvae fed on DP451B/RR completed development faster and exhibited better survival than those fed on NuCOTN 33B owing to lower levels of delta-endotoxin detected in DP451B/RR as compared to NuCOTN 33B (Adamczyk *et al.,* 2000).

Turner *et al.* (2000) tested both genetically modified (GM) and non-modified baculoviruses alone and in combination with an insecticide for the control of cotton bollworm, *H. zea* (Boddie), in Bt and conventional cotton. GM viruses, engineered for faster kill (Dupont), were compared with a conventional virus (Dupont) to Asana™ [fenvalerate] in Bt and conventional cotton. In conventional cotton, the bollworm population was significantly lower in all treatments when compared to untreated controls, however, only the Asana™ standard gave adequate control. In Bt cotton, the GM virus IC917-11 with IC848-14 (Dupont) and the pyrethroid Asana™ provided significantly better control over untreated check.

The effect of Bt transgenic cotton on the cotton bollworm, *H. zea,* nucleopolyhedrovirus interaction and the effectiveness of lower rates of Gemstar [based on *H. zea* nuclear polyhedrosis virus] against cotton bollworms on Bt transgenic cotton was studied by Streett *et al.* (2000). The Bt treated diet reduced the growth rate of cotton bollworms and caused an additive mortality response when combined with Gemstar under laboratory conditions, In general, the larval counts of *H. zea* in field studies were significantly lower on Bt cotton, while *H. zea* larvae in all virus treatments showed lower survival.

The effects of transgenic *B. thuringiensis* (Bt) cotton on cotton bollworm (*H. armigera*) biology were studied in China. Upon feeding Bt cotton, larval weight, pupation rate, eclosion rate, pupal weight and body length of larvae decreased. Except for the third instar larvae, which were mainly distributed on plant reproductive parts in both non-Bt and Bt cotton, the preferred microhabitats of cotton bollworms in transgenic Bt cotton were in the order of blossom > boll > bud as compared to non-Bt cotton preference of bud > boll > blossom (Wang *et al.*, 2003).

2. 3 Persistence of NPV in Soils

Baculoviruses are very stable in soil environment and some activity persists for years or decades under normal environmental conditions. The

Trichoplusia ni NPV applied to soil by drenching retained most of its activity even after 95 weeks (Jaques, 1964).

The interactivity of both types of boxed soils treated with polyhedra declined more rapidly when kept in the greenhouse than outside. The persistence of the virus in both types of soil was similar either dry or when watered weekly, indicating that leaching of the polyhedra may not be an important factor. After a heavy rain, the cabbage plants grown on treated soils were lethally infective against more test larvae (38%) than the plants grown on non-treated soil (4%), showing that virus in soil can be splashed to foliage and cause infection indicating the role of contaminated soil in dissemination of this disease (Jaques, 1964).

Analysis of intact and whole inclusion bodies of the corn earworm, *H. zea* (Boddie) NPV was studied by Estes and Faust (1966). They showed a silicon content of 0.12 per cent of the total body weight. The presence of silicon in the polyhedral protein and the absence of this element in the virus rods could be well demonstrated. However, the possibility remained that under in vitro conditions of virus liberation, the silicon might have been present in the virus but lost prior to assay. Evidence gathered by Estes and Faust (1966) support the idea that dissolution of the polyhedral protein material was primarily dependant upon solubilization of the silicates present. A framework or skeletal function was thus assigned to these silicates. Hence high silicon content might contribute to its resistance to break down.

According to Jaques (1967), approximately 25 per cent of the *T.* ni NPV activity remained after five years. This source of virus served as a reservoir among generations of *T. ni* because active virus laden soil was splashed on to the plants by rain. When the plots were planted with cabbage, the *T. ni* NPV increased to higher concentrations, which further reduced by preparatory tillage during spring. On the other hand, the *T. ni* GV did not persist for a long after application and traces could be detected after two years. In contrast, the *P. brassicae* GV retained most of its activity in soil and sand even after two years. The *P. rapae* GV also persisted at a high concentration following epizootics.

Jaques (1967) determined the viral activity of soil treated with polyhedra of the cabbage looper, (*T. ni*) by bioassay at different intervals. The plots treated at a rate of 6.4 x 10^{10} polyhedra per square meter of soil surface retained about 28 per cent of the original infective virus after 5 years. The viral activity of soil under artificial conditions decreased more rapidly.

Sunlight is known to inactivate virus (Bullock, 1967; Cantwell, 1967; Morris, 1971 and Ignoffo, 1973). Schmid (1974) demonstrated that, apart from ultra violet (UV) radiation; visible light was also able to inactivate a Granulosis virus (GV). It is obvious that this susceptibility limits these of viruses in insect control under field conditions. Several measures to alleviate this problem have been tested and the results were quite encouraging. The occlusion bodies of insect viruses are known to persist in soil for longer time without loss of infectivity. This property is of much practical significance as the soil borne virus can initiate epizootics in the subsequent seasons.

A nuclear polyhedrosis virus of the soybean looper, *Pseudoplusia includens* was applied to soil at 0, 2470, 24700 and 247000 LE/ha in soybean during spring in Arkansas, USA. Epizootics occurred in soybean loopers in all treatments. Cent per cent mortality was observed with higher level of NPV at 6 weeks of application. Bioassay studies indicated that the virus concentrations increased as the epizootic progressed (Young and Yearian, 1974).

The activity of viruses in forest soil and duff appears to be better than in crops fields. A low level of *Orgyia pseudotsugata* NPV was present in soil of forest in which the outbreak of *O. pseudotsugata* was recorded 40 years back.

The pest outbreak had been eliminated by a disease that had symptoms recognized as NPV (Thompson *et al.,* 1981).

Young and Yearian (1986) reported that the *A. gemmatalis* NPV applied to soil persisted during summer months. This source of NPV initiated disease outbreaks in larvae on soybean as the activity on plants was enhanced after rainfall or tillage, moving virus-laden soil to plants. Several workers have reported the remarkable stability of baculoviruses in soil. Studies on soil biology demonstrated that appreciable quantities of active virus accumulated in the upper 1 cm layer of the soil in plots where virus was sprayed at 10, 20 and 100 LE/acre. The accumulation of virus continued after completion of spraying and considerable quantities of active virus persisted throughout winter (Thomas *et al.,* 1972; Thompson and Scott, 1979 and Evans and Harrap, 1982). Strong adsorption of virus inclusion bodies to soil particles was also reported (Hukhara and Namura, 1971 and Hukhara and Wada, 1972). These studies confirmed the remarkable resistance of polyhedra against decomposition in a chemically and microbiologically complex environment like soil.

Nair and Jacob (1988) studied the persistence of NPV of *Spodoptera mauritia* (Boisduval) in soil. The virus when incorporated in soil under field conditions exhibited a half-life of 6.64 months whereas, the half-life was extended upto 16.03 months when the soil was kept under laboratory conditions. A comparison of the LT_{50} values showed that the virus lost its virulence more rapidly when the virus treated soil was kept outdoors. However, the virus stored in a refrigerator showed only slight loss in original activity even after 18 months.

The effect of leaching on the persistence of nuclear polyhedrosis virus (NPV) of *H. armigera* in a column of black soil using polyhedra pre-labelled with an isotope 32^{p} was studied by Narayanan and Jayaraj (1988). The study revealed that the virus persisted in the upper layer was capable of contaminating leaves of crop plants and re-infecting the larvae. The top surface of soil column at 0.6 cm depth recorded 59.35 per cent radio activity and decreased with depth.

Adsorption of the polyhedra in two types of soils was studied by Narayana and Jayaraj (1988) and it was found that the adsorption rate was higher in alfisol than in vertisols. The NPV of *H. armigera* from black soil when applied at 5 x 10^9pre-labelled polyhedra at the top of the soil column, retained the polyhedra upto 10.2 cm after continous leaching for 18 days.

Hugar (1993) studied the persistence of NPV of *Mythimna separata* in soil. When the MsNPV obtained from the black soil and fed to the larvae of *M. separata*, the larval mortality declined from 84.46 to 1.00 per cent in 25 months when the treated soil was kept outdoor, whereas, in case of red soil, the mortality declined from 84.46 to 18.72 per cent. The rate of adsorption of virus particles was higher in alfisol than in vertisols. The virus retained 50 per cent of its activity even upto nine months when MsNPV was added to black soil kept outdoors while, the same soil when kept indoor retained its 50 per cent activity upto 12 months. Though the activity of virus could be observed upto 25 months, half life the viral activity could be retained only upto 13 months in red soil kept indoors.

The computed half-life of *M. separata* NPV in treated black soil kept outdoors and indoors revealed at 9 and 12.50 months, respectively. For the treated red soil it was 13.00 and 13.20 months when kept outdoors and indoors, respectively.

2. 4 Assessment of quality standards of HaNPV produced by private firms

In India, there has been a rapid development in the production and use of biocontrol products and particularly, nucleopolyhedrosis viruses (NPVs) in the last decade. The Indian Government has supported a policy of promoting biocontrol agents as part of IPM programmes, particularly against *H. armigera.*

Although, there was a rapid expansion in the number of products being produced during early 1990s, this was accompanied by reports of problems with regards to product quality. In a survey comprising of NPV being either sold or distributed to farmers during 1994, none of the 17 samples examined showed the declared level of NPV. Surprisingly, in the five samples no NPV Occlusion Bodies (OB) could be traced. Of those containing NPV none of them contained more than 10 per cent of the expected NPV levels but had less than 1 per cent in most of the cases. In the samples of HaNPV, instead of nominal 1×10^9 OB/ml, the mean OB count was 2×10^4 OB /ml, while for SlNPV the mean count was 4×10^8 /ml) (Kennedy *et al.,* 1999).

The causes of the problem of quality lay many deficiencies in production techniques and poor quality control producers in the absence of clearly understood standards for NPV production (Anon., 1993). At the time, India lacked any regulatory standard for baculoviral control agents as they are outside the remit of the Pesticides Act. Also, while interest in Baculoviruses (BVs) has encouraged both researchers and companies in their development, a widespread understanding of effective quality control techniques has been lacking.

Contributing to this problem, the widespread use of the term larval equivalent (LE) as the accepted standard measure of NPV content in place of actual OB counts, is still practiced even in some scientific work. A larval equivalent is described as the average amount of NPV/GV produced by a single infected larva. However, the number of OBs produced in larva dependents upon a variety of factors, including the quality of the inoculum, rearing condition, size and instar of the larvae as well as the production system. Even if healthy larvae are fed with NPV, the amount of NPV produced can vary depending upon the age and size of the larvae at inoculation, rearing conditions and inoculum dose. Another factor influencing the quality was that many producers (nearly 70%) use wild-collected insects, many of which were either the wrong (non- susceptible) species or at inappropriate stages for effective NPV propagation.

In some of the products, the NPV content was accompanied by high counts of microsporidian spores, bacterial spores and insect debris. Although, the products have been produced from proper method, but the inocula and/or insects used for BV production could be highly contaminated. Also, in some cases, quality control staff were unable to identify or, count OBs precisely.

To deal with these problems, the Indian Council of Agricultural Research (ICAR) in collaboration with NRI and Tamil Nadu Agricultural University (TNAU), initiated NPV training courses in 1994 comprising of quality control and production techniques for researchers, regulators and producers. In addition to providing training, the distribution of properly purified and characterized NPV as inocula was also identified as an important element in improving production quality. Most producers readily accepted both the training and new inocula to improve quality control and production practices. However, a minority of producers showed their unwillingness either to admit the problem or to attempt to improve product quality.

Anonymous (2000) reported that among 45 samples of both HaNPV and SlNPV received from different sources in Karnataka, all the samples have been characterized as standard samples since they could meet the required quality standard of $1x10^9$ POB per ml. The main producers of NPV in Karnataka included, Bio-pest management (BPM), Bio Pest Laboratories (BPL) and Pest Control India (PCI).

2. 5 Assessment of HaNPV isolates in the IPM of *Helicoverpa armigera* on chickpea

Patel *et al.* (1968) were the first to evaluate the HaNPV against *H. armigera* in chickpea ecosystem. In a field trial with synthetic insecticides and HaNPV, Makode (1978) reported that reduction in pod damage was due to NPV. Although NPV was effective in controlling the pest, it failed to give higher yields as compared to chemical treatments (Backwad, 1979). Narayanan (1979) obtained similar effect with HaNPV @ 125 LE/ha and 250 LE/ha sprayed three times in the evening hours at an interval of seven to ten days when the pest was in the very early stage. The higher NPV dose was comparable to 0.07 per cent endosulfan. NPV spray three times at 250 LE/ha along with 2.5 per cent cane sugar effectively checked the larval population, reduced damage to leaflets, flowers and pod, and increased the total number of pods and yield (Narayanan, 1980).

According to Santharam and Balasubramanian (1982) two sprays of NPV at 375 LE/ha gave maximum protection to the crop and resulted in higher yield of chickpea. Lowest pod damage (7.28%) and highest grain yield was recorded with single spray of either insecticide or NPV (@ 375 LE/ha). Whereas, five sprays of HaNPV @ 250 LE/ha at weekly interval gave satisfactory control of pests and resulted in increase of grain yield by 28 per cent during 1980-81 and 47 per cent during 1981-82 (Mishra *et al.,* 1984). Similarly, several workers from Tamil Nadu have reported the field efficacy of NPV against *H. armigera* (Ethiraju, 1986; Sathiah, 1987; Muthiah, 1988 and Mahesh Babu, 1990).

The results of Dhamdhere and Khaire (1986) showed that two applications of HaNPV @ 450 LE/ha at 10 days interval were the most effective in reducing the pod damage thereby recording highest yield. Whereas, Abdally *et al.*(1987) did not find any significant control of *H. armigera* on chickpea with NPV alone @ $6X10^{11}$POB's per ha as against the chemical spray with monocrotophos.

Rabindra and Jayaraj (1986) and Santharam and Balasubramanian (1982) reported that high volume application of NPV was quite effective in controlling *H. armigera* on chickpea.

Jayaraj *et al.* (1987 and 1989) noticed significant reduction in larval population with NPV application of 250 LE per ha when applied during evening hours. The virus applied @ 1.5 $X10^{12}$ POB's per ha sprayed three to four times during evening hours at an interval of seven to ten days when the pest is in the early stages effectively controlled the pest (Rabindra and Jayaraj, 1988a and 1988b).

Balasubramanian *et al.* (1989) undertook spraying of HaNPV @ 250 LE per ha when the population of *H. armigera* was at early stage of development. To obtain better control of the pest, the chickpea could be well protected against *H. armigera* with two sprays of HaNPV @ 500 LE/ha (Jagadeesh Babu *et al.,* 1992) or two sprays of HaNPV @ 250 LE per ha (Pawar and Thombre, 1992 and Srinivasan *et al.,* 1994).

The larval mortality of *H. armigera* caused by nuclear polyhedrosis virus (NPV) was significantly higher on *H. armigera*-susceptible varieties of chickpea than the resistant accessions (ICC-506 and ICC-10187). Intermediate mortality was observed on the moderately susceptible accession Co-2. The leaf consumption was significantly higher on the susceptible varieties, which correlated positively with larval mortality rates. Probit analysis showed that the virus recorded a shorter LT_{50} on the susceptible variety than the tolerant or resistant varieties. Results of a field trial conducted in Tamil Nadu showed that control of *H. armigera* with NPV was significant on the highly susceptible or moderately susceptible (Co2) varieties. However, on the tolerant variety ICC 506, even though virus application significantly reduced the larval number, pod damage and yield did not differ significantly from the untreated control (Rabindra *et al.,* 1992).

NPV sprayed at 250 LE/ha in 350 to 400 litres of spray fluid for 5 to 6 times recorded significantly higher per cent mortality (90.00%). Nearly 47 per cent increase in yield was recorded which was attributed to the effectiveness of NPV (Anon., 1993)

Application of nuclear polyhedrosis virus (NPV) at 300 LE per ha mixed with endosulfan at 500 ml/ha resulted in minimum numbers of larvae per meter row length (2.40 larvae) in chickpea. The minimum pod damage (39.86 %) and maximum grain yield (20.15 q/ha) were obtained in this treatment. Simultaneously, repeated application of HaNPV at 300 LE/ha significantly reduced the larval population and resulted in reduced pod damage and increased yield. However, two applications of HaNPV at 250 LE/ha, at pod initiation stage and after 10 days interval also enhanced grain yield of chickpea (Kumar *et al.,* 1998).

Field evaluation with eight treatments comprising of HaNPV, a commercial formulation of *B. thuringiensis* subsp. *krustaki* (Dipel), neem seed extract and endosulfan either alone or in combination of two products was carried out against *H. armigera* in chickpea. It was noticed that all the treatments were effective in reducing larval populations as well as pod damage which resulted in greater grain yields as compared to the untreated control (Wanjari *et al.,* 1998).

Singh *et al.* (1999) evaluated microbial pesticides and NPV (250 LE/ha) alone and in-combination with endosulfan for their effectiveness against *H. armigera* on chickpea in the field in Bihar during 1995-96. These biopesticides when used alone or in combination with endosulfan proved superior to the untreated control by reducing the pod damage and enhancing grain yield. However, when used in combination with endosulfan, they were still effective resulting in relatively low pod damage, with an average of 4.21 (Delpin), 5.65 (Dipel) and 6.66 per cent (NPV) and recorded increased grain yield of 49.7, 47.2 and 46.7 per cent, respectively.

The role of nuclear polyhedrosis virus (NPV) for managing *H. armigera* was studied in chickpea at Saravanampatti, Coimbatore district, during 1997-98. Treatments were applied 3 times at 7-10 days intervals in the evening to reduce the inactivation of the virus by ultraviolet radiation. The results indicated that *H. armigera* could be controlled successfully through the use of NPV. Grain yield was highest (1029.60 kg/ha) when NPV was applied with 5 per cent crude sugar +5 per cent starch followed by 5 per cent crude sugar + 15 per cent chickpea seed extract (972.39 kg/ha) (Chand *et al.,* 1999).

Field trials revealed that 2 applications of HaNPV alone or HaNPV followed by insecticides resulted in effective control of *H. armigera* infesting chickpea which resulted in significant increase in grain. Mean pod damage in treated plots ranged between 4.2 and 6.7 per cent as compared to 10.9 per cent in untreated check. In the treated plots the yield varied between 1750 kg/ha and 1953 kg/ha, whereas in the untreated plot it was as low as 1567 kg/ha. The results of trials conducted at 10 different locations under AICPIP confirmed these findings (Ahmad *et al.,* 1999).

The bio-efficacy of five IPM modules was evaluated for the management of *H. armigera* (Hub.) in chickpea for three consecutive years from 1995-96 to

1997-98 under irrigated as well as rain-fed conditions in Rajasthan. The IPM modules, except for M4 and M5, consisted of three insecticide spray application while, module 4 and 5 received two spray applications. All the IPM modules proved significantly superior over the untreated control, both in terms of protection and production. Minimum pod damage and maximum gain yield were observed in the module 1 {HaNPV, Btk (*B. thuringiensis* spp. *kurstaki*) and endosulfan} both under irrigated (3.4% and 16.81 q/ha) and unirrigated (4.6% and 11.55 q/ha) conditions. The untreated control plots registered maximum pod damage (irrigated 26.04% and un-irrigated 24.8%) and minimum pod yield (7.96 q/ha and 5.39 q/ha). Module 2 (in which the second spray of Btk of module 1 was replaced with nimbecidine) was the second best in controlling *H. armigera* and gave maximum benefit-cost (B: C ratio) due to low cost of nimbecidine (Singh *et al.,* 2000).

In order to evaluate the efficacy of Spicturin (R) (*B. thuringiensis* spp. *Galleriae*) and HaNPV (*H. armigera* nuclear polyhedrosis virus) against *H. armigera* on chickpea. Two field experiments were carried out in Coimbatore district, Tamil Nadu during summer 1997. In both the field trails, chlorpyrifos @ 1.00 litre/ha proved superior by recording the lowest larval population and pod damage, followed by Spicturin @ 2.0, 1.50 and 1.00 litre/ha, Spicturin @ 2.00 litres/ha + HaNPV and Spicturin @ 1.50 litres+HaNPV, which were on par with each other as compared to untreated check. The cost:benefit ratio was higher with chlorpyrifos at 1.00 litre/ha (1:3.50) followed by HaNPV (@ 1.5×10^{12} POB/ha) alone (1:3.32), Spicturin @ 1.00 litre/ha (1:2.18) and Spicturin (@ 1.00 litre/ha) + HaNPV (1: 2.07) (Loganathan *et al.*, 2000).

Treating chickpea with *H. armigera* NPV at 1.5×10^{12} PIB per ha proved effective in controlling *H. armigera* larvae and resulted in increased yield over untreated control as well as standard check, endosulfan or *B. thuringiensis*, in two successive years. The high virus production costs make the viral treatments uncompetitive compared with the chemical treatment. Several formulations of virus were tested including an emulsifiable concentrate, a ULV suspension and a microencapsulated preparation, but none showed consistent effectiveness over filtered but unpurified aqueous suspension of HaNPV. HaNPV proved to be slow acting with an average survival time (AST) of 5.5 days as compared to 3.2 and 4.3 days recorded in *B. thuringiensis* and endosulfan, respectively (Cherry *et al.*, 2000).

Material and Methods

III. MATERIAL AND METHODS

The studies on *Helicoverpa armigera* nuclear polyhedrosis virus (HaNPV) isolates were carried out during 2001-02 under laboratory conditions in the Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad (UASD) and their assessment in the integrated management of *H. armigera* in chickpea eco-system during 2002-03 and 2003-04 at the Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad.

Dharwad is situated in the transitional tract of Karnataka (Zone-8) at 15^0 26′ North latitude and 75^0 07′ East longitude with latitude of 678 m above the mean sea level. The mean annual rainfall of Dharwad is about 890 mm distributed all over the period of seven to eight months with two prominent peaks occurring in July and October. The meteorological observations during the experimentation period are presented in the Appendix-1.

The details of the materials used and methodology adopted during the course of investigation are described hereunder.

3.1 Characterization of *Helicoverpa armigera* nuclear polyhedrosis virus (HaNPV) isolates

The experiment was conducted under laboratory condition in the Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad.

3. 1. 1 Collection of *H. armigera* NPV isolates

HaNPV isolates were collected from ten locations in India during 2001-2002. The isolates collected and the source is presented in Table-1. The subculture of these isolates was prepared by infecting the NPV to the larvae of *H. armigera* maintained in the laboratory.

Table 1. Details of HaNPV isolates collected for the study

Sl. No.	Product Name	Source
1	Heliocide	Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad-580 005.
2	*Heiothis* NPV	Agricultural Research Station, Gulbarga –585 101
3	Heliokill	Department of Agricultural Entomology, College of Agriculture, Raichur-584 101.
4	HaNPV	Agriculture Research Station, Guntur, Andra Pradesh - 522 101
5	HaNPV	Department of Agricultural Entomology, Tamil Nadu Agriculture University, Coimbatore -641 003.
6	HaNPV	Project Directorate of Biological Control (PDBC), Post Bag No. 2491, H.A. Farm Post, Hebbal, Bellary Road, Bangalore – 560 024.
7	Helicide	Pest control India Pvt. Ltd. (PCI), P. B. No. 6426, Yelahanka, Bangalore-560 064.
8	Helinash LC	Bio-Pest Management Pvt. Ltd. (BPM), Opp. Jakkur Aerodrome, Jakkur Post, Bangalore-560 064.
9	*Helicoverpa* NPV	Bio-Pest Laboratories Pvt. Ltd. (BPL), No. 81, Narasimhalu Layout, 5th main, Nandini Layout, Bangalore-560 096.
10	NPV of *H. armigera*	Kalpavruksha Bio-systems. No. 1909, III cross, Prashantha Nagar, Bangalore-560 079.

3. 1. 2 Laboratory rearing of *H. armigera*

To initiate the pure culture of *H. armigera,* large number of larvae were collected from field and were reared in individual glass vials (5X2cm) containing the semisynthetic diet developed by Jayaraj and Sathiah (1993) till pupation (Plate1). During rearing, the larvae were kept in quarantine to maintain healthy culture by eliminating parasitization and removing unhealthy larvae. After discarding the feeble and diseased pupae, the healthy ones were subjected to sterilization by dipping in 0.25 per cent sodium hypochlorite solution. The sterilized pupae were taken in petriplate (10 cm diameter) containing fine sawdust and kept in earthen pots. After a week, pupae were taken outside and transferred separately to the moth emergence cage (1'X1'X1') for eclosion. The pupae were observed at 12 h intervals for adult emergence. The freshly emerged male and female moths in 13:10 ratio (Gopali, 1998) were released inside the earthern pots (45 X 30 cm). The earthen pots were placed in plastic basins surrounded by moist sand to half the height of basin. The sand bed was moistened daily with one per cent sodium hypochlorite solution to avoid fungal growth. The top portion of pots was covered with black muslin cloth fastened by rubber band for oviposition. Fresh honey solution (10.0%) enriched with vitamins in cotton wad was provided as adult food daily till the death of moths. Eggs were collected by changing the black cloth daily in the morning. The eggs of 24 h old were surface sterilized with sodium hypochlorite (1.0%) solution. Immediately after hatching of eggs, the neonate larvae were reared for four days on chickpea seedlings. Afterwards, the grown up larvae were reared individually in vials containing artificial diet. Ingredients of the artificial diet are mentioned in Appendix-2. The second instar larvae of five day old were used for further experimentation.

3. 1. 3 Production of subculture of HaNPV isolates

Mass production of HaNPV isolates was undertaken by rearing *Helicoverpa* larvae on large scale in collaboration with the GOI project on Mass Production of Quality Bio-agents/Bio-pesticides in the laboratory, Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad. The larvae starved for eight hours were fed with soaked bengalgram seeds treated with stock NPV suspension of different isolates. Thereafter the larvae were reared in separate vials on soaked bengalgram seeds until death or pupation. The dead larvae showing typical symptoms of virus infection were collected in distilled water and allowed for further putrification.

3. 1. 3. 1 Purification

Diseased larvae were allowed to putrify in distilled water for seven days and were ground in a blender; sediment containing POB's was collected and suspended in distilled water, which was centrifuged for one minute at 500 rpm in order to remove larval debris. Supernatant containing POB's was taken and it was subjected for centrifugation at 4500-5000 rpm. The virus pellets were collected in distilled water after discarding the supernatant. The differential centrifugation was repeated till the pure POB's were obtained. This purification procedure was followed for all the isolates under study.

3. 1. 3. 2 Enumeration of polyhedral occlusion Bodies

Enumeration of polyhedra in the viral suspension was done with the help of modified Neubauer's haemocytometer, which comprised of a glass slide carrying calibrations in two replicates.

3. 1. 3. 3 Standardization of polyhedral occlusion bodies in each isolate

One ml of purified stock suspension was made to 1000 ml through serial dilution using 0.1 per cent teepol. Teepol ensured thorough mixing and uniform

Plate 1: Rearing of *Helicoverpa armigera*

Plate 2: Standardization of HaNPV isolates

distribution of polyhedra. As 1 ml of virus suspension was made up to 1000 ml by serial dilution, 1000 was taken as dilution factor.

For counting number of polyhedra, Thomawhite cell pipette provided with haemocytometer was used. One ml of the diluted virus suspension containing 0.1 per cent teepol was drawn in to pipette. Then the pipette was shaken vigorously closing the rubber tube tightly. First three drops were discarded and fourth drop was put in the groove of haemocytometer and then standard cover slip was covered over the slide, care was taken to see that haemocytometer was clean and only required quantity of suspension was put to fill the calibrated area. After allowing the polyhedra to settle down for two minutes, the polyhedra count was taken in about 25 squares of the 1/400 sq. mm area at random with the help of phase contrast microscope under 10 x 40 magnification. Proper care was taken to avoid duplication of counts of polyhedra on the lines of calibration. This was done by counting polyhedra inside the square as well as the polyhedra on the top and left side lines only. This procedure was followed for the suspensions of all HaNPV isolates (Plate 2).

3. 1. 3. 4 Assessment of Number of Polyhedra

Firstly the polyhedra count was taken from 25 squares of 1/400 sq. mm area. Then POB's per ml were determined by taking dilution factor into consideration. A virus suspension of known concentration was prepared from the stock solution by suitable dilution with distilled water. Thus, the standardization of polyhedra in each suspension of all isolates was determined and kept for further bioassay and field experimentations.

3. 1. 4 Collection of *H. armigera* from different locations

H. armigera larvae from different crop eco-systems were collected from different places *viz.*, Dharwad, Gulbarga, Raichur, Nagpur and Guntur. The maintenance of these populations under laboratory condition to get the F1 progeny, was made as explained in chapter 3. 1. 2.

3.1.5 Genetic diversity of HaNPV isolates

This study was carriedout in the molecular biology laboratory, Institute of Agri-Biotechnology, University of Agricultural Sciences, Dharwad.

3.1.5.1 *Isolation and DNA restriction endonuclease analysis of HaNPV isolates*

An aliquot of 240µl of purified virus suspension containing $2X10^9$ POB was taken in an eppendorf tube and 50µl of 0.5M EDTA and 6 µl of proteinase K (20mg/ml) were added and incubated at 37^0C for 2.0 h. To this, 150µl of 1 Na_2CO_3 was added and incubated at 37^0C for 1 h to release the virions from the POB. After adding 25µl of 10 per cent sodium dodecyl sulphate, the samples were incubated at 37^0C for 1 h. and centrifuged at 10,000 rpm for 10 min to remove any undissolved polyhedra. The supernatant was extracted with an equal volume of 25:24:1 Tris-saturated phenol: Chloroform: Isoamyal alcohol for deprotinization. Finally, the DNA was extracted with an equal volume of 24:1 Chloroform: Isoamyal alcohol. The extracted DNA was dissolved in T_{10} E_1 after 2-3 PCI treatments. Quality and quantity of DNA was assessed through agarose electrophoresis. Isolation was repeated with fresh sample till good quality DNA was obtained. DNA samples of each isolate were double digested in three combinations with restriction enzymes *viz.*, EcoR1, Saw 3A1, BamH1 and Sac1 along with appropriate buffers given by the manufactures at 37^0C for 5 h and the DNA fragments so obtained by restriction were electrophoresed using 0.7 per cent agarose gel at 35v and 50mA current for 5 h in Hoefer Electrophorosis unit. The DNA banding profile (stained with ethidium bromide) so obtained were photographed in UVi Tech transilluminator. The appropriate size of fragments was calculated using the option available in UVi-doc software.

The restricted products were scored as 1 for presence and 0 for absence. Measurement of genetic similarity for the ten isolates studied was based on Jaccard's similarity co-efficient. A similarity matrix and a dendrogram were constructed via clustering analysis following UPGMA method using NTSYSPC version 1:8 (Rohlf, 1993).

3. 1. 6 Efficacy of HaNPV isolates on *H. armigera* populations collected from different localities

Treatments Details

T_1 - UAS, Dharwad isolate

T_2 - Gulbarga isolate

T_3 - Raichur isolate

T_4 - Guntur isolate

T_5 - Coimbatore isolate

T_6 - PDBC, isolate

T_7 - PCI isolate

T_8 - BPM isolate

T_9 - BPL isolate

T_{10} - Kalpavruksha isolate

The *invitro* infectivity of different HaNPV isolates was investigated against the second instar larvae of *H. armigera* collected from different locations. The population was obtained from five different locations *viz*., Dharwad, Raichur, Nagpur, Gulbarga and Guntur. The population obtained from Dharwad and

Gulbarga was basically from chickpea and pigeonpea eco systems respectively, whereas, the population obtained from Raichur, Nagpur and Guntur was from cotton ecosystem.

The POB count of different isolates was estimated using modified Neubaur's haemocytometer. Minimum concentration of 10^6 POB's per ml, which is required to cause infection, was prepared. The standardization was done through serial dilution. Chickpea (Annigeri-1) shoots of uniform size with 6-8 compound leaves were washed thoroughly in water containing 0.1 per cent teepol, surface sterilized with 0.1 per cent sodium hypochlorite, rinsed in sterilized water and were shade dried. In each treatment 20 uniform sized second instar (5 day old) larvae were used. These shoots were treated with this concentration of HaNPV isolates for a minute and fed to the larvae. Subsequent days larvae were provided with fresh leaves till the death or completion of larval period. The observations on mortality of larvae due to HaNPV was observed from 3rd day to 10 day of the treatment and the LT_{50} values were calculated.

Further to know the LC_{50} value of HaNPV isolates, viral suspension of 10^4, 10^5, 10^8, 10^7, 10^8, and 10^9 POB's/ml were prepared using distilled water by serial dilution technique. The chickpea shoots were treated with these concentrations and in each concentration 20 second instar larvae were treated and the mortality of larvae due to virus was recorded at suitable intervals and the per cent mortality was worked out by using the following formula. The LC_{50} values were calculated for all the HaNPV isolates against the *H. armigera* population collected from Raichur, Gulbarga, Nagpur, Guntur and Dharwad. The LT_{50} and LC_{50} values were calculated by probit analysis (Finney, 1964).

$$\text{Per cent mortality} = \frac{\text{Number of larvae dead due to NPV}}{\text{Total number treated}} \times 100$$

3.1.7 Assessment of POB yielding capacity of different isolates of HaNPV

All the ten HaNPV isolates were observed for their polyhedral occlusion body yielding capacity in different larval stages. The 2nd, 3rd, 4th and 5th instar larvae of *H. armigera* were selected from the stock culture maintained separately under laboratory conditions (Dharwad population). The larvae were transferred to the multicavity trays with artificial diet inoculated with 10^8 POB per ml concentration, used for commercial production of NPV. In each HaNPV isolate 60 larvae were used. The virosed larvae were harvested after death. After harvesting, larvae were kept separately and further processing and centrifugation was made following standard procedures as explained earlier. The POB count was made with the help of modified Neubaur's haemocytometer as indicated in 3.1.3.4.

The total viral yield from the 60 larvae treated, POBs per larva and total number of larvae required to yield one Larval equivalent ($LE = 6X10^9$) was calculated. The same procedure was followed for all the ten isolates against 2nd, 3rd, 4th and 5th instar larvae of *H. armigera.*

3.2 Influence of cotton species on HaNPV isolates

The experiment was conducted under cage house conditions in the Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad.

3. 2. 1 Raising of cotton species under cage house condition

The different cotton genotypes belonging to different species included in the study are as under

Details of the treatments

T_1 - Sahana *(Gossypium hirsutum)*

T_2 - B-82 (*G. barbadense)*

T_3 - Jayadhar (*G. herbaceum)*

T_4 - DHH-11 (Intra-specific hybrid)

T_5 - MECH -12Bt

T_6 - MECH –12 NBt

T_7 - MECH –184 Bt

T_8 - MECH –184 NBt

T_9 - MECH –162 Bt

T_{10} - MECH –162 NBt

T_{11} - RCH- 2 Bt

T_{12} - RCH- 2 NBt

T_{13} - RCH- 20 Bt

T_{14} - RCH- 20 NBt

T_{15} - RCH- 144Bt

T_{16} - RCH –144 NBt

T_{17} - Control (Larvae reared on artificial diet)

An effort was made to know the impact of different cotton species including Bt cotton varieties with their non-Bt versions on the efficacy of

MECH-162Bt

MECH-162NBt

RCH-2Bt

RCH-2NBt

RCH-20Bt

RCH-20NBt

RCH-144Bt

RCH-144NBt

Plate 4: Different cotton genotypes tested in the investigation

Sahana

B-82

Jayadhar

DHH-11

MECH-12Bt

MECH-12NBt

MECH-184Bt

MECH-184NBt

Plate 3: Different cotton genotypes tested in the investigation

HaNPV isolates against *H.* armigera under laboratory conditions. Different cotton species *viz.*, Sahana *(Gossypium hirsutum)*, B-82 (*G. barbadense)*, and Jayadhar (*G. herbaceum)*, DHH-11 (Intra-specific hybrid), three Bt cotton hybrids produced by Mahyco seeds Ltd. (MECH-12Bt, MECH-184Bt and MECH-162Bt with their non-Bt versions) and three Bt cotton hybrids produced by Rasi seeds Ltd. (RCH-2Bt, RCH-20Bt and RCH-144Bt with their non-Bt versions) were included in the study (Plate 3 & 4).

The different cotton species including different Bt with their Non-Bt versions have been raised under cage house conditions in earthern pots (45X30 cm). All the package of practices were followed except plant protection measures for raising the plants. When the plants attained 65-70 days, the leaf pH of the plants was estimated. Twenty, second instar larvae of uniform age (5 days) were used per plant. On the first day, the floral parts of each plant were brought to the laboratory and fed to the larvae in multicavity trays. From second day onwards, the larvae were fed with the floral parts of the plants sprayed with NPV suspension of $1X10^9$ POB's per ml (Teepol 0.1 % and Jaggery 0.5 % was used as adjuvants). The food was changed every alternate day and fresh floral parts from the same sprayed plants was given till the death or completion of larval period. The dead larvae counted at each interval were analyzed for the presence of NPV by crushing the larvae and observing the extract under phase contrast microscope. The total larval mortality and the mortality due to NPV were calculated and converted in to percentage. The data were analyzed statistically.

3.3 Persistence of HaNPV isolates in soil

Persistence of the two virulent HaNPV isolates in soil was investigated under laboratory conditions in the Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad.

Soils selected for the study included

1. Red soil

2. Black soil

Two virulent isolates used for the study included

1. Gulbarga isolate

2. Coimbatore isolate

Two soil samples were collected from Main Agricultural Research Station, University of Agricultural Sciences, Dharwad having pH. 7.7, electrical conductivity 0.18 ds/m for black soil and 8.15 and 0.43 ds/m for red soil and were used for the experiment.

The soils were dried in the sun for three days, finally ground and passed through 20 mesh sieves. The sieved soil was divided in to nine lots of one kg each. Each lot was placed in a rectangular glass troughs (20 X 20 X 10cm). The soils in six troughs was mixed with polyhedral suspension containing 1.0 $X10^9$ POB's per ml @ 200ml/trough. Three troughs were kept in open field (Plate-4) and other three troughs were kept under laboratory condition. Moisture content of the soil in all the troughs was kept at field capacity by daily addition of required quantity of sterilized water.

Soil samples were drawn from the trough at monthly intervals up to 12 months; the first sample was drawn on the first day of the experiment. On each sampling occasion, 5 g soil was drawn from each trough. Each soil was suspended in 10 ml of sterile distilled water, stirred thoroughly and allowed to stand for 30 minutes to enable the particles to settle down. The clear supernatant was used for further bioassay studies of viral activity against second instar larvae (5 day old) of *H. armigera* following chickpea shoot dip method. The chickpea shoots were dipped in this aqueous suspension

HaNPV isolates incorporated soils kept outdoors

HaNPV isolates incorporated soils stored in refrigerator

HaNPV isolates incorporated soils kept indoors

Plate 5: Persistence of HaNPV isolates in different soils

containing 0.1 per cent teepol, air-dried and fed to the second instar larvae of *H. armigera*. There were three replications of 20 larvae in each. The observations were recorded daily on larval mortality, time required for mortality, per cent pupation and per cent adult emergence.

Soil sample containing virus suspension of 1.0 x 10^9 POB's / ml was also kept in a refrigerator at 4 ^{0}C and was bio-assayed against the second instar larvae of *H. armigera* at the beginning of the experiment and thereafter at three monthly intervals and was continued up to one year for comparison with viral activity of the virus treated soil kept under laboratory and field condition. The observations on larval mortality, mean time for mortality, per cent pupation and per cent adult emergence was made. The data were subjected to regression analysis.

Similar procedure was followed for both Gulbarga and Coimbatore isolates for red and black soils kept both in field as well as under laboratory conditions (Plate 5).

3. 4 Assessment of quality of HaNPV products produced by private firms

The study was carried out for three years during 2001-03 under laboratory conditions in the Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad. The HaNPV supplied by various private entrepreneurs to different offices of the Assistant Director of Agriculture was referred for quality standards to the Referral Laboratory, Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad. The HaNPV samples obtained were analyzed for their quality standards by counting the number of polyhedral occlusion body count per ml of suspension.

One ml of purified stock suspension was made upto 1000 ml through serial dilution using 0.1 per cent teepol. Teepol ensured thorough mixing and uniform distribution of polyhedra. As 1 ml of virus suspension was made up to 1000 ml by serial dilution, 1000 was taken as dilution factor.

For counting number of polyhedral occlusion bodies in each sample, the procedure outlined under section 3.1.3.3 was followed. This procedure was followed for all the HaNPV samples received during three years. The number of POBs per ml was estimated and the evaluation report of the same was sent to the respective ADA offices.

3. 5 Assessment of HaNPV isolates in the IPM of *H. armigera* on chickpea

Field experiments were conducted during 2002-03 and 2003-04, at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad (Plate-5). Annigeri-1 variety of chickpea was grown by following recommended package of practices except plant protection measures with a spacing of 30 cm between row and 10 cm between the plants. Randomized block design was followed with the following treatments replicated thrice.

Treatments

T_1 – UAS, Dharwad Isolate

T_2 - Gulbarga Isolate

T_3 – Coimbatore Isolate

T_4 - BPM Isolate

T_5 - PCI Isolate

T_6 - Recommended Package of Practice

T_7 - Untreated control

The recommended Package of Practices comprise the following schedule of sprays-

1st spray- Profenophos 50 EC @ 0.10 per cent per litre at 30 days after sowing (DAS)

2nd spray – NSKE 5 per cent at 45 DAS

3rd spray –HaNPV 100 LE/ac at 60 DAS

4th spray - Monocrotophos 36 SL @ 0.045 per cent at 75 DAS

Ten days after emergence, the crop was thinned to maintain uniform plant population. The first spray was initiated at 30 days after sowing. The HaNPV isolates were sprayed @ 100 LE per acre, and four such sprays were undertaken at an interval of 15 days between two sprays.

Observation on number of larvae per plant on ten randomly selected plants was made one day before spraying and 5th, 10th and 15th day after spraying at each spray.

The pod damage was assessed on ten randomly selected plants by counting the pods damaged by the pod borer larvae and healthy pods. The yield was recorded per plot and further it was converted in to q/ha. The data were analyzed statistically using RBD design.

Experimental Results

IV. EXPERIMENTAL RESULTS

Results of both laboratory and field experiments conducted from 2002-2004 at the Department of Agricultural Entomology, College of Agriculture, Dharwad and Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad on " Studies on the HaNPV isolates and their assessment in the IPM of *Helicoverpa armigera* (Hubner)" are elucidated in the following pages.

4.1 Characterization of HaNPV isolates

4.1.1 Genetic diversity in HaNPV isolates

There exist variation in the DNA profiling of different HaNPV isolates (Plate 6 & 7). The dendrogram constructed using symmetric matrix of different isolates resulted into two major clusters (Fig. 1). The first major cluster comprised of Dharwad, Kalpavruksha, Coimbatore, BPM, PDBC and BPL isolates. Within the first major cluster, there are two sub-clusters in which the first subcluster has two sub-sub clusters. Dharwad and Kalpavruksha isolates formed one sub sub-cluster with a genetic similarity co-efficient of around 0.65 whereas, Coimbatore and BPM formed the second sub sub-cluster with a genetic similarity value of 0.63. PDBC and BPL formed the second sub-cluster in the first major cluster with a genetic similarity of 0.61.

The second major cluster constituted three isolates *viz.,* Gulbarga, Raichur and Guntur isolates, which separated from first major cluster at the similarity value of around 0.54. In the second major cluster, there are two sub-clusters. The first sub-cluster has two sub sub-clusters with a co-efficient of 0.77 forming Gulbarga as a separate sub sub-cluster and another sub sub-cluster constitute of Raichur and Guntur with a similarity co-efficient of 0.82. The second sub-cluster constitute of PCI alone that diverged from all other

Table 2. Similarity Matrix of different isolates.

Isolates	Gulbarga	Coimbatore	Guntur	Raichur	PDBC	PCI	BPM	BPL	KLP	Dharwad
Gulbarga	1.00									
Coimbatore	0.65	1.00								
Guntur	0.55	0.76	1.00							
Raichur	0.53	0.77	0.82	1.00						
PDBC	0.58	0.53	0.56	0.69	1.00					
PCI	0.56	0.51	0.50	0.49	0.62	1.00				
BPM	0.40	0.61	0.47	0.54	0.54	0.56	1.00			
BPL	0.63	0.52	0.38	0.58	0.63	0.56	0.53	1.00		
KLP	0.56	0.51	0.50	0.41	0.47	0.61	0.51	0.51	1.00	
Dharwad	0.65	0.54	0.45	0.52	0.56	0.63	0.55	0.60	0.59	1.00

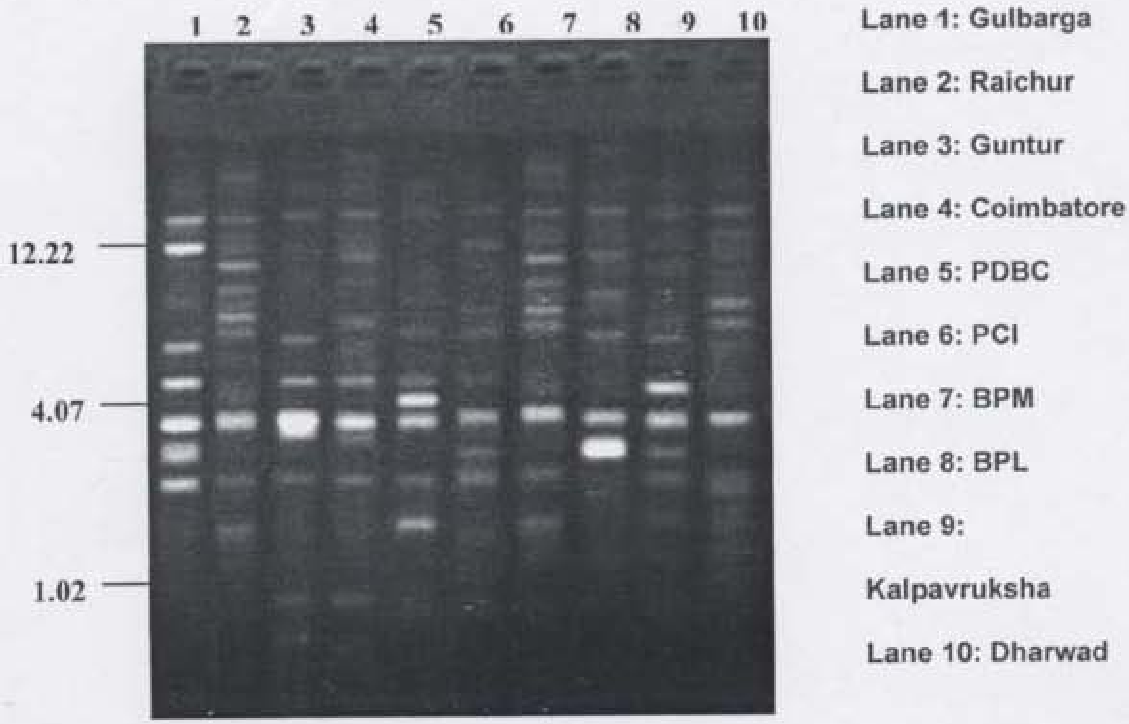

Plate 6: Characterization of DNA of HaNPV isolates with EcoR1 and Saw 3A1 double digest

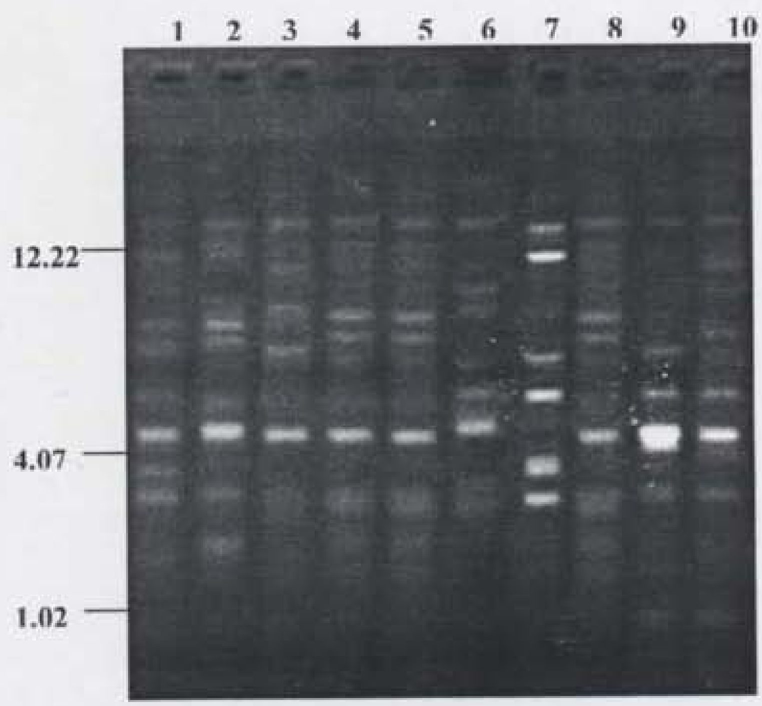

Plate 7: Characterization of DNA of HaNPV isolates with BamH1 and Sac1 double digest

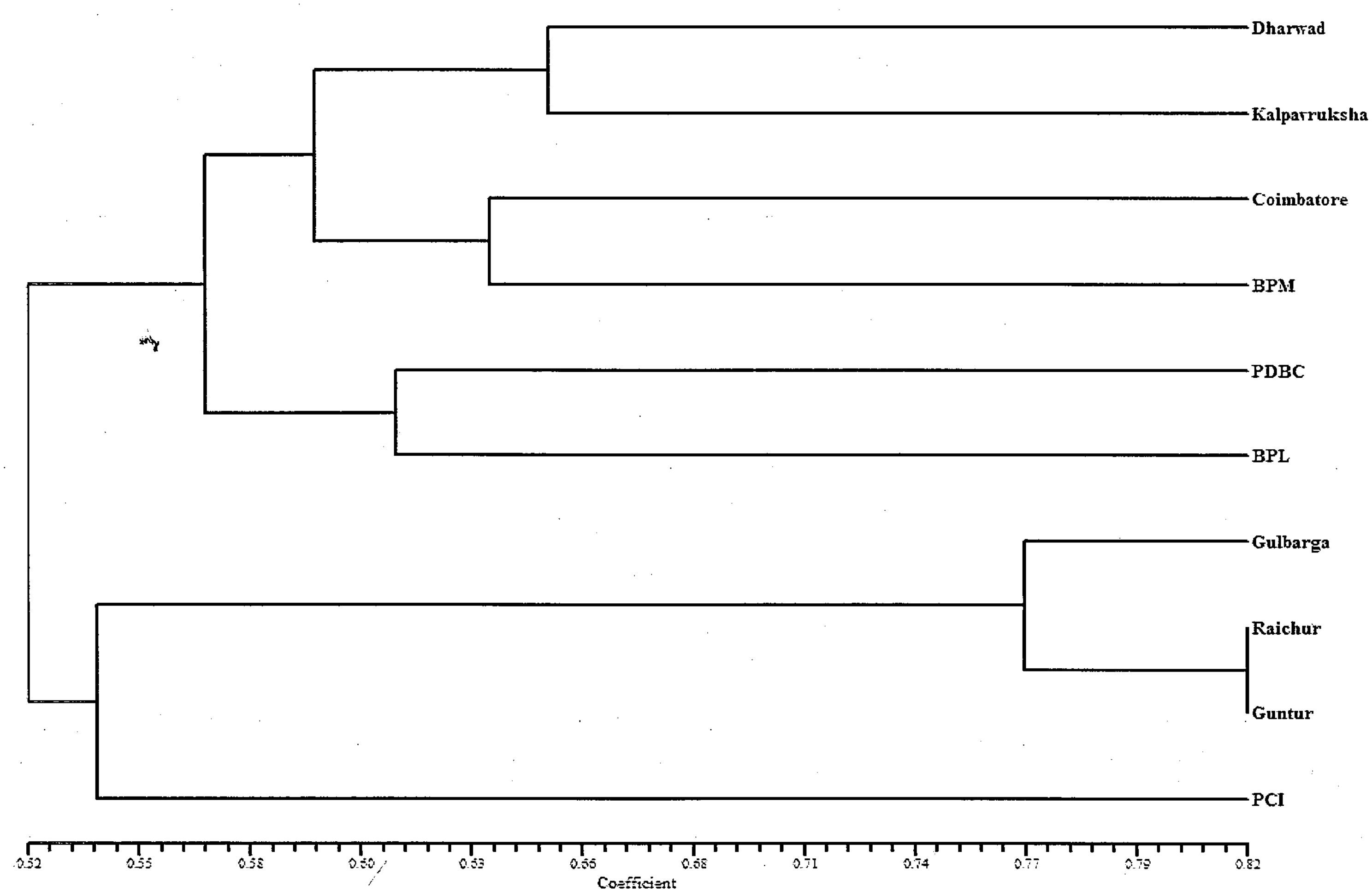

Fig 1. Dendrogram of different HaNPV isolates

clusters at similarity co-efficient of 0.54. Raichur and Guntur isolates found to possess highest similarity compared to others.

The similarity matrix pertaining to different isolates revealed that the similarity co-efficient ranged from 0.38 to 0.82 (Table 2). The highest genetic similarity index of 0.82 was seen between the isolates from Raichur and Guntur followed by 0.77 between the isolates from Coimbatore and Raichur. The minimum genetic similarity of 0.38 was found between the isolates from PCI and Dharwad.

4.1.2 Biological activity

The biological activity of different HaNPV isolates interms of LT_{50} and LC_{50} values against the different geographical populations of *H. armigera* was made and presented hereunder.

4.1.2.1 Dharwad population:

The time and dose mortality response of different HaNPV isolates against the Dharwad population of *H. armigera* is presented in Table 3. On the basis of LT_{50} values, HaNPV isolate obtained from Coimbatore (105.41 h) and Gulbarga (105.86 h) had quick knock down effect on the larvae, whereas, the HaNPV produced by Kalpavruksha Biosystems was the least effective with a LT_{50} value of 181.32 h.

Similarly, the virulence of Coimbatore and Gulbarga isolates was further evidenced from the least LC_{50} values (1.89×10^4 and 2.10×10^4 POB ml^{-1}), which were more virulent than other isolates. The HaNPV produced by two private firms namely, Kalpavruksha Biosystems (1.23×10^5 POB ml^{-1}) and Bio Pest Laboratories (1.03×10^5 POB ml^{-1}) have registered highest LC_{50} values, indicating their least infectivity against the host. There was approximately five

Table 3. Efficacy of different HaNPV isolates against Dharwad population of *Helicoverpa armigera*

Sl. No	Isolates	Time mortality response			Dose mortality response		
		LT_{50} (h)	Fiducial Limits (h)		LC_{50} (POB/ml)	Fiducial Limits (POB/ml)	
			Lower	Upper		Lower	Upper
1	Dharwad	140.36	136.14	144.58	5.60×10^4	2.30×10^4	8.97×10^4
2	Gulbarga	105.86	100.86	111.33	1.89×10^4	1.23×10^4	7.46×10^4
3	Raichur	149.98	140.53	153.67	7.50×10^4	5.35×10^4	1.03×10^5
4	Guntur	140.09	134.49	147.59	7.69×10^4	4.53×10^4	1.160×10^5
5	Coimbatore	105.41	98.36	109.24	2.10×10^4	1.02×10^4	4.77×10^4
6	PDBC	159.00	151.35	164.58	7.97×10^4	6.45×10^4	1.94×10^5
7	PCI, Ltd.	161.35	154.23	167.39	9.31×10^4	5.68×10^4	1.39×10^5
8	BPM, Ltd.	159.93	155.30	168.51	8.90×10^4	6.79×10^4	1.11×10^5
9	BPL, Ltd.	162.39	155.17	169.39	1.03×10^5	9.8×10^4	2.41×10^5
10	Kalpavruksha, Ltd.	181.32	177.54	191.05	1.23×10^5	1.10×10^5	4.89×10^5

PDBC: Project Directorate of Biological Control, Bangalore
PCI: Pest Control India Ltd, Bangalore
BPM: Bio-Pest Management, Ltd, Bangalore
BPL: Bio Pest Laboratories, Ltd, Bangalore

fold increase in the infectivity of Gulbarga and Coimbatore isolates over Kalpavruksha and BPL isolates.

4.1.2.2 Raichur population

Comparatively, all the isolates were more virulent against Raichur population than Dharwad population of *H. armigera.* Gulbarga isolate excelled over other isolates by registering least LT_{50} (98.09 h) and LC_{50} ($1.59X10^4$ POB ml^{-1}) values, compared to other isolates in the study. Coimbatore isolate was found to be closely related to Gulbarga isolate in its virulence interms of LT_{50} (101.35 h) and LC_{50} ($1.78X10^4$ POB ml^{-1}) values. Among the isolates, the Kalpavruksha and BPL NPV emerged as least virulent by recording highest LT_{50} (181.00 h and 162.48 h) and LC_{50} ($1.15X10^5$ and $1.01X10^5$ POB ml^{-1}) values, indicating their inferiority in rendering the larvae to death. Kalpavruksha isolate required 7 folds more concentration than Gulbarga isolate to cause 50 per cent mortality of the population. Similarly, it took approximately 80 hours more time than Gulbarga isolate to induce mortality of larvae (Table 4).

4.1.2.3 Nagpur population

Similar trend was observed in time and dose mortality response of different isolates against Nagpur population of *H. armigera.* Coimbatore isolate induced quick mortality in the larvae, which is followed by Gulbarga isolate with LT_{50} value of 99.41 h and 10.343 h respectively. Kalpavruksha and PCI isolates emerged as least effective with highest LT_{50} values of 186.40 and 161.35 h respectively.

Similarly, the LT_{50} and LC_{50} values for Gulbarga and Coimbatore isolates were least ($1.83X10^4$ POB $ml^{-1}$and $2.00X10^4$ POB ml^{-1} respectively) and maximum for Kalpavruksha isolate ($1.36X10^5$ POB ml^{-1}) (Table 5).

Table 4. Efficacy of different HaNPV isolates against Raichur population of *Helicoverpa armigera*

Sl. No	Isolates	Time mortality response			Dose mortality response		
		LT_{50} (h)	Fiducial Limits (h)		LC_{50} (POB/ml)	Fiducial Limits (POB/ml)	
			Lower	Upper		Lower	Lower
1	Dharwad	138.50	130.59	153.49	5.33×10^4	2.93×10^4	1.09×10^5
2	Gulbarga	98.09	93.19	117.23	1.59×10^4	9.31×10^3	4.76×10^5
3	Raichur	150.08	139.36	155.60	7.69×10^4	1.24×10^4	1.05×10^5
4	Guntur	140.60	133.29	155.67	8.00×10^4	2.99×10^4	1.10×10^5
5	Coimbatore	101.35	93.49	114.01	1.78×10^4	8.01×10^3	9.38×10^4
6	PDBC	159.90	148.58	166.03	8.71×10^4	3.89×10^4	1.09×10^5
7	PCI, Ltd.	158.62	145.09	161.13	8.99×10^4	1.57×10^4	1.89×10^5
8	BPM, Ltd.	160.09	150.53	173.49	8.61×10^4	3.36×10^4	1.21×10^5
9	BPL, Ltd.	162.48	153.37	176.09	1.01×10^5	3.98×10^4	3.15×10^5
10	Kalpavruksha, Ltd.	181.00	170.17	198.23	1.15×10^5	4.50×10^4	8.36×10^5

PDBC: Project Directorate of Biological Control, Bangalore
PCI: Pest Control India Ltd, Bangalore
BPM: Bio-Pest Management, Ltd, Bangalore
BPL: Bio Pest Laboratories, Ltd, Bangalore

Table 5. Efficacy of different HaNPV isolates against the Nagpur population of *Helicoverpa armigera*

Sl. No	Isolates	Time mortality response			Dose mortality response		
		LT_{50} (h)	Fiducial Limits (h)		LC_{50} (POB/ml)	Fiducial Limits (POB/ml)	
			Lower	Upper		Lower	Upper
1	Dharwad	138.41	128.18	145.09	5.36×10^4	1.98×10^4	1.03×10^5
2	Gulbarga	103.43	95.53	119.03	1.83×10^4	8.05×10^3	1.31×10^5
3	Raichur	148.98	134.89	154.32	7.34×10^4	3.09×10^4	1.67×10^5
4	Guntur	140.67	130.02	155.00	7.08×10^4	1.67×10^4	1.30×10^5
5	Coimbatore	99.41	91.55	117.37	2.00×10^4	1.35×10^4	2.57×10^5
6	PDBC	156.00	145.23	167.67	8.17×10^4	1.97×10^4	2.89×10^5
7	PCI, Ltd.	161.35	153.28	169.58	8.96×10^4	2.45×10^4	1.65×10^5
8	BPM, Ltd.	157.37	147.17	166.83	7.94×10^4	3.36×10^4	2.07×10^5
9	BPL, Ltd.	159.51	151.28	168.10	1.10×10^5	6.70×10^4	1.44×10^6
10	Kalpavruksha, Ltd.	186.40	174.14	194.03	1.36×10^5	5.30×10^4	9.34×10^5

PDBC: Project Directorate of Biological Control, Bangalore
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BPL: Bio Pest Laboratories, Ltd, Bangalore

4.1.2.4 Gulbarga population

The activity of different HaNPV isolates against the Gulbarga population of *H. armigera* is depicted in Table 6. Coimbatore isolate established its superiority, interms of lowest LT_{50} and LC_{50} values, which registered 100.35 h and $2.01X10^4$ POB ml^{-1}, LT_{50} and LC_{50} values, respectively. Gulbarga isolate was the next best isolate (LT_{50} values of 107.67 h and LC_{50} value of $3.94X10^4$ POB ml^{-1}, respectively). The isolates obtained from private firms were least virulent as evidenced from their highest LT_{50} and LC_{50} values.

4.1.2.5 Guntur population

The data on LT_{50} and LC_{50} values recorded by different isolates against Guntur population of *H. armigera* is presented in Table 7. The results revealed the superiority of Gulbarga and Coimbatore isolates, which recorded lowest LT_{50} (98.04 hr and 101.59 h) and LC_{50} ($1.86X10^4$ POB ml^{-1} and $1.99X10^4$ POB ml^{-1}) values. The inferiority of Kalpavruksha and Bio Pest Laboratories isolates was further confirmed against Guntur population with highest LT_{50} and LC_{50} values.

Overall, the pooled data indicated in Table-8 revealed that irrespective of the population tested, Coimbatore and Gulbarga isolates have emerged as superior ones with lowest average LT_{50} (101.62 h and 102.62 h) and LC_{50} ($1.98X10^4$ POB $ml^{-1}$and $2.02X10^4$ POB ml^{-1}) values. Further, irrespective of the isolates screened, the population colleted from Gulbarga was more susceptible to NPV infection followed by the population from Guntur (141.68 &142.07 h LT_{50} value and $7.17X10^4$ and $7.18\ X10^4$ POB ml^{-1} LT_{50} value) (Table 9).

Table 6. Efficacy of different HaNPV isolates against the Gulbarga population of *Helicoverpa armigera*

Sl. No	Isolates	Time mortality response			Dose mortality response		
		LT_{50} (h)	Fiducial Limits (h)		LC_{50} (POB/ml)	Fiducial Limits (POB/ml)	
			Lower	Upper		Lower	Upper
1	Dharwad	133.35	126.23	140.92	4.98×10^4	1.05×10^4	9.34×10^4
2	Gulbarga	107.67	100.30	117.09	2.94×10^4	5.64×10^3	4.76×10^4
3	Raichur	143.21	136.91	148.79	7.55×10^4	3.49×10^4	1.70×10^5
4	Guntur	138.56	133.52	149.00	7.31×10^4	4.65×10^4	2.09×10^5
5	Coimbatore	100.35	96.61	116.59	2.01×10^4	1.93×10^4	1.43×10^5
6	PDBC	153.79	1470.08	162.32	7.33×10^4	2.93×10^4	1.74×10^5
7	PCI, Ltd.	149.31	138.59	160.20	9.94×10^4	4.43×10^4	2.09×10^5
8	BPM, Ltd.	150.74	144.08	161.11	8.99×10^4	3.49×10^4	1.85×10^5
9	BPL, Ltd.	168.07	158.13	173.27	1.00×10^5	3.57×10^4	8.57×10^5
10	Kalpavruksha, Ltd.	171.79	165.80	177.01	1.16×10^5	5.83×10^4	6.36×10^5

PDBC: Project Directorate of Biological Control, Bangalore
PCI: Pest Control India Ltd, Bangalore
BPM: Bio-Pest Management, Ltd, Bangalore
BPL: Bio Pest Laboratories, Ltd, Bangalore

Table 7. Efficacy of different HaNPV isolates against the Guntur population of *Helicoverpa armigera*

Sl. No	Isolates	Time mortality response			Dose mortality response (POB/ml)		
		LT_{50} (h)	Fiducial Limits (h)		LC_{50} (POB/ml)	Fiducial Limits	
			Lower	LC_{50} (POB/ml)		Lower	Upper
1	Dharwad	137.71	128.19	157.81	$4.67 X10^4$	$1.05 X10^4$	$2.09 X10^5$
2	Gulbarga	98.04	87.12	119.21	$1.86 X10^4$	$9.47 X10^3$	$1.66 X10^5$
3	Raichur	144.48	136.21	157.98	$7.06 X10^4$	$8.64 X10^3$	$1.73 X10^5$
4	Guntur	141.30	132.10	154.90	$8.97 X10^4$	$7.92 X10^3$	$2.32 X10^5$
5	Coimbatore	101.59	94.40	121.22	$1.99 X10^4$	$8.79 X10^3$	$2.69 X10^5$
6	PDBC	155.09	144.88	169.91	$7.09 X10^4$	$1.09 X10^4$	$3.39 X10^5$
7	PCI, Ltd.	154.39	145.09	169.11	$1.01 X10^5$	$7.91 X10^4$	$1.51 X10^6$
8	BPM, Ltd.	149.00	132.32	164.01	$9.90 X10^4$	$3.46 X10^4$	$1.33 X10^5$
9	BPL, Ltd.	164.77	152.29	170.56	$1.01 X10^5$	$9.57 X10^4$	$9.09 X10^5$
10	Kalpavruksha, Ltd.	174.33	166.89	180.01	$1.01 X10^5$	$8.01 X10^4$	$1.31 X10^6$

PDBC: Project Directorate of Biological Control, Bangalore
PCI: Pest Control India Ltd, Bangalore
BPM: Bio-Pest Management, Ltd, Bangalore
BPL: Bio Pest Laboratories, Ltd, Bangalore

Table 8. Efficacy of different isolates on *Helicoverpa armigera* (Pooled of all Populations)

Sl. No.	Isolate	LT_{50} (h)	LC_{50} (POB/ml X10^4)
1	Dharwad	137.67	5.18
2	Gulbarga	102.62	2.02
3	Raichur	147.35	7.43
4	Guntur	140.24	7.81
5	Coimbatore	101.62	1.98
6	PDBC	156.76	7.85
7	PCI, Ltd.	157.00	9.46
8	BPM, Ltd.	155.43	8.86
9	BPL, Ltd.	163.46	10.34
10	Kalpavruksha, Ltd.	178.97	11.80

Table 9. Response of different Populations of *Helicoverpa armigera* to HaNPV (Pooled of all isolates)

Sl. No	Population	LT_{50} (h)	LC_{50} (POB/ml)
1	Dharwad	146.58	7.36×10^4
2	Raichur	145.10	7.37×10^4
3	Nagpur	145.15	7.33×10^4
4	Gulbarga	141.68	7.17×10^4
5	Guntur	142.07	7.18×10^4

4.1.3 Polyhedral Occlusion Body (POB) yielding potential of HaNPV isolates

The Polyhedral Occlusion Body (POB) yielding capacity of different HaNPV isolates in different larval instars of *H. armigera* was studied under laboratory conditions and the same is presented hereunder.

4.1.3.1 Second instar larvae

In an experiment to estimate the polyhedral occlusion body yield of different HaNPV isolates, the per cent larval mortality was recorded at 5, 7 and 10 days after virus inoculation (DAI). The per cent mortality recorded and the yield of virus obtained is presented in Table 10. The per cent mortality of second instar larvae recorded at different intervals revealed that, at 5 DAI, Gulbarga isolate of HaNPV recorded highest mortality (70.00 %) compared to the other isolates under the study, which is statistically significant over other isolates and is closely followed by Coimbatore isolate, which recorded 60.00 per cent mortality at 5DAI. These two isolates consistently recorded higher mortality at 7 and 10 DAI. Cent percent mortality of the larvae was recorded at 7 DAI when the larvae were fed with the HaNPV obtained from Gulbarga and Coimbatore. At 10 DAI, in addition to Gulbarga and Coimbatore isolates, Dharwad isolate also recorded cent per cent mortality and is on par with virulent isolates.

The POB yield obtained by the infection of different isolates in second instar larvae revealed variability in the POB counts in *H. armigera.* The POB yield of different isolates ranged from 7.00 $X10^{9}$ to 1.76 $X10^{10}$ POBs. Among the isolates tested, Coimbatore and Gulbarga isolates of HaNPV recorded highest POB yield (1.76$X10^{10}$ and 1.68 $X10^{10}$ POBs per 60 larvae, respectively), indicating their superiority in producing the progeny virus in the host. The HaNPV isolates obtained from Bio-Pest laboratories (BPL), Kalpavruksha bio-

Table 10. POB yielding capacity of different HaNPV isolates in second instar larvae of *Helicoverpa armigera*

Sl. No.	Isolates	Larval mortality (%)			Total viral yield		POBs/larva	No. of larvae to yield 1 LE
		5 DAI	7 DAI	10 DAI	POB	LE		
1	Dharwad	46.67cd (43.11)	60.00bc (50.77)	100.00a (90.00)	1.49×10^{10}	2.48	2.48×10^{8}	24.19
2	Gulbarga	70.00a (56.79)	100.00a (90.00)	100.00a (90.00)	1.68×10^{10}	2.80	2.81×10^{8}	21.43
3	Raichur	41.67de (40.22)	58.33bc (49.78)	83.33b (65.88)	1.20×10^{10}	2.00	2.40×10^{8}	25.00
4	Guntur	48.33c (44.03)	56.67bc (48.88)	81.67b (64.67)	9.30×10^{9}	1.56	1.90×10^{8}	31.41
5	Coimbatore	60.00b (50.77)	100.00a (90.00)	100.00a (90.00)	1.76×10^{10}	2.93	2.93×10^{8}	20.48
6	PDBC	46.67cd (43.11)	61.67b (51.77)	83.33b (65.88)	1.09×10^{10}	1.83	2.19×10^{8}	27.32
7	PCI, Ltd.	38.33e (38.23)	58.33bc (49.78)	66.67d (54.76)	1.05×10^{10}	1.75	2.63×10^{8}	22.86
8	BPM, Ltd.	36.67ef (37.29)	61.67b (51.77)	75.00c (60.00)	1.02×10^{10}	1.70	2.27×10^{8}	26.48
9	BPL, Ltd.	33.33f (35.24)	55.00cd (47.87)	70.00cd (56.79)	7.00×10^{9}	1.18	1.69×10^{8}	35.59
10	Kalpavruksha, Ltd.	38.33e (38.23)	53.33d (46.89)	63.33d (52.71)	8.70×10^{9}	1.46	2.30×10^{8}	26.03

PDBC: Project Directorate of Biological Control, Bangalore; PCI: Pest Control India Ltd, Bangalore; BPM: Bio-Pest Management, Ltd, Bangalore; BPL: Bio Pest Laboratories, Ltd, Bangalore

DAI: Days After Inoculation One larval equivalent (LE) $= 6 \times 10^{9}$ POBs

Figures in the parenthesis are the arc sine transformed values

Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.01)

systems and Guntur yielded least number of POBs (7.00 $X10^9$, 8.70 $X10^9$and 9.30$X10^9$ POBs, respectively).

The number of POBs per larvae was calculated and the results clearly indicate that the larvae inoculated with Coimbatore and Gulbarga isolates recorded highest number of POBs (2.93$X10^8$ and 2.81$X10^8$ POBs per larvae). Least number of viral bodies were harvested in case of BPL and Guntur isolates of HaNPV (1.69$X10^8$ and 1.90$X10^8$ POBs per larvae, respectively). The observation on total number of second instar larvae to yield one Larval Equivalent (LE) revealed the superiority of Coimbatore and Gulbarga isolate in which, one LE virus yield was harvested from 20.48 and 21.43 larvae, respectively. Maximum numbers of 35.59 and 31.41 larvae were required to yield one LE POB yield when the larvae were infected with BPL and Guntur isolates of HaNPV indicating their reduced efficacy in producing the progeny virus in the host larvae.

4.1.3.2 Third instar larvae

The per cent larval mortality and the POB yield obtained when the third instar larvae of *H. armigera* infected with different HaNPV isolates is presented in Table 11. The larval mortality recorded at 5 DAI revealed the superiority of Gulbarga isolate which recorded highest mortality of 60.00 per cent and remained statistically superior over the other isolates which is followed by Coimbatore isolate, which recorded 50 per cent mortality in third instar at 5 DAI. At 7 DAI, Gulbarga and Coimbatore isolates recorded 95.00 and 80.00 per cent mortality, respectively indicating their superiority over other isolates. Theses two isolates consistently shown encouraging performance and recorded cent per cent larval mortality at 10 days after the virus is inoculated and statistically on par in their virulence.

Table 11. POB yielding capacity of different HaNPV isolates in third instar larvae of ***Helicoverpa armigera***

Sl. No.	Isolates	Larval mortality (%)			Total viral yield		POBs/larva	No. of larvae to yield 1 LE
		5 DAI	7 DAI	10 DAI	POB	LE		
1	Dharwad	36.67cd (37.29)	53.33cd (46.89)	83.33b (65.88)	7.50×10^{10}	12.50	1.50×10^{9}	4.00
2	Gulbarga	60.00a (50.77)	95.00a (77.08)	100.00a (90.00)	1.28×10^{11}	21.17	2.13×10^{9}	2.83
3	Raichur	35.00de (36.27)	50.00de (45.00)	76.67c (61.14)	4.50×10^{10}	7.50	9.80×10^{8}	6.13
4	Guntur	40.00cd (39.23)	50.00cd (45.00)	66.67d (54.76)	4.12×10^{10}	6.87	1.03×10^{9}	5.82
5	Coimbatore	50.00b (45.00)	80.00b (63.44)	100.00a (90.00)	1.08×10^{11}	18.00	1.79×10^{9}	3.33
6	PDBC	41.67c (40.22)	55.00c (47.87)	75.00c (60.00)	4.00×10^{10}	6.67	9.00×10^{8}	6.75
7	PCI, Ltd.	33.33ef (35.24)	46.67ef (43.11)	58.33e (49.78)	2.60×10^{10}	4.33	7.60×10^{8}	8.08
8	BPM, Ltd.	35.00de (36.27)	55.00c (47.87)	75.00c (60.00)	3.96×10^{10}	6.60	8.80×10^{8}	6.82
9	BPL, Ltd.	31.67ef (34.27)	46.67ef (43.11)	60.00de (50.77)	3.46×10^{10}	5.77	9.60×10^{8}	6.24
10	Kalpavruksha, Ltd.	30.00f (33.21)	38.33e (38.23)	61.67de (51.77)	3.03×10^{10}	5.05	8.20×10^{8}	7.33

PDBC: Project Directorate of Biological Control, Bangalore; PCI: Pest Control India Ltd, Bangalore; BPM: Bio-Pest Management, Ltd, Bangalore; BPL: Bio Pest Laboratories, Ltd, Bangalore

DAI: Days After Inoculation One larval equivalent (LE) $= 6 \times 10^{9}$ POBs

Figures in the parenthesis are the arc sine transformed values

Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.01)

Variation in the viral yield of different HaNPV isolates in third instar larvae of *H. armigera* revealed that the POB yield ranged between 2.60 $X10^{10}$ to 1.28 $X10^{11}$ POBs, indicating the variation in the multiplying ability of isolates in third instar larvae. The larvae infected with the NPV obtained from Gulbarga and Coimbatore produced maximum number of POBs (1.28 $X10^{11}$ and 1.08 $X10^{11}$ POBs). Further, theses two isolates produced more number of LE's (21.17 and 18.00 respectively) compared to other isolates. The HaNPV produced by Pest Control India (PCI) yielded less number of larval equivalents (4.33) which is almost five times less than the total virus obtained from Gulbarga isolate. As per the POB yield per larvae is concerned, more number of viral particles per larvae were harvested in the larvae treated with Gulbarga isolate (2.13 $X10^{9}$ POBs) followed by Coimbatore isolate (1.79 $X10^{9}$). Only 7.60 $X10^{8}$ POBs per larvae were harvested when the larvae were fed with PCI isolate which is found to be the least among the isolates.

Comparatively, few numbers of larvae were required to yield one LE POB yield in case of Gulbarga and Coimbatore isolates (2.83 and 3.33 larvae to yield one LE). Maximum of 8.08 larvae were required to produce the same quantum of virus yield, when the larvae were infected with the HaNPV obtained from PCI, Ltd, Bangalore, indicating the least efficacy of the isolate to multiply in the host.

4.1.3.3 Fourth instar larvae

The per cent larval mortality caused by different HaNPV isolates and their POB yielding capacity in fourth instar larvae is presented in Table 12. Overall, the per cent mortality was reduced in fourth instar compared to second and third instar as evident from the data indicated. At 5 DAI, the per cent larval mortality ranged from 16.67 to 55.00 per cent as against 33.33 to 70.00 per cent and 30.00 to 60.00 per cent mortality at 5 DAI in second and third instar

Table 12. POB yielding capacity of different HaNPV isolates in fourth instar larvae of *Helicoverpa armigera*

Sl. No.	Isolates	Larval mortality (%)			Total viral yield		POBs/larva	No. of larvae to yield 1 LE
		5 DAI	7 DAI	10 DAI	POB	LE		
1	Dharwad	23.33cd (28.86)	33.33cd (35.24)	65.00b (53.73)	$8.19X10^{10}$	13.65	$2.10X10^{9}$	2.86
2	Gulbarga	55.00a (47.87)	73.33a (58.89)	93.33a (75.00)	$1.52X10^{11}$	25.32	$2.73X10^{9}$	2.21
3	Raichur	20.00de (26.56)	33.33cd (35.24)	50.00de (45.00)	$5.16X10^{10}$	8.60	$1.72X10^{9}$	3.49
4	Guntur	23.33cd (28.86)	33.33cd (35.24)	55.00cd (47.87)	$5.45X10^{10}$	9.08	$1.65X10^{9}$	3.64
5	Coimbatore	46.67b (43.11)	66.67b (54.76)	91.67a (73.26)	$1.33X10^{11}$	22.17	$2.42X10^{9}$	2.48
6	PDBC	25.00c (30.00)	38.33c (38.23)	60.00bc (50.77)	$7.20X10^{10}$	12.00	$2.00X10^{9}$	3.00
7	PCI, Ltd.	16.67f (24.12)	33.33cd (35.24)	46.67ef (43.11)	$5.63X10^{10}$	9.38	$2.01X10^{9}$	2.99
8	BPM, Ltd.	21.67cd (27.76)	35.00cd (36.27)	50.00de (45.00)	$6.00X10^{10}$	10.00	$2.00X10^{9}$	3.00
9	BPL, Ltd.	18.33ef (25.33)	30.00d (33.21)	45.00ef (42.13)	$5.08X10^{10}$	8.47	$1.86X10^{9}$	3.19
10	Kalpavruksha, Ltd.	18.33ef (25.33)	30.00d (33.21)	41.67f (40.22)	$4.08X10^{10}$	6.80	$1.63X10^{9}$	3.68

PDBC: Project Directorate of Biological Control, Bangalore; PCI: Pest Control India Ltd, Bangalore; BPM: Bio-Pest Management, Ltd, Bangalore; BPL: Bio Pest Laboratories, Ltd, Bangalore

DAI: Days After Inoculation One larval equivalent (LE) $= 6X10^{9}$ POBs

Figures in the parenthesis are the arc sine transformed values

Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.01)

larvae, respectively. Maximum mortality (55.00 %) was recorded when the larvae were treated with Gulbarga isolate of HaNPV which is closely followed by Coimbatore isolate causing 46.67 per cent larval mortality.

The superiority of Gulbarga isolate in recording higher mortality was consistent over days that is evident from the results which recorded 73.33 and 93.33 per cent mortality at 7 and 10 days after viral inoculation. Coimbatore isolate recorded 66.67 and 91.67 per cent mortality at 7 and 10 DAI, respectively, and remained statistically on par with Gulbarga isolate at 10 DAI. Least mortality was recorded by the isolate obtained from Kalpavruksha bio-systems (18.33, 30.00 and 41.67 per cent larval mortality at 5, 7 and 10 DAI, respectively).

The viral yield harvested from the total virosed larvae was highest in Gulbarga isolate ($1.52 X10^{11}$POBs) followed by Coimbatore isolate ($1.33 X10^{11}$POBs). Dharwad isolate is the next best isolate v/s Gulbarga and Coimbatore isolates, which recorded $8.19X10^{10}$ POBs. Kalpavruksha isolate of HaNPV produced least amount of progeny virus in fourth instar larvae of *H. armigera.*

The number of polyhedra produced in single virosed larvae also revealed the superiority of Gulbarga isolate, which recorded highest POB count per larvae ($2.73X10^{9}$), followed by Coimbatore isolate ($2.42X10^{9}$). The HaNPV isolate obtained from Kalpavruksha Biosystems yielded least number of viral counts per larvae ($1.63 X10^{9}$). The total number of fourth instar larvae required to contribute to one LE virus was least in Gulbarga isolate (2.21), which was followed by Coimbatore NPV (2.48). As the POB yield per larvae was least in Kalpavruksha HaNPV isolate, more number of virosed larvae (3.68) were required to obtain the same yield.

4.1.3.4 Fifth instar larvae

The larval mortality caused by different HaNPV isolates and their capacity to produce progeny virus in the fifth instar larvae of *H. armigera* is detailed in Table 13. The larval mortality caused by different isolates in fifth instar of *H. armigera* was found very less compared to second, third and fourth instar. The per cent larval mortality at 5 DAI ranged between 3.33 to 13.33 when infected with Gulbarga isolate recording the highest and Kalpavruksha isolate recording the lowest number of virosed larvae. At 7 and 10 DAI, similar trend was seen with maximum of 16.67 and 30.00 per cent larval mortality by Gulbarga isolate and 16.67 and 23.00 per cent larval mortality by Coimbatore isolate, respectively.

The total viral yield produced by the virosed larvae revealed the superiority of Gulbarga and Coimbatore isolates. The viral yield obtained in fifth instar was very less compared to the yield obtained in fourth instar larvae. The viral yield ranged from $1.17X10^{10}$ to $5.96X10^{10}$ POBs. Maximum yield was recorded in the treatment with Gulbarga isolate ($5.96X10^{10}$) followed by Coimbatore isolate ($4.17X10^{10}$ POBs). Least viral count was recorded by the larvae infected with PCI isolate ($1.17X10^{10}$ POBs). The total number of polyhedra produced by a single fifth instar larva ranged from $2.19\ X10^{9}$ to $3.31\ X10^{9}$ POBs, which was considerably high compared to the yield obtained in fourth instar larvae. Maximum numbers of POBs per larvae were obtained in the larvae infected with Gulbarga HaNPV ($3.31X10^{9}$ POBs per larva) followed by Coimbatore HaNPV ($2.98X10^{9}$ POBs/larva) The total number of larvae required to yield one LE virus yield was least in Gulbarga isolate (1.81) followed by Coimbatore isolate (2.01). The HaNPV collected from Kalpavruksha Biosystems required maximum number of larvae (2.92) to produce one LE yield.

Table 13. POB yielding capacity of different HaNPV isolates in fifth instar larvae of *Helicoverpa armigera*

Sl. No.	Isolates	Larval mortality (%)			Total viral yield		POBs/larva	No. of larvae to yield 1 LE
		5 DAI	7 DAI	10 DAI	POB	LE		
1	Dharwad	10.00b (18.44)	10.00bc (18.44)	18.33c (25.33)	$3.23X10^{10}$	5.38	$2.94X10^{9}$	2.04
2	Gulbarga	13.33a (21.39)	16.67a (24.12)	30.00a (33.21)	$5.96X10^{10}$	9.93	$3.31X10^{9}$	1.81
3	Raichur	6.67c (15.00)	10.00bc (18.44)	15.00de (22.79)	$2.22X10^{10}$	3.70	$2.46X10^{9}$	2.43
4	Guntur	8.33b (16.74)	11.67b (20.00)	16.67cd (24.12)	$2.39X10^{10}$	3.98	$2.39X10^{9}$	2.51
5	Coimbatore	10.00b (18.44)	16.67a (24.12)	23.33b (28.93)	$4.17X10^{10}$	6.95	$2.98X10^{9}$	2.01
6	PDBC	6.67c (15.00)	8.33cd (16.74)	15.00de (22.79)	$2.16X10^{10}$	3.60	$2.39X10^{9}$	2.50
7	PCI, Ltd.	3.33e (10.47)	6.67d (15.00)	8.33 h (16.74)	$1.17X10^{10}$	1.95	$2.33X10^{9}$	2.56
8	BPM, Ltd.	6.67c (15.00)	8.33cd (16.74)	13.33ef (21.39)	$1.77X10^{10}$	2.95	$2.21X10^{9}$	2.71
9	BPL, Ltd.	5.00d (12.92)	6.67d (15.00)	10.00gh (18.44)	$1.32X10^{10}$	2.20	$2.19X10^{9}$	2.73
10	Kalpavruksha, Ltd.	3.33e (10.47)	6.67d (15.00)	11.67fg (20.00)	$1.68X10^{10}$	2.40	$2.40X10^{9}$	2.92

PDBC: Project Directorate of Biological Control, Bangalore; PCI: Pest Control India Ltd, Bangalore; BPM: Bio-Pest Management, Ltd, Bangalore; BPL: Bio Pest Laboratories, Ltd, Bangalore

DAI: Days After Inoculation One larval equivalent (LE) $= 6X10^{9}$ POBs

Figures in the parenthesis are the arc sine transformed values

Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.01)

The polyhedral yield of different instars obtained by the infection of different isolates is averaged and presented in Table 14.The average POB yield varied between 8.91X10^{10} to 2.42X10^{10} POBs. Gulbarga isolate excelled over other isolates in producing maximum yield which recorded an average of 8.91X10^{10} POBs followed by Coimbatore isolate with 7.51X10^{10} POBs. Further, Gulbarga isolate recorded four times more POBs yield compared to Kalpavruksha isolate as evident from the total viral yield in terms of LE. The average POB yield per larvae ranged from 2.11X10^{9} to 1.21X10^{9} POBs. Gulbarga isolate excelled over other isolates in producing highest number of POBs per larvae (2.11X10^{9} POBs), followed by Coimbatore isolate (1.87X10^{9}POBs). The average number of larvae to yield one larval equivalent ranged between 7.07 to 11.94 larvae. Gulbarga isolate required on an average 7.09 virosed larvae to produce 6.00X10^{9} POBs as against BPL isolate which required on an average 11.94 larvae to yield the same amount of virus.

4.2 Influence of cotton species on the virulence of HaNPV isolates against *H. armigera*

4.2.1 Larval mortality without HaNPV spray

The pH estimated for all the cotton plants indicated that the pH range was between 9.00 to 9.50 indicting that there was no much variation among the species. The per cent larval mortality on different cotton species without HaNPV spray was recorded and presented in Table 15. The larval mortality recorded at 5 DAS ranged from zero per cent to 45.00 per cent. Among the treatments, except the six Bt hybrids, all the cotton species and non-Bt versions recorded no mortality in released larvae at 5, 7 and 10 days after the release (DAR) and are on par with each other. Among the various Bt cotton hybrids, larvae fed with MECH-184 Bt recorded significantly higher mortality (45.00%) and is found statistically superior over the other treatments. MECH-

Table 14. POB yield of different HaNPV isolates (Average of all instars)

Sl. No.	Isolates	Total viral yield		POBs/larva ($X10^9$)	No. of larvae to yield 1 LE
		POB yield $X 10^{10}$	LE		
1	Dharwad	5.10	8.50	1.69	8.28
2	Gulbarga	8.91	14.81	2.11	7.07
3	Raichur	3.27	5.45	1.35	9.26
4	Guntur	3.22	5.38	1.32	10.85
5	Coimbatore	7.51	12.51	1.87	7.08
6	PDBC	3.61	6.03	1.38	9.89
7	PCI, Ltd.	2.61	4.35	1.34	9.12
8	BPM, Ltd.	3.19	5.31	1.33	9.75
9	BPL, Ltd.	2.64	4.41	1.29	11.94
10	Kalpavruksha, Ltd.	2.42	3.93	1.27	10.06

PDBC: Project Directorate of Biological Control, Bangalore; PCI: Pest Control India Ltd, Bangalore; BPM: Bio-Pest Management, Ltd, Bangalore; BPL: Bio Pest Laboratories, Ltd, Bangalore
Inoculation dose = 10^8POBs One larval equivalent (LE) = $6X10^9$ POBs

Table 15. Influence of cotton species without NPV spray against *Helicoverpa armigera*

Sl. No.	Cotton Species	Average leaf pH	Larval mortality (%)		
			5 DAR	7 DAR	10 DAR
1	Sahana	9.50	00.00d (00.00)	00.00e (00.00)	00.00e (00.00)
2	B-82	9.50	00.00d (00.00)	00.00e (00.00)	00.00e (00.00)
3	Jayadhar	9.50	00.00d (00.00)	00.00e (00.00)	00.00e (00.00)
4	DHH-11	9.50	00.00d (00.00)	00.00e (00.00)	00.00e (00.00)
5	MECH-12 Bt	9.50	40.00b (39.21)	45.00c (41.79)	55.00c (47.88)
6	MECH-12 NBt	9.50	00.00d (00.00)	00.00e (00.00)	00.00e (00.00)
7	MECH-184 Bt	9.50	45.00a (41.79)	51.65a (45.00)	65.00a (53.76)
8	MECH-184 NBt	9.50	00.00d (00.00)	00.00e (00.00)	00.00e (00.00)
9	MECH-162 Bt	9.50	40.00b (39.21)	50.00ab (45.00)	60.00b (50.79)
10	MECH-162 NBt	9.50	00.00d (00.00)	00.00e (00.00)	00.00e (00.00)
11	RCH-2 Bt	9.00	38.35c (37.58)	48.35ab (44.04)	53.35c (46.91)
12	RCH-2 NBt	9.00	00.00d (00.00)	00.00e (00.00)	00.00e (00.00)
13	RCH-20 Bt	9.50	38.35c (38.24)	48.35ab (44.04)	53.35c (46.91)
14	RCH-20 NBt	9.50	00.00d (00.00)	00.00e (00.00)	00.00e (00.00)
15	RCH-144 Bt	9.50	40.00b (39.21)	48.35ab (44.04)	55.00c (47.88)
16	RCH-144 NBt	9.50	00.00d (00.00)	00.00e (00.00)	00.00e (00.00)
17	Control		00.00d (00.00)	03.33d (6.29)	03.33d (00.00)

Figures in the parenthesis are the arc sine transformed values
Means followed by same alphabet do not differ significantly by DMRT (P=0.01)
DAR: Days after release
Control: Larvae reared on artificial diet

162Bt, MECH-12Bt and RCH-144Bt have recorded 40.00 per cent larval mortality and were found statistically on par with each other. Similar trend in larval mortality was observed at 7 and 10 days after the release of the larvae. MECH-184Bt consistently recorded higher larval mortality at 7 and 10 DAR and remained superior among the Bt hybrids included in the study. MECH-162Bt and all the Bt versions of Rasi were statistically on par with each other at 7 DAR. MECH-12Bt (55.00%), RCH-144Bt (55.00%), RCH-2Bt (53.35%) and RCH-20Bt (53.35%) were statistically on par in expressing the Bt toxin, which caused on par lethality in the larvae at 10 DAR.

4.2.2 Larval mortality with HaNPV spray

4.2.2.1 UAS, Dharwad isolate of HaNPV

The influence of different cotton species on the virulence of Dharwad isolate of HaNPV against second instar larvae of *H. armigera* was investigated and the larval mortality obtained at different intervals after the spray is presented in Table 16. The total larval mortality recorded when the larvae were fed on the HaNPV sprayed cotton plants ranged from zero per cent to 76.65 per cent at 5 days after the spray (DAS). Among the different treatments, the larvae fed on the squares and bolls of MECH-184Bt registered highest larval mortality (76.65%), which is followed by MECH-162Bt (75.00%) and MECH-12Bt (73.35%), which were statistically on par with each other in their efficacy.

Similar trend was observed at 7 and 10 days after the spray. All the Mahyco Bt cotton hybrids excelled over others in recording higher mortality (90.00%) at 7 DAS. At 10 DAS, all the Mahyco Bt cotton hybrids recorded cent per cent larval mortality and emerged as superior treatments. Among the Rasi Bt cotton hybrids, RCH-20Bt and RCH-144Bt recorded cent per cent larval mortality and were on par with the Mahyco Bt cotton hybrids at 10 DAS.

Table 16. Influence of cotton species on the efficacy of Dharwad HaNPV against *Helicoverpa armigera*

Sl. No.	Cotton Species	Larval mortality (%)					
		5 DAS		7 DAS		10 DAS	
		TM	MV	TM	MV	TM	MV
1	Sahana	16.25e (24.05)	16.25ef (24.05)	26.65d (31.07)	26.65c (31.07)	30.00cd (33.16)	30.00ef (33.16)
2	B-82	23.35d (28.85)	23.35cd (28.85)	30.00d (33.16)	30.00c (33.16)	35.00c (36.24)	35.00d (36.24)
3	Jayadhar	15.00e (22.74)	15.00f (22.74)	25.00d (30.00)	25.00c (30.00)	30.00cd (33.16)	30.00ef (33.16)
4	DHH-11	20.00de (26.45)	20.00de (26.45)	28.35d (32.14)	28.35c (32.14)	33.35cd (35.25)	33.35de (35.25)
5	MECH-12 Bt	73.35ab (58.93)	30.00ab (33.13)	90.00a (71.96)	38.35ab (38.24)	100.00a (90.00)	45.00b (42.12)
6	MECH-12 NBt	20.00de (26.56)	20.00de (26.56)	26.65d (31.07)	26.65c (31.07)	30.00cd (33.16)	30.00ef (33.16)
7	MECH-184 Bt	76.65a (61.21)	31.65a (34.23)	90.00a (71.96)	43.35a (41.16)	100.00a (90.00)	50.00a (45.00)
8	MECH-184 NBt	18.35de (25.30)	18.35ef (25.30)	25.00d (30.00)	25.00c (30.00)	30.00cd (33.16)	30.00ef (33.16)
9	MECH-162 Bt	75.00ab (60.08)	30.00ab (33.16)	90.00a (71.96)	40.00ab (39.21)	100.00a (90.00)	45.00b (42.12)
10	MECH-162 NBt	20.00de (26.56)	20.00de (26.56)	28.35d (32.14)	28.35c (32.14)	30.00cd (33.16)	30.00ef (33.16)
11	RCH-2 Bt	66.65c (54.75)	25.00c (29.92)	85.00c (67.41)	36.65b (37.26)	98.35b (90.00)	40.00c (39.21)
12	RCH-2 NBt	16.65e (24.05)	16.65ef (24.05	26.65d (31.07)	26.65c (31.07)	28.65d (32.16)	28.65f (32.16)
13	RCH-20 Bt	70.00bc (56.84)	25.00c (30.00)	86.65bc (68.68)	38.35ab (38.24)	100.00a (90.00)	45.00b (42.12)
14	RCH-20 NBt	20.00de (26.56)	20.00de (26.56)	28.35d (32.14)	28.35c (32.14)	30.00cd (33.16)	30.00ef (33.16)
15	RCH-144 Bt	70.00bc (56.84)	26.65bc (31.07)	88.35ab (70.12)	40.00ab (39.21)	100.00a (90.00)	43.45bc (41.16)
16	RCH-144 NBt	18.35de (25.30)	18.35ef (25.30)	28.35d (32.14)	28.35c (32.14)	30.00cd (33.16)	30.00ef (33.16)
17	Control	00.00f (00.00)	00.00g (00.00)	5.00e (12.92)	05.00d (12.92)	05.00e (12.92)	05.00g (12.92)

DAS: Days after spraying　　Control: Larvae reared on artificial diet
TM-Total mortality　　MV-Mortality due to virus
Figures in the parenthesis are the arc sine transformed values
Means followed by same alphabet do not differ significantly by DMRT (P=0.01)

An effort was made to know the number of larvae succumbed to viral infection from the total larvae killed. The dead larvae in different treatments were further investigated for the presence of viral bodies in their mid gut. The numbers of larvae died due to viral infection were presented as mortality due to virus (MV) in the table.

In all the three cotton varieties (Sahana, B-82 and Jayadhar), intra-specific hybrid (DHH-11) and in all the non-Bt version of Bt cotton hybrids, all the dead larvae displayed the typical viral symptoms, indicating that the total larval mortality is due to NPV only.

The per cent larval mortality due to viral infection ranged from Zero to 31.65, 5.00 to 43.35 and 5.00 to 50.00 per cent at 5, 7 and 10 days after NPV spray (DAS), respectively. Among the various treatments, the HaNPV sprayed on Bt cotton registered more viral infection. Among the Bt cotton hybrids, the larvae fed on MECH-184Bt encountered higher number of virosed larvae followed by MECH-12Bt (30.00%) and MECH-162Bt (30.00%), which are statistically on par with each other.

Similar trend in larval mortality was observed among the various treatments at 7 and 10 days after the spray, where all the Bt hybrids encouraged the virus to multiply and cause death in the larvae fed on them.

4.2.2.2 Gulbarga isolate of HaNPV

The results on impact of different cotton species on the efficacy of Gulbarga isolate of HaNPV are presented in Table 17.

Among the various treatments, the Bt hybrids excelled in recording highest mortality over days. AT 5 DAS, MECH-184Bt registered highest larval mortality (88.35%) and found superior over others. AT 7 DAS, all the Mahyco Bt hybrids including RCH-144Bt recorded cent per cent mortality and were statistically on

Table 17. Influence of cotton species on the efficacy of Gulbarga HaNPV against *Helicoverpa armigera*

Sl. No.	Cotton Species	Larval mortality (%)					
		5 DAS		7 DAS		10 DAS	
		TM	MV	TM	MV	TM	MV
1	Sahana	26.65ef (31.07)	26.65de (31.07)	31.35ef (34.23)	31.35ef (34.23)	38.35d (38.24)	38.35e (38.24)
2	B-82	30.00e (33.16)	30.00bc (33.16)	38.35d (38.24)	38.35cd (38.24)	45.00b (42.13)	45.00bc (42.13)
3	Jayadhar	25.00f (29.92)	25.00ef (29.92)	26.65g (31.07)	26.65f (31.07)	33.35e (35.25)	33.35f (35.25)
4	DHH-11	30.00e (33.16)	30.00bc (33.16)	35.00de (36.27)	35.00de (36.27)	40.00cd (39.23)	40.00de (39.23)
5	MECH-12 Bt	80.00bc (63.56)	31.65ab (34.23)	100.00a (90.00)	48.35ab (34.23)	100.00a (90.00)	48.35ab (44.04)
6	MECH-12 NBt	23.35f (28.85)	23.35f (28.85)	30.00fg (33.16)	30.00ef (33.16)	40.00cd (39.23)	40.00de (39.23)
7	MECH-184 Bt	88.35a (70.12)	35.00a (36.24)	100.00a (90.00)	50.00a (45.00)	100.00a (90.00)	50.00a (45.00)
8	MECH-184 NBt	25.00f (29.92)	25.00ef (29.92)	30.00fg (33.16)	30.00ef (33.16)	38.35d (38.24)	38.35e (38.24)
9	MECH-162 Bt	83.35b (65.93)	33.35ab (35.25)	100.00a (90.00)	48.35ab (44.04)	100.00a (90.00)	48.35ab (44.04)
10	MECH-162 NBt	26.65ef (31.07)	26.65de (31.07	33.35ef (35.25)	33.35e (35.25)	43.35bc (41.16)	43.35cd (41.16)
11	RCH-2 Bt	75.00d (60.08)	28.35cd (32.14)	96.65c (81.39)	43.35bc (41.16)	100.00a (90.00)	45.00bc (42.13)
12	RCH-2 NBt	25.00f (29.92)	25.00ef (29.92)	31.35ef (34.23)	31.35ef (34.23)	41.65bc (40.20)	41.65cd (40.20)
13	RCH-20 Bt	78.35cd (62.29)	30.00bc (33.16)	98.35b (85.69)	43.35bc (41.16)	100.00a (90.00)	45.00bc (42.13)
14	RCH-20 NBt	25.00f (29.92)	25.00ef (29.92)	33.35ef (35.25)	33.35e (35.25	40.00cd (39.23)	40.00de (39.23)
15	RCH-144 Bt	80.00cd (63.56)	30.00bc (33.16)	100.00a (90.00)	45.00ab (42.12)	100.00a (90.00)	45.00bc (42.13)
16	RCH-144 NBt	25.00f (29.92)	25.00ef (29.92)	30.00fg (33.16)	30.00ef (33.16)	40.00cd (39.23)	40.00de (39.23)
17	Control	00.00g (00.00)	00.00g (00.00)	00.00h (00.00)	00.00g (00.00)	03.33f (6.29)	03.33g (6.29)

DAS: Days after spraying — Control: Larvae reared on artificial diet

TM-Total mortality — MV-Mortality due to virus

Figures in the parenthesis are the arc sine transformed values

Means followed by same alphabet do not differ significantly by DMRT (P=0.01)

par with each other. All the six Bt cotton hybrids recorded cent per cent mortality at 10 DAS and were statistically on par in their effect.

The number of virosed larvae observed after the post mortality investigation revealed the same trend that noticed in Dharwad isolate. All the Bt hybrids excelled over others in registering more number of virosed larvae. Among the different species, the larvae fed on the floral parts of B-82 were more susceptible to viral infection. Of the various Bt hybrids, the NPV sprayed on MECH-184Bt was more virulent, which could record highest number of larvae succumbed to viral infection with a larval mortality of 50.00 per cent at 7 DAS and is statistically on par with MECH-12Bt, MECH-162Bt and RCH-144Bt. Similar trend was observed at 10 DAS.

4.2.2.3 Raichur isolate of HaNPV

The total larval mortality and mortality due to viral infection are elucidated in Table 18.

All the treatments established superiority in recording higher larval mortality over the control. The total larval mortality, noticed, when the NPV sprayed floral parts of different cotton species were fed to the second instar larvae of *H. armigera* ranged from zero to 70.00, zero to 80.00 and 5.00 to 100.00 per cent at 5, 7 and 10 DAS, respectively. The larval mortality in various treatments steadily increased over days from 5th to 10th day, which indicated the combined effect of Bt toxin and NPV infection.

Among the various treatments, maximum larval mortality was recorded when the larvae were fed with NPV sprayed Bt cotton hybrids *viz.*, MECH-184Bt, MECH-162Bt, MECH-12Bt and RCH-144 with a larval mortality of 70.00, 70.00, 68.35 and 65.00 per cent at 5 DAS. All the Mahyco Bt hybrids recorded 80.00 per cent larval mortality and 78.35 per cent by RCH-144Bt at

Table 18 Influence of cotton species on the efficacy of Raichur HaNPV against *Helicoverpa armigera*

Sl. No.	Cotton Species	Larval mortality (%)					
		5 DAS		7 DAS		10 DAS	
		TM	MV	TM	MV	TM	MV
1	Sahana	15.00d (22.60)	15.00d (22.60)	18.35cd (25.30)	18.35bc (25.30)	23.35e (28.85)	23.35cd (28.85)
2	B-82	16.65d (24.04)	16.65b (24.04)	21.65c (27.71)	21.65b (27.71)	30.00d (33.16)	30.00b (33.16)
3	Jayadhar	13.35d (21.34)	13.35cd (21.34)	15.00d (22.60)	15.00c (22.60)	20.00ef (26.45)	20.00de (26.45)
4	DHH-11	15.00d (22.60	15.00d (22.60	18.35cd (25.30)	18.35bc (25.30)	25.00e (30.00)	25.00c (30.00)
5	MECH-12 Bt	68.35ab (55.77)	25.00a (30.00)	80.00a (63.56)	31.65a (34.23)	98.35b (85.69)	40.00a (39.21)
6	MECH-12 NBt	15.00d (22.60)	15.00d (22.60)	20.00c (26.45)	20.00b (26.45)	23.35e (28.85)	23.35cd (28.85)
7	MECH-184 Bt	70.00a (56.84)	26.65a (31.07)	80.00a (63.56)	33.35a (35.25)	100.00a (90.00)	45.00a (42.12)
8	MECH-184 NBt	15.00d (22.60)	15.00d (22.60)	18.35cd (25.30)	18.35bc (25.30)	21.65ef (27.71)	21.65cd (27.71)
9	MECH-162 Bt	70.00a (56.84)	26.65a (31.07)	80.00a (63.56)	33.35a (35.25)	100.00a (90.00)	43.35a (41.16)
10	MECH-162 NBt	16.65d (24.04)	16.65d (24.04)	20.00c (26.45)	20.00b (26.45)	23.35e (28.85)	23.35cd (28.85)
11	RCH-2 Bt	60.00bc (52.74)	20.00bc (26.56)	75.00b (60.08)	30.00a (33.16)	95.00c (81.55)	40.00a (39.21)
12	RCH-2 NBt	13.35d (21.34)	13.35e (21.34)	18.35cd (25.30)	18.35bc (25.30)	23.35e (28.85)	23.35cd (28.85)
13	RCH-20 Bt	61.65c (51.76)	21.65b (27.71)	75.00b (60.08)	30.00a (33.16)	95.00c (81.55)	41.65a (40.20)
14	RCH-20 NBt	13.35d (21.34)	13.35e (21.34)	15.00d (22.60)	15.00c (22.60)	21.65ef (27.71)	21.65cd (27.71
15	RCH-144 Bt	65.00ab (53.76)	25.00a (30.00)	78.35ab (62.29)	31.65a (34.23)	96.65c (81.38)	41.65a (40.20)
16	RCH-144 NBt	15.00d (22.60)	15.00d (22.60)	18.35cd (25.30)	18.35bc (25.30)	20.00f (26.45)	20.00e (26.45)
17	Control	00.00e (00.00)	00.00f (00.00)	00.00e (00.00)	00.00d (00.00)	05.00g (12.92)	05.00f (12.92)

DAS: Days after spraying　　Control: Larvae reared on artificial diet

TM-Total mortality　　MV-Mortality due to virus

Figures in the parenthesis are the arc sine transformed values

Means followed by same alphabet do not differ significantly by DMRT (P = 0.01)

10 DAS. This indicates the joint action of Bt and NPV in inhibiting the larval survival.

The post mortality studies of the dead larvae recorded in different treatments revealed that all the sprayed plants recorded virosed larvae. Among the various treatments, the total larvae killed by feeding on the three cotton species, one Intra specific hybrid and all the non Bt versions encountered viral bodies in their mid gut, which is the only cause for their mortality. This trend in recording the virosed larvae continued even up to 10th day after the spray. Among these treatments, B-82 registered more number of virus infected larvae indicating the virulence of Raichur isolate on B-82 plants compared to other two species and the intra specific hybrid.

The Raichur HaNPV sprayed on Bt cotton hybrids emerged more virulent than on the non-Bt hybrids and cotton varieties. Among the Bt hybrids, MECH-184Bt, MECH-162Bt, MECH-12Bt and RCH-144Bt encouraged the virus to cause mortality in the larvae fed on them by recording higher larval mortality at 5, 7 and 10 DAS. Maximum larval mortality due to viral infection in Bt hybrids was about 25.00, 33.00 and 45.00 per cent, respectively at 5, 7 and 10 DAS. This indicates that on Mahyco Bt cotton hybrids the NPV is more potent against *H. armigera*.

4.2.2.4 Guntur isolate of HaNPV

The different cotton species sprayed with Guntur isolate of HaNPV were evaluated for their influence on larval survival and is presented in Table 19. The total larval mortality recorded on different cotton plants indicated the superiority of Bt cotton hybrids at 5, 7 and 10 days after the spray. Among the various Bt hybrids, all the three Mahyco Bt hybrids and one Rasi Bt hybrid (RCH-144Bt) registered more larval mortality by their combined action of Bt and virus. MECH-184Bt recorded 70.00, 81.65 and 100.00 per cent mortality

Table 19 Influence of cotton species on the efficacy of Guntur HaNPV against *Helicoverpa armigera*

Sl. No.	Cotton Species	Larval mortality (%)					
		5 DAS		7 DAS		10 DAS	
		TM	MV	TM	MV	TM	MV
1	Sahana	16.65c (24.05)	16.65bc (24.05)	21.65c (27.71)	21.65cd (27.71)	25.00cd (30.00)	25.00cd (30.00)
2	B-82	18.35c (25.30)	18.35b (25.30)	23.35c (28.85)	23.35c (28.85)	30.00c (33.16)	30.00b (33.16)
3	Jayadhar	15.00c (22.60)	15.00c (22.60)	20.00c (26.56)	20.00cd (26.56)	25.00cd (30.00)	25.00cd (30.00)
4	DHH-11	18.35c (25.30)	18.35b (25.30)	20.00c (26.56)	20.00cd (26.56)	28.35cd (32.14)	28.35bc (32.14)
5	MECH-12 Bt	70.00a (56.84)	26.65a (31.07)	80.00ab (63.56)	30.00b (33.16)	100.00a (90.00)	41.65a (40.20)
6	MECH-12 NBt	15.00c (22.60)	15.00c (22.60)	18.35c (25.30)	18.35d (25.30)	23.35de (28.85)	23.35cd (28.85)
7	MECH-184 Bt	70.00a (56.84)	26.65a (31.07)	81.65a (64.71)	33.35ab (35.25)	100.00a (90.00)	43.35a (41.16)
8	MECH-184 NBt	15.00c (22.60)	15.00c (22.60)	20.00c (26.56)	20.00cd (26.56)	25.00cd (30.00)	25.00cd (30.00)
9	MECH-162 Bt	70.00a (56.84)	28.35a (32.14)	81.65a (64.71)	31.65ab (34.23)	100.00a (90.00)	43.35a (41.16)
10	MECH-162 NBt	16.65c (24.05)	16.65bc (24.05)	18.35c (25.30)	18.35d (25.30)	23.35de (28.85)	23.35cd (28.85)
11	RCH-2 Bt	65.00b (53.76)	25.00a (30.00)	75.00b (60.08)	35.00a (36.24)	95.00b (79.55)	40.00a (39.21)
12	RCH-2 NBt	16.65c (24.05)	16.65bc (24.05	20.00c (26.56)	20.00cd (26.56)	21.65e (27.71)	21.65d (27.71)
13	RCH-20 Bt	65.00b (53.76)	25.00a (30.00)	76.65ab (61.15)	30.00b (33.16)	96.65b (81.39)	41.65a (40.20)
14	RCH-20 NBt	15.00c (22.60)	15.00c (22.60)	21.65c (27.71)	21.65cd (27.71)	23.35de (28.85)	23.35cd (28.85
15	RCH-144 Bt	68.35ab (55.70)	25.00a (30.00)	80.00ab (63.56)	33.35ab (35.25)	96.65b (81.39)	40.00a (39.21)
16	RCH-144 NBt	15.00c (22.60)	15.00c (22.60)	20.00c (26.56)	20.00cd (26.56)	25.00cd (30.00)	25.00cd (30.00)
17	Control	00.00d (00.00)	00.00d (00.00)	03.33d (6.29)	03.33e (6.29)	05.00f (12.92)	05.00e (12.92)

DAS: Days after spraying Control: Larvae reared on artificial diet
TM-Total mortality MV-Mortality due to virus
Figures in the parenthesis are the arc sine transformed values
Means followed by same alphabet do not differ significantly by DMRT (P=0.01)

at 5, 7 and 10 days after the spray followed by MECH-162Bt, MECH-12Bt which are equally effective. At 10 DAS, all the Mahyco Bt cotton hybrids recorded cent per cent larval mortality and are statistically on par with each other.

The trend of virosed larvae of the total larvae recorded in different treatments revealed the same as that observed in the earlier isolates. Here also, all the dead larvae in all the treatments except in Bt versions revealed the presence of POBs in the haemolymph of the larvae, indicating virus as the only cause for their death. Also the Bt hybrids enhanced the efficacy of the virus to infect and multiply in the host larvae compared to the other treatments. Among the Bt hybrids, the virus sprayed on MECH Bt series was highly potent in causing the infection in the fed larvae followed by the Rasi Bt hybrids. All the six Bt hybrids recorded on par mortality due to virus at 5, 7 and 10 days after the spray. Among the three varieties, B-82 excelled over others in retaining the virulence of the virus.

4.2.2.5 Coimbatore isolate of HaNPV

The per cent mortality recorded when the larvae were fed with the squares and bolls of different cotton species sprayed with Coimbatore isolate of HaNPV is envisaged in Table 20. All the treatments established superiority in recording higher larval mortality over the control. Among the various treatments, the larvae fed on Bt hybrids MECH-184, 162 and 12Bt encountered higher larval mortality by recording 83.35, 80.00 and 80.00 per cent at 5 DAS, respectively, and were statistically on par with each other. The superiority of all the three MECH-Bt hybrids was appreciable at 10 DAS, in which all the fed larvae were succumbed to death indicating the higher expression of Bt toxin in combination with Coimbatore NPV. In contrast, all the Rasi Bt hybrids took 7 days to cause 100.00 per cent larval mortality in the

Table 20. Influence of cotton species on the efficacy of Coimbatore HaNPV against *Helicoverpa armigera*

Sl. No.	Cotton Species	Larval mortality (%)					
		5 DAS		7 DAS		10 DAS	
		TM	MV	TM	MV	TM	MV
1	Sahana	25.00cd (30.00)	25.00cd (30.00)	33.35cd (35.25)	33.35cd (35.25)	38.35b (38.24)	38.35c (38.24)
2	B-82	28.35c (32.14)	28.35bc (32.14)	36.65c (37.26)	36.65c (37.26)	40.00b (39.21)	40.00bc (39.21)
3	Jayadhar	23.35cd (28.85)	23.35de (28.85)	30.00d (33.16)	30.00d (33.16)	36.65b (37.26)	36.65c (37.26)
4	DHH-11	25.00cd (30.00)	25.00cd (30.00)	35.00c (36.24)	35.00c (36.24)	40.00b (39.21)	40.00bc (39.21)
5	MECH-12 Bt	80.00ab (63.56)	30.00ab (33.16)	100.00a (90.00)	45.00ab (42.12)	100.00a (90.00)	45.00ab (42.12)
6	MECH-12 NBt	20.00e (26.45)	20.00ef (26.45)	30.00d (33.16)	30.00d (33.16)	36.65b (37.26)	36.65c (37.26)
7	MECH-184 Bt	83.35a (65.97)	33.65a (35.25)	100.00a (90.00)	50.00a (45.00)	100.00a (90.00)	50.00a (45.00)
8	MECH-184 NBt	20.00e (26.45)	20.00de (26.45)	31.65cd (34.23)	31.65cd (34.23)	36.65b (37.26)	36.65c (37.26)
9	MECH-162 Bt	80.00ab (63.56)	33.65a (35.25)	100.00a (90.00)	46.65ab (43.09)	100.00a (90.00)	46.65a (43.09)
10	MECH-162 NBt	21.65de (27.71)	21.65de (27.71)	33.35cd (35.25)	33.35cd (35.25)	36.65b (37.26)	36.65c (37.26)
11	RCH-2 Bt	78.35b (62.29)	30.00ab (33.16)	96.65b (81.39)	41.65b (40.20)	100.00a (90.00)	45.00ab (42.12)
12	RCH-2 NBt	20.00e (26.45)	20.00f (26.45)	35.00cd (36.24)	35.00cd (36.24)	38.35b (38.24)	38.35c (38.24)
13	RCH-20 Bt	75.00b (60.00)	30.00ab (33.16)	95.00b (79.55)	43.35b (41.16)	100.00a (90.00)	46.65a (43.09)
14	RCH-20 NBt	23.35cd (28.85)	23.35de (28.85)	33.35cd (35.25)	33.35cd (35.25)	38.35b (38.24)	38.35c (38.24)
15	RCH-144 Bt	78.35b (62.29)	31.65ab (34.23)	95.00b (79.55)	43.35b (41.16)	100.00a (90.00)	46.65a (43.09)
16	RCH-144 NBt	21.65de (27.71)	21.65de (27.71)	35.00cd (37.26)	35.00cd (36.24)	36.65b (37.26)	36.65c (37.26)
17	Control	00.00f (00.00)	00.00g (00.00)	00.00e (00.00)	00.00e (00.00)	03.33c (6.29)	03.33d (6.29)

DAS: Days after spraying — Control: Larvae reared on artificial diet

TM-Total mortality — MV-Mortality due to virus

Figures in the parenthesis are the arc sine transformed values

Means followed by same alphabet do not differ significantly by DMRT (P=0.01)

larvae fed on them. At 10 DAS, all the six Bt hybrids recorded cent per cent mortality and remained on par with each other.

Similar trend was observed in the larvae succumbed to NPV infection as that of earlier isolates. Among the various treatments, the NPV sprayed on Bt hybrid cotton plants caused more viral infection in the fed larvae as compared to the other treatments. There was no statistical difference among the Bt hybrids in recording the larval mortality due to NPV infection, where all the six Bt hybrids recorded on par results at 5 and 10 DAS.

4.2.2.6 PDBC isolate of HaNPV

Table 21 indicates the results recorded when PDBC isolate of HaNPV was sprayed on different cotton species and the larval mortality obtained at 5, 7 and 10 DAS.

The results obtained revealed the same trend in the larval mortality when different cotton plants sprayed with PDBC isolate, in which the superiority of Bt hybrid cottons in recording higher mortality confirmed over days. The larval mortality obtained on the plants sprayed with PDBC isolate was comparatively less than that obtained with Gulbarga, Coimbatore and Dharwad isolates. All the Bt hybrids except RCH-20Bt, recorded highest larval mortality compared to other treatments at 5 DAS and were on par with each other. More or less similar trend in their effect in reducing the larval population was observed over days. Only MECH series of Bt cottons recorded cent per cent larval mortality at 10 DAS.

The trend in the count of virosed larvae in different treatments due to PDBC HaNPV remained the same. All the Bt hybrids encouraged the NPV to cause viral infection in the host larvae by recording more number of larvae succumbed to viral infection. The number of virosed larvae due to PDBC isolate

Table 21. Influence of cotton species on the efficacy of PDBC HaNPV against *Helicoverpa armigera*

Sl. No.	Cotton Species	Larval mortality (%)					
		5 DAS		7 DAS		10 DAS	
		TM	MV	TM	MV	TM	MV
1	Sahana	15.00c (22.60)	15.00ef (22.60)	18.35cd (25.30)	18.35cd (25.30)	23.35de (28.85)	23.35cd (28.85)
2	B-82	16.65c (24.05)	16.65cd (24.05)	21.65c (27.71)	21.65c (27.71)	30.00d (33.16)	30.00b (33.16)
3	Jayadhar	13.35c (21.34)	13.35e (21.34)	16.65cd (24.05)	16.65de (24.05)	20.00f (26.45)	20.00d (26.45)
4	DHH-11	15.00c (22.60)	15.00ef (22.60)	20.00cd (26.45)	20.00cd (26.45)	28.35de (32.14)	28.35bc (32.14)
5	MECH-12 Bt	65.00ab (53.76)	23.35ab (28.85)	78.35ab (62.29)	31.65ab (34.23)	100.00a (90.00)	41.65a (40.20)
6	MECH-12 NBt	13.35c (21.34)	13.35f (21.34)	20.00cd (26.45)	20.00cd (26.45)	23.35ef (28.85)	23.35cd (28.85)
7	MECH-184 Bt	68.35a (55.77)	25.00a (30.00)	81.65a (64.71)	35.00a (36.24)	100.00a (90.00)	43.35a (41.16)
8	MECH-184 NBt	15.00c (22.60)	15.00ef (22.60)	18.35cd (25.30)	18.35cd (25.30)	25.00de (30.00)	25.00cd (30.00)
9	MECH-162 Bt	68.35a (55.77)	25.00a (30.00)	80.00ab (63.56)	35.00a (36.24)	100.00a (90.00)	43.35a (41.16)
10	MECH-162 NBt	16.65c (24.05)	16.65cd (24.05)	18.35cd (25.30)	18.35cd (25.30)	23.35ef (28.85)	23.35cd (28.85)
11	RCH-2 Bt	60.00ab (52.74)	20.00bc (26.45)	75.00b (60.00)	33.35ab (35.25)	95.00b (79.55)	40.00a (39.21)
12	RCH-2 NBt	13.35c (21.34)	13.35f (21.34)	15.00d (22.60)	15.00e (22.60)	20.00f (26.45)	20.00d (26.45)
13	RCH-20 Bt	61.65b (51.76)	20.00cd (26.45)	75.00b (60.00)	30.00b (33.16)	93.35c (75.24)	40.00a (39.21)
14	RCH-20 NBt	13.35c (21.34)	13.35f (21.34)	16.65cd (24.05)	16.65de (24.05)	21.65f (27.71)	21.65d (27.71)
15	RCH-144 Bt	65.00ab (53.76)	23.35ab (28.85)	78.35ab (62.29)	33.35ab (35.25)	96.65b (81.39)	41.65a (40.20)
16	RCH-144 NBt	15.00c (22.60)	15.00de (22.60	16.65cd (24.05)	16.65de (24.05)	23.35ef (28.85)	23.35cd (28.85)
17	Control	00.00d (00.00)	00.00g (00.00)	03.33e (6.29)	03.33f (6.29)	05.00g (12.92)	05.00e (12.92)

DAS: Days after spraying   Control: Larvae reared on artificial diet
TM-Total mortality   MV-Mortality due to virus
Figures in the parenthesis are the arc sine transformed values
Means followed by same alphabet do not differ significantly by DMRT (P=0.01)

on different cotton plants was considerably less compared to the number of virosed larvae recorded by Gulbarga, Coimbatore and Dharwad isolates.

4.2.2.7 PCI isolate of HaNPV

The PCI isolate of HaNPV was sprayed on different cotton species and the larvae of *H. armigera* were allowed to feed on the squares and bolls of plants. The results on per cent larval mortality recorded and the larvae virosed observed is presented in Table 22. The per cent larval mortality recorded was considerably less compared to that of Gulbarga, Coimbatore and Dharwad isolates of NPV. The total mortality on different cotton pants ranged between zero to 61.65, 3.33 to 70.00 and 5.00 to 85.00 per cent, respectively at 5, 7 and 10 days after the spray. Among the various treatments, the Bt plants recorded highest mortality in the larva and emerged as the best treatment. At 5 DAS, all Mahyco hybrids recorded higher mortality and remained on par with each other. MECH-184Bt, MECH-162Bt and RCH-144Bt caused lethality in 85.00, 83.35 and 81.65 percentage of the total larvae used and were statistically on par to each other. Among all the Bt cotton hybrids, none of them recorded cent per cent mortality at 10 DAS, indicating the inferiority of PCI isolate in combination with Bt compared to other isolates like Gulbarga, Coimbatore and Dharwad, in which most of the Bt hybrids recorded cent per cent mortality at 10 DAS.

Though, the larval mortality observed due to virus was considerably less, the dead larvae analyzed for the presence of POBs in different treatments indicated the same trend that observed in other isolates. The superiority of Bt cottons is further confirmed in recording more number of virosed larvae. Maximum number of virosed larvae were observed in the treatments with MECH-184 (36.65%) which is followed by RCH-144 (33.35%) and RCH-20Bt

Table 22. Influence of cotton species on the efficacy of PCI HaNPV against *Helicoverpa armigera*

Sl. No.	Cotton Species	Larval mortality (%)					
		5 DAS		7 DAS		10 DAS	
		TM	MV	TM	MV	TM	MV
1	Sahana	13.35c (21.34)	13.35de (21.34)	18.35cd (25.30)	18.35cd (25.30)	20.00e (26.45)	20.00d (26.45)
2	B-82	15.00c (22.60)	15.00cd (22.60)	21.65c (27.71)	21.65bc (27.71)	25.00d (30.00)	25.00c (30.00)
3	Jayadhar	13.35c (21.34)	13.35de (21.34)	18.35cd (25.30)	18.35cd (25.30)	20.00e (26.45)	20.00d (26.45)
4	DHH-11	13.35c (21.34)	13.35de (21.34)	20.00cd (26.45)	20.00cd (26.45)	23.35de (28.85)	23.35cd (28.85)
5	MECH-12 Bt	53.35b (46.91)	18.35ab (25.30)	63.35b (51.76)	25.00ab (30.00)	80.00bc (63.56)	30.00b (33.16)
6	MECH-12 NBt	13.35c (21.34)	13.35de (21.34)	20.00cd (26.45)	20.00cd (26.45)	23.35de (28.85)	23.35cd (28.85)
7	MECH-184 Bt	61.65a (51.76)	20.00a (26.45)	70.00a (56.84)	28.35a (32.14)	85.00a (67.41)	36.65a (37.26)
8	MECH-184 NBt	11.65c (19.89)	11.65e (19.89)	18.35cd (25.30)	18.35cd (25.30)	25.00d (30.00)	25.00c (30.00)
9	MECH-162 Bt	56.65a (48.84)	20.00a (26.45)	65.00b (53.76)	26.65a (31.07)	83.35ab (65.97)	30.00b (33.16)
10	MECH-162 NBt	11.65c (19.89)	11.65e (19.89)	20.00cd (26.45)	20.00cd (26.45)	23.35de (28.85)	23.35cd (28.85)
11	RCH-2 Bt	51.65b (45.96)	16.65bc (24.04)	60.00b (50.79)	25.00ab (30.00)	80.00bc (63.56)	30.00b (33.16)
12	RCH-2 NBt	10.00c (21.34)	10.00de (21.34)	18.35cd (25.30)	18.35cd (25.30)	23.35de (28.85)	23.35cd (28.85)
13	RCH-20 Bt	53.35b (46.91)	18.35ab (25.30)	60.00b (50.79)	25.00ab (30.00)	78.35c (62.29)	31.65ab (34.23)
14	RCH-20 NBt	11.65c (19.89)	11.65e (19.89)	18.35cd (25.30)	18.35cd (25.30)	25.00d (30.00)	25.00c (30.00)
15	RCH-144 Bt	56.65a (48.84)	20.00a (26.45)	63.35b (51.76)	26.65a (31.07)	81.65ab (64.71)	33.35ab (35.25)
16	RCH-144 NBt	13.35c (21.34)	13.35de (21.34)	16.65d (24.04)	16.65d (24.04)	23.35de (28.85)	23.35cd (28.85)
17	Control	00.00d (00.00)	00.00f (00.00)	03.33e (6.29)	03.33e (6.29)	05.00f (12.92)	05.00e (12.92)

DAS: Days after spraying — Control: Larvae reared on artificial diet

TM-Total mortality — MV-Mortality due to virus

Figures in the parenthesis are the arc sine transformed values

Means followed by same alphabet do not differ significantly by DMRT (P=0.01)

(31.65%), at 10 DAS, respectively. B-82 again enhanced the potency of NPV, which harbourd number of virosed larvae to its credit (25% at 10 DAS).

4.2.2.8 BPM isolate of HaNPV

The trend in recording larval mortality by the various treatments remained unchanged (Table 23). The total larval mortality observed in this isolate on different cotton plants was found to be very less as compared to Gulbarga, Coimbatore and Dharwad isolates. It is evident from the details given in the table that the total mortality ranged from zero to 25.00, 3.33 to 35.00 and 5.00 to 41.65 per cent, respectively, at 5, 7 and 10 DAS indicating the inferiority of BPM isolate.

The number of virosed larvae obtained in different plants sprayed with BPM isolate was less than that of Gulbarga, Coimbatore and Dharwad isolates. The per cent larval mortality due to viral infection ranged from zero to 25.00, 3.33 to 35.00 and 5.00 to 41.65 per cent at 5, 7 and 10 DAS, respectively.

4.2.2.9 BPL isolate of HaNPV

The plants treated with BPL isolate of HaNPV recorded very less larval mortality, which is lesser than that observed in BPM isolate (Table 24). At 10 DAS, not even a single hybrid Bt cotton recorded cent per cent mortality. The highest mortality at 10 DAS being 83.35 per cent recorded by MECH-184Bt followed by MECH-162Bt with 80.00 per cent mortality.

There was drastic reduction in the number of virosed larvae harvested from various treatments. The larval mortality caused by NPV ranged from 3.33 to 26.65, 5.00 to 30.00 and 5.00 to 36.65 per cent at 5, 7 and 10 DAS, respectively. Among the treatments, Bt plants topped the list in recording more number of virosed larvae. B-82 and Jayadhar emerged best among the species.

Table 23. Influence of cotton species on the efficacy of BPM HaNPV against *Helicoverpa armigera*

Sl. No.	Cotton Species	Larval mortality (%)					
		5 DAS		7 DAS		10 DAS	
		TM	MV	TM	MV	TM	MV
1	Sahana	15.00cd (22.60)	15.00bc (22.60)	18.35de (25.30)	18.35cd (25.30)	25.00ef (30.00)	25.00cd (30.00)
2	B-82	16.65c (24.04)	16.65b (24.04)	23.35c (28.85)	23.35b (28.85)	33.35d (35.25)	33.35b (35.25)
3	Jayadhar	13.35d (21.34)	13.35c (21.34)	15.00e (22.60)	15.00d (22.60)	20.00f (26.45)	20.00e (26.45)
4	DHH-11	15.00cd (22.60)	15.00bc (22.60)	20.00cd (26.45)	20.00bc (26.45)	20.00f (26.45)	20.00e (26.45)
5	MECH-12 Bt	65.00ab (53.76)	23.35a (28.85)	78.35ab (62.29)	33.65a (35.25)	95.00b (79.55)	40.00a (39.21)
6	MECH-12 NBt	13.35d (21.34)	13.35c (21.34)	18.35de (25.30)	18.35cd (25.30)	21.65ef (27.71)	21.65de (27.71)
7	MECH-184 Bt	68.35a (55.77)	25.00a (30.00)	80.00a (63.56)	35.00a (36.24)	100.00a (90.00)	41.65a (39.53)
8	MECH-184 NBt	15.00cd (22.60)	15.00bc (22.60)	18.65de (25.62)	18.65cd (25.62)	23.35ef (28.85)	23.35cd (28.85)
9	MECH-162 Bt	65.00ab (53.76)	23.35a (28.85)	80.00a (63.56)	33.35a (35.25)	100.00a (90.00)	43.35a (41.16)
10	MECH-162 NBt	16.65c (24.04)	16.65b (24.04)	20.00cd (26.45)	20.00bc (26.45)	25.00ef (30.00)	25.00cd (30.00)
11	RCH-2 Bt	61.65b (51.76)	25.00a (30.00)	75.00b (60.00)	33.35a (35.25)	93.35c (75.24)	40.00a (39.21)
12	RCH-2 NBt	16.65c (24.04)	16.65b (24.04)	20.00cd (26.45)	20.00bc (26.45)	26.65e (31.07)	26.65c (31.07)
13	RCH-20 Bt	63.35b (52.74)	23.35a (28.85)	76.65ab (61.15)	31.65a (34.23)	95.00b (79.55)	40.00a (39.21)
14	RCH-20 NBt	15.00cd (22.60)	15.00bc (22.60)	21.65cd (27.71)	21.65bc (27.71)	26.65e (31.07)	26.65f (31.07)
15	RCH-144 Bt	65.00ab (53.76)	21.65a (27.71)	78.35ab (62.29)	31.65a (34.23)	95.00b (79.55)	38.35a (38.24)
16	RCH-144 NBt	13.35d (21.34)	13.35c (21.34)	15.00e (22.60)	15.00d (22.60)	21.65ef (27.71)	21.65de (27.71)
17	Control	00.00e (00.00)	00.00d (00.00)	03.33f (6.29)	03.33e (6.29)	05.00g (12.92)	05.00g (12.92)

DAS: Days after spraying — Control: Larvae reared on artificial diet
TM-Total mortality — MV-Mortality due to virus
Figures in the parenthesis are the arc sine transformed values
Means followed by same alphabet do not differ significantly by DMRT (P=0.01)

Table 24. Influence of cotton species on the efficacy of BPL HaNPV against *Helicoverpa armigera*

Sl. No.	Cotton Species	Larval mortality (%)					
		5 DAS		7 DAS		10 DAS	
		TM	MV	TM	MV	TM	MV
1	Sahana	13.35b (21.34)	13.35c (21.34)	18.35d (25.30)	18.35b (25.30)	20.00c (26.45)	20.00c (26.45)
2	B-82	15.00b (22.60)	15.00bc (22.60)	20.00d (26.45)	20.00b (26.45)	25.00c (30.00)	25.00cd (30.00)
3	Jayadhar	13.35b (21.34)	13.35c (21.34)	16.65d (24.04)	16.65b (24.04)	20.00c (26.45)	20.00d (26.45)
4	DHH-11	15.00b (22.60)	15.00bc (22.60)	20.00d (26.45)	20.00b (26.45)	23.35c (28.85)	23.35d (28.85)
5	MECH-12 Bt	60.00a (50.79)	25.00a (30.00)	66.65ab (54.75)	28.35a (32.14)	78.35b (62.29)	33.35ab (35.25)
6	MECH-12 NBt	15.00b (22.60)	15.00bc (22.60)	20.00d (26.45)	20.00b (26.45)	23.35c (28.85)	23.35d (28.85)
7	MECH-184 Bt	60.00a (50.79)	26.65a (31.07)	70.00a (56.84)	30.00a (33.16)	83.35a (65.97)	36.65a (37.26)
8	MECH-184 NBt	13.35b (21.34)	13.35c (21.34)	16.65d (24.04)	16.65b (24.04)	21.65c (27.71)	21.65d (27.71)
9	MECH-162 Bt	60.00a (50.79)	26.65a (31.07)	68.35a (55.77)	28.35a (32.14)	80.00ab (63.56)	33.35ab (35.25)
10	MECH-162 NBt	15.00b (22.60)	15.00bc (22.60)	18.35d (25.30)	18.35b (25.30)	21.65c (27.71)	21.65d (27.71)
11	RCH-2 Bt	58.35a (49.80)	25.00a (30.00)	60.00c (50.79)	26.65a (31.07)	76.65b (61.15)	30.00bc (33.16)
12	RCH-2 NBt	16.65b (24.04)	16.65b (24.04)	20.00d (26.45)	20.00b (26.45)	23.35c (28.85)	23.35d (28.85)
13	RCH-20 Bt	58.35a (49.80)	25.00a (30.00)	63.35bc (52.74)	28.35a (32.14)	78.35b (62.29)	33.35a (35.25)
14	RCH-20 NBt	15.00b (22.60)	15.00bc (22.60)	18.35d (25.30)	18.35b (25.30)	23.35c (28.85)	23.35d (28.85)
15	RCH-144 Bt	60.00a (50.79)	25.00a (30.00)	63.35bc (52.74)	28.35a (32.14)	78.35b (62.29)	35.00ab (36.24)
16	RCH-144 NBt	13.35b (21.34)	13.35c (21.34)	18.35d (25.30)	18.35b (25.30)	25.00c (30.00)	25.00cd (30.00)
17	Control	03.33c (6.29)	03.33d (6.29)	05.00e (12.92)	05.00c (12.92)	05.00d (12.92)	05.00e (12.92)

DAS: Days after spraying — Control: Larvae reared on artificial diet

TM-Total mortality — MV-Mortality due to virus

Figures in the parenthesis are the arc sine transformed values

Means followed by same alphabet do not differ significantly by DMRT (P=0.01)

4.2.2.10 Kalpavruksha isolate of HaNPV

Among the ten HaNPV isolates being screened for their efficacy on different cotton species against *H. armigera*, Kalpavruksha isolate of HaNPV emerged as the least virulent isolate as per the total mortality and the mortality induced by the virus is concerned (Table 25). At 5, 7 and 10 DAS, the total mortality ranged from zero to 61.65, 5.00 to 68.35 and 5.00 to 83.35 per cent respectively. However, the Bt hybrids topped the list in recording more per cent larval mortality against the other treatments.

As per the number of larvae virosed is concerned, it was found to be least in case of Kalpavruksha isolate, which failed to induce higher mortality on different cotton plants compared to the other isolates included in the investigation. Though the number of larvae succumbed to viral infection were very less, the NPV sprayed on Bt hybrids was more potent. The per cent virosed larvae ranged from zero to 25.00, 5.00 to 28.35 and 5.00 to 35.00 per cent at 5, 7 and 10 DAS, respectively.

The average mean total larval mortality (Table 26) revealed that irrespective of the cotton species, Gulbarga isolate recorded 52.24 per cent larval mortality followed by Coimbatore (51.08%) and Dharwad (45.85%) isolate. PCI isolate recorded lowest mean total larval mortality (34.50 %) followed by BPL (35.20%) and KPL (35.66). Further, irrespective of the HaNPV isolates, the larvae fed on the floral parts of MECH-184Bt recorded highest mortality (82.72%) followed by MECH-162Bt (81.56%), MECH-12Bt (80.22%) and RCH-144Bt (79.14%).

The average mean mortality caused by the virus on different cotton plants (Table 27) indicated that irrespective of the cotton species, Gulbarga isolate recorded highest per cent larval mortality (33.92) followed by Coimbatore (32.98) and Dharwad isolate (28.47). Further, irrespective of the isolates

Table 25. Influence of cotton species on the efficacy of Kalpavruksha HaNPV against *Helicoverpa armigera*

Sl. No.	Cotton Species	Larval mortality (%)					
		5 DAS		7 DAS		10 DAS	
		TM	MV	TM	MV	TM	MV
1	Sahana	13.35e (21.34)	13.35d (21.34)	18.35d (25.30)	18.35e (25.30)	20.00f (26.45)	20.00e (26.45)
2	B-82	15.00de (22.60)	15.00cd (22.60)	20.00d (26.45)	20.00de (26.45)	25.00e (30.00)	25.00d (30.00)
3	Jayadhar	13.35e (21.34)	13.35d (21.34)	18.35d (25.30)	18.35e (25.30)	20.00f (26.45)	20.00e (26.45)
4	DHH-11	15.00de (22.60)	15.00cd (22.60)	20.00d (26.45)	20.00de (26.45)	23.35e (28.85)	23.35de (28.85)
5	MECH-12 Bt	58.35ab (49.80)	23.35a (28.85)	65.00ab (53.76)	26.65ab (31.07)	80.00bc (63.56)	30.00bc (33.16)
6	MECH-12 NBt	16.65d (24.04)	16.65c (24.04)	20.00d (26.45)	20.00de (26.45)	25.00e (30.00)	25.00d (30.00)
7	MECH-184 Bt	61.65a (51.76)	25.00a (30.00)	68.35a (55.77)	28.35a (32.14)	83.35a (65.97)	35.00a (36.24)
8	MECH-184 NBt	15.00de (22.60)	15.00cd (22.60)	20.00d (26.45)	20.00de (26.45)	26.65e (31.07)	26.65cd (31.07)
9	MECH-162 Bt	60.00ab (50.79)	25.00a (30.00)	68.35a (55.77)	28.35a (32.14)	81.65ab (64.71)	31.65ab (34.23)
10	MECH-162 NBt	16.65d (24.04)	16.65c (24.04)	21.65d (27.71)	21.65cd (27.71)	25.00e (30.00)	25.00d (30.00)
11	RCH-2 Bt	58.35b (49.80)	23.35a (28.85)	63.35bc (52.74)	25.00ab (30.00)	80.00bc (63.56)	30.00bc (33.16)
12	RCH-2 NBt	13.35e (21.34)	13.35d (21.34)	20.00d (26.45)	20.00de (26.45)	26.65e (31.07)	26.65cd (31.07)
13	RCH-20 Bt	58.35b (49.80)	23.35a (28.85)	63.35c (52.74)	25.00ab (30.00)	76.65d (61.15)	30.00bc (33.16)
14	RCH-20 NBt	15.00de (22.60)	15.00cd (22.60)	18.35d (25.30)	18.35e (25.30)	23.35e (28.85)	23.35de (28.85)
15	RCH-144 Bt	55.00c (47.87)	20.00b (26.45)	61.65bc (51.76)	23.35bc (28.85)	78.35cd (62.29)	31.65ab (34.23)
16	RCH-144 NBt	15.00de (22.60)	15.00cd (22.60)	21.65d (27.71)	21.65cd (27.71)	25.00e (30.00)	25.00d (30.00)
17	Control	00.00f (00.00)	00.00e (00.00)	05.00e (12.92)	05.00f (12.92)	05.00g (12.92)	05.00f (12.92)

DAS: Days after spraying — Control: Larvae reared on artificial diet
TM-Total mortality — MV-Mortality due to virus
Figures in the parenthesis are the arc sine transformed values
Means followed by same alphabet do not differ significantly by DMRT (P=0.01)

Table 26. Influence of cotton species on the efficacy of HaNPV isolates against *Helicoverpa armigera* (Average of all isolates)

Sl. No	Cotton Species	Mean Total mortality recorded on different Cotton Plants (%)										
		UASD	GLB	RCHR	GNTR	CBE	PDBC	PCI	BPM	BPL	KLP	Mean
1	Sahana	24.30	32.12	18.90	21.10	32.23	18.90	17.23	19.45	17.23	17.23	**21.87**
2	B-82	29.45	37.78	22.77	23.90	35.00	22.77	20.55	24.45	20.00	20.00	**25.67**
3	Jayadhar	23.33	28.33	16.12	20.00	30.00	16.67	17.23	16.12	16.67	17.23	**20.17**
4	DHH-11	27.23	35.00	19.45	22.23	33.33	21.12	18.90	18.33	19.45	19.45	**23.45**
5	MECH-12 Bt	87.78	93.33	82.23	83.33	93.33	81.12	65.57	79.45	68.33	67.78	**80.22**
6	MECH-12 NBt	25.55	31.12	19.45	18.90	28.88	18.90	18.90	17.78	19.45	20.55	**21.95**
7	MECH-184 Bt	88.88	96.12	83.33	83.88	94.45	83.33	72.22	82.78	71.12	71.12	**82.72**
8	MECH-184 NBt	24.45	31.12	18.33	20.00	29.43	19.45	18.33	19.00	17.22	20.55	**21.79**
9	MECH-162 Bt	88.33	94.45	83.33	83.88	93.33	82.78	68.33	81.67	69.45	70.00	**81.56**
10	MECH-162 NBt	26.12	34.45	20.00	19.45	30.55	19.45	18.33	20.55	18.33	21.10	**22.83**
11	RCH-2 Bt	83.33	90.55	76.67	78.33	91.67	76.67	63.88	76.67	65.00	67.23	**76.95**
12	RCH-2 NBt	23.98	32.67	18.35	19.43	31.12	16.12	17.23	21.10	20.00	20.00	**21.10**
13	RCH-20 Bt	85.55	92.23	77.22	79.43	90.00	76.67	63.90	78.33	66.68	66.12	**77.61**
14	RCH-20 NBt	26.12	32.78	16.67	20.00	31.68	17.22	18.33	21.10	18.90	18.90	**22.17**
15	RCH-144 Bt	86.12	93.33	80.00	81.67	91.12	80.00	67.22	79.45	67.23	65.00	**79.14**
16	RCH-144 NBt	25.57	31.67	17.78	20.00	31.10	18.33	17.78	16.67	18.90	20.55	**21.84**
17	Control	3.33	01.11	01.67	02.77	01.10	02.78	02.78	02.78	04.43	03.33	**02.61**
	Mean	**45.85**	**52.24**	**39.55**	**41.07**	**51.08**	**39.55**	**34.51**	**39.75**	**35.20**	**35.66**	-

UASD-University of Agricultural Sciences, Dharwad; GLB-Gulbarga; RCHR-Raichur; GNTR-Guntur; CBE-Coimbatore; PDBC-Project Directorate of Biological Control; PCI-Pest Control India; BPM- Bio Pest Management; BPL- Bio Pest Laboratories; KLP- Kalpavruksha Bio Systems Control: Larvae reared on artificial diet

Table 27. Influence of cotton species on the efficacy of HaNPV isolates against *Helicoverpa armigera* (Average of all isolates))

Sl. No	Cotton Species	Mean mortality caused by the virus on different Cotton Plants (%)										
		UASD	GLB	RCHR	GNTR	CBE	PDBC	PCI	BPM	BPL	KLP	Mean
1	Sahana	24.30	32.12	18.90	21.10	32.23	18.90	17.23	19.45	17.23	17.23	**21.87**
2	B-82	29.45	37.78	22.77	23.90	35.00	22.77	20.55	24.45	20.00	20.00	**25.67**
3	Jayadhar	23.33	28.33	16.12	20.00	30.00	16.67	17.23	16.12	16.67	17.23	**20.17**
4	DHH-11	27.23	35.00	19.45	22.23	33.33	21.12	18.90	18.33	19.45	19.45	**23.45**
5	MECH-12 Bt	37.78	42.78	32.22	32.77	40.00	32.22	24.45	32.33	28.90	26.67	**33.01**
6	MECH-12 NBt	25.55	31.12	19.45	18.90	28.88	18.90	18.90	17.78	19.45	20.55	**21.95**
7	MECH-184 Bt	41.67	45.00	35.00	34.45	44.55	34.45	28.33	33.88	31.10	29.45	**35.80**
8	MECH-184 NBt	24.45	31.12	18.33	20.00	29.43	19.45	18.33	19.00	17.22	20.55	**21.79**
9	MECH-162 Bt	38.33	43.35	34.45	34.45	42.32	34.45	25.55	33.35	29.45	28.33	**34.40**
10	MECH-162 NBt	26.12	34.45	20.00	19.45	30.55	19.45	18.33	20.55	18.33	21.10	**22.83**
11	RCH-2 Bt	33.88	38.90	30.00	33.33	38.88	31.12	23.88	32.78	27.22	26.12	**31.61**
12	RCH-2 NBt	23.98	32.67	18.35	19.43	31.12	16.12	17.23	21.10	20.00	20.00	**21.10**
13	RCH-20 Bt	36.12	39.45	31.10	32.22	40.00	30.00	25.00	31.67	28.90	26.12	**32.06**
14	RCH-20 NBt	26.12	32.78	16.67	20.00	31.68	17.22	18.33	21.10	18.90	18.90	**22.17**
15	RCH-144 Bt	36.70	40.00	32.77	32.78	40.55	32.78	26.67	30.55	29.45	25.00	**32.73**
16	RCH-144 NBt	25.57	31.67	17.78	20.00	31.10	18.33	17.78	16.67	18.90	20.55	**21.84**
17	Control	3.33	01.11	01.67	02.77	01.10	02.78	02.78	02.78	04.43	03.33	**02.61**
	Mean	**28.47**	**33.92**	**22.65**	**23.99**	**32.98**	**22.75**	**19.97**	**23.05**	**21.51**	**21.21**	-

UASD-University of Agricultural Sciences, Dharwad; GLB-Gulbarga; RCHR-Raichur; GNTR-Guntur; CBE-Coimbatore; PDBC-Project Directorate of Biological Control; PCI-Pest Control India; BPM- Bio Pest Management; BPL- Bio Pest Laboratories; KLP- Kalpavruksha Bio Systems   Control: Larvae reared on artificial diet tested, the NPV sprayed on MECH-184Bt recorded highest mean per cent virosed larvae (35.80) followed by MECH-162Bt (34.40), MECH-12Bt (33.01), RCH-144Bt (32.73) and RCH-20Bt (32.06).

4.3 Persistence of HaNPV isolates in soils

The occlusion bodies of the insect viruses are known to persist in soil for longer period without loosing much of the activity and infectivity. This property is of immense significance as the soil born viruses can form inoculum to initiate epizootics in subsequent seasons.

With this background, two promissing isolates of HaNPV (Gulbarga and Coimbatore), which have shown encouraging results and emerged as virulent isolates, were further investigated for their persistence in both red and black soils kept out door and indoor conditions.

4.3.1 Persistence of Gulbarga isolate of HaNPV in soils

4.3.1.1 Persistence of HaNPV in black soil kept outdoors

The per cent larval mortality, mean time for mortality, per cent pupation and adult emergence due to the virus infection of Gulbarga isolate of HaNPV kept outdoor is presented in Table 28. The per cent larval mortality declined from 88.35 to 38.35 per cent in a period of 12 months when the virus treated black soil was kept outdoors. The virus treated black soil kept outdoors retained more than 50.00 per cent of its infectivity up to ten months. The mean per cent larval mortality declined steadily as the period of storage of virus in soil advanced and they were found to be inversely proportional to each other.

The mean time required for larval mortality in virus suspension obtained from the soil treated on the day was 4 days and after 12 months of treatment, it rose to 7.33 days to manifest the symptoms of virus infection. As the period of storage of virus in soil advanced, the mean time required for mortality was

Table 28. Persistence of Gulbarga isolate of HaNPV in black soil kept outdoors

Period (Months)	Larval mortality (%)	Mean time for mortality (days)	Pupation (%)	Adult emergence (%)
0	88.35	4.00	11.65	8.35
1	85.00	4.33	15.00	10.00
2	80.00	5.00	20.00	15.00
3	80.00	5.33	20.00	16.65
4	76.65	5.67	23.35	20.00
5	73.35	6.00	26.65	23.35
6	71.65	6.33	28.35	25.00
7	66.65	6.67	33.35	28.35
8	60.00	6.67	40.00	35.00
9	58.35	7.00	41.65	36.65
10	51.65	7.00	48.35	41.65
11	48.35	7.33	51.65	45.00
12	38.35	7.33	61.65	50.00

also found to be increased. Hence, the period of storage and mean time for mortality (in days) were directly proportional.

When the virus suspension obtained from the soil treated on the same day and fed to larvae resulted in the mean per cent pupation of 11.65 per cent, that steadily increased and reached 61.65 per cent after 12 months of virus storage. As the period of storage of virus in soil increased, the rate of pupation also increased and were directly proportional to each other.

The rate of emergence of adults ranged from 8.35 per cent to 50.00 per cent. When the virus suspension obtained from the soil treated on the same day resulted in only 8.35 per cent adult emergence and this reached to 50.00 per cent after 12 months of virus storage in black soil kept outdoors.

4.3.1.2 Persistence of HaNPV in black soil kept indoors

The results on the persistence of Gulbarga isolate of HaNPV in black soil kept indoors is indicated in Table 29. The results revealed that the larval mortality due to NPV declined from 90.00 per cent to 51.65 per cent in a period of 12 months. The maximum mortality of 90.00 per cent was observed when the virus suspension obtained from the soil was treated on the same day while, minimum of 51.65 per cent mortality was recorded when the virus suspension obtained from the soil was treated after 12 months. More than 50.00 per cent mortality of the larvae was recorded after one year when the NPV treated black soil was kept indoors. As the period of storage advanced, the rate of mortality decreased steadily and both were found to be inversely proportional.

The mean time required to cause mortality in virus suspension obtained from the black soil kept indoors treated with NPV on the same day was 4.00 days, while it was 8.00 days after 12 months. As the duration of virus storage

Table 29. Persistence of Gulbarga isolate of HaNPV in black soil kept indoors

Period (Months)	Larval mortality (%)	Mean time for mortality (days)	Pupation (%)	Adult emergence (%)
0	90.00	4.00	10.00	8.35
1	88.35	4.00	11.67	10.00
2	88.35	4.33	11.67	10.00
3	83.35	4.67	16.85	13.35
4	81.65	5.00	18.35	15.00
5	81.65	5.67	18.35	16.65
6	75.00	6.33	25.00	20.00
7	70.00	6.67	30.00	25.00
8	68.35	7.00	31.65	30.00
9	65.00	7.33	35.00	33.35
10	56.65	7.33	43.35	36.65
11	55.00	7.33	45.00	40.00
12	51.65	8.00	48.35	43.35

progressed, the mean time required for mortality was also increased, indicating the direct relationship between the two.

With regards to the rate of pupation, it was 10.00 per cent when the NPV suspension obtained from the black soil was treated on the same day. This steadily increased and attained 48.35 per cent at the end of 12th month, indicating that the period of storage of virus and the rate of pupation were directly related to each other.

The rate of adult emergence ranged from 8.35 to 43.35 per cent, when the larvae were exposed to virus extracted from the soil treated on the same day and after 12 months of soil treatment, respectively. The adult emergence gradually increased in a peak of 43.35 per cent after 12 months of the storage of virus treated soil.

4.3.1.3 Persistence of HaNPV in red soil kept outdoors

The results of Gulbarga isolate of HaNPV in red soil kept outdoors has been presented in Table 30. The second instar larvae of *H. armigera* fed on the virus suspension obtained on the same day of virus inoculation recorded 88.35 per cent larval mortality. The rate of mortality declined steadily over months and it reached 85.00, 80.00, 66.65 and 60.00 per cent on 3rd, 6th, 9th and 12th month after the virus incorporation in to the red soil kept under field conditions.

The mean time required to display the typical viral symptoms and to cause mortality when the NPV obtained from red soil kept outdoors ranged from 4.00 to 7.33 days.

The virus suspension obtained from the red soil, which is kept outdoors and fed to the larvae showed the rate of pupation of 11.65 per cent and it reached 20.00 per cent after 5 months of virus inoculation. However, the pupation rate of 40.00 per cent was registered after 12 months of storage. As

Table 30. Persistence of Gulbarga isolate of HaNPV in red soil kept outdoors

Period (Months)	Larval mortality (%)	Mean time for mortality (days)	Pupation (%)	Adult emergence (%)
0	88.35	4.00	11.65	10.00
1	86.65	4.00	13.35	10.00
2	85.00	4.33	15.00	11.65
3	85.00	4.33	15.00	13.35
4	81.65	4.67	18.35	15.00
5	80.00	5.00	20.00	15.00
6	80.00	5.67	20.00	18.35
7	73.35	5.67	26.65	20.00
8	70.00	6.33	30.00	23.35
9	66.65	6.67	33.35	26.65
10	63.35	7.00	36.65	28.35
11	60.00	7.00	40.00	31.65
12	60.00	7.33	40.00	33.35

the period of storage progressed, the rate of pupation also increased and both were directly proportional to each other. Further, the rate of adult emergence ranged from 10.00 per cent to 33.35 per cent, when the extracted virus from such soil was fed to the larvae. The percentage of adult emergence increased gradually with the advancement of storage period.

4.3.1.4 Persistence of HaNPV in red soil kept indoors

The virus obtained from the HaNPV treated red soil kept under laboratory conditions revealed that the mortality of the larvae due to virus infection declined from 90.00 to 63.35 per cent in 12 months period upon storage (Table 31). The NPV treated red soil, which was kept indoors, retained almost 75 per cent of its infectivity up to 7 months. As the storage duration prolonged, the rate of larval mortality declined and there was inverse relation between storage period and larval mortality.

When the larvae exposed to virus extracted from red soil, the mean time required for mortality was 4 days, and it increased to 5.00 days after 4th month and further it increased to 7.00 days after 12th month of storage. Hence, it is evident from the table that, the period of virus storage and mean time for mortality were directly proportional.

The virus suspension extracted from treated red soil on the same day kept under laboratory condition recorded 10.00 per cent pupation and further it increased steadily to 20.00 per cent after 6 months. It was further extended to 36.65 per cent after one year. This clearly indicated that the rate of pupation and the storage period have direct relationship. When the virus obtained from the treated red soil on the same day and fed to the larvae, the rate of adult emergence was 10.00 per cent, which reached to 16.65 per cent after six months. It was further extended to 26.65 per cent after 12 months, indicating the increased emergence as the period of virus storage advanced.

Table 31. Persistence of Gulbarga isolate of HaNPV in red soil kept indoors

Period (Months)	Larval mortality (%)	Mean time for mortality (days)	Pupation (%)	Adult emergence (%)
0	90.00	4.00	10.00	10.00
1	88.35	4.00	11.65	10.00
2	88.35	4.33	11.65	11.65
3	86.65	4.33	13.35	11.65
4	85.00	5.00	15.00	13.35
5	83.35	5.00	16.65	15.00
6	80.00	5.33	20.00	16.65
7	75.00	5.33	25.00	20.00
8	71.65	5.67	28.35	20.00
9	68.35	6.00	31.65	23.35
10	68.35	6.00	31.65	25.00
11	63.35	6.33	36.65	25.00
12	63.35	7.00	36.65	26.65

4.3.1.5 Persistence of HaNPV in soil stored in refrigerator

The results on the persistence of HaNPV treated black and red soil kept in refrigerator condition is presented in Table 32. The data revealed that there was no much loss in the infectivity of the virus stored in refrigerator. When the black soil incorporated with HaNPV was extracted and fed to the larvae on the same day of incorporation recorded 88.35 per cent larval mortality. It slowly declined to 83.35 per cent at 12 months of storage. There was no much loss in the infectivity of the virus over months. The mean time required to cause mortality was four days when the suspension was fed to the larvae on the same day and it was slowly raised to 5.35 days at the end of 12th month. The per cent pupation and per cent adult emergence also followed the same trend. There was slight increase in the pupation and adult emergence over months, which ranged from 11.65 to 16.65 per cent and 10.00 to 15.00 per cent, respectively from the day of inoculation to 12 months after the storage.

Similar trend was observed in the larval mortality, mean time for mortality, per cent pupation and per cent adult emergence, when the HaNPV treated red soil was stored in refrigerator. However, the per cent larval mortality observed was slightly high i.e. 90.00 per cent, which declined to 86.00 per cent. The mean time required to cause mortality was comparatively less in case of red soil than black soil. The per cent pupation and per cent adult emergence ranged from 10.00 to 14.00 per cent and 10.00 to 13.00 per cent when the NPV treated red soil was stored in refrigerator.

The computed half life of Gulbarga isolate of HaNPV kept in out doors, indoors and in refrigerator is presented in Table 33. The computed half life of the Gulbarga isolate of HaNPV in black soil was 10.57 months, while it was 12.92 months for the same soil kept out doors. But, the half life of HaNPV was 15.70 months when the virus treated red soil was kept outdoors while, it

Table 32. Persistence of Gulbarga HaNPV in soil stored in refrigerator

Period (Months)	Larval mortality (%)	Mean time for mortality (days)	Pupation (%)	Adult emergence (%)
Black soil				
0	88.35	4.00	11.65	10.00
3	87.00	4.00	13.00	11.65
6	86.65	4.33	13.35	12.35
9	85.00	4.67	15.00	13.65
12	83.35	5.35	16.65	15.00
Red soil				
0	90.00	4.00	10.00	10.00
3	88.35	4.00	11.65	10.00
6	88.35	4.00	11.65	10.00
9	86.65	4.33	13.35	11.35
12	86.00	5.00	14.00	13.00

Table 33. Half life of Gulbarga isolate of HaNPV in soil kept outdoors and indoors

Sl. No.	Treatments	Regression equation	Half Life (Months)
1	HaNPV treated black soil kept outdoor	Y= 90.69-3.85X	10.57
2	HaNPV treated black soil kept indoor	Y= 93.81-3.39X	12.92
3	HaNPV treated red soil kept outdoor	Y= 90.97-2.61X	15.70
4	HaNPV treated red soil kept indoor	Y= 92.82-2.50X	17.13
5	HaNPV treated black soil kept under refrigerator	Y= 88.47-0.40X	96.17
6	HaNPV treated red soil kept under refrigerator	Y= 89.81-0.32X	124.40

increased to 17.13 months for the same soil kept indoors. This indicates the extended period of storage of virus in red soil and increased persistence.

Further, the computed half life for the same isolate in black and red soil stored in refrigerator was 96.17 and 124.40 months, respectively. Between the two soils, the NPV treated red soil retained the virulence over a long period (124.40 months) compared to the black soil (96.17 months). This indicated that there was some change in the virulence of HaNPV stored in refrigerator.

4.3.2 Persistence of Coimbatore isolate of HaNPV in soils

The persistence of Coimbatore isolate of HaNPV incorporated in black and red soil kept in both outdoors and indoors is presented in Table 34 to 37.

The results on the persistence of Coimbatore isolate of HaNPV in both the soil types kept under field and lab conditions revealed the same trend that was encountered in Gulbarga isolate of HaNPV. However, compared to Gulbarga isolate, the per cent larval mortality recorded over months was less. The larval mortality declined from 85.00 to 35.00 and 85.00 to 50.00 per cent, when the Coimbatore isolate treated black soil was kept outdoors and indoors. Whereas, the larval mortality ranged from 85.00 to 50 and 85.00 to 53.35 per cent, when the HaNPV treated red soil was kept outdoors and indoors, respectively.

The mean time required for mortality increased as the storage period advanced when Coimbatore isolate treated soil suspension was fed to the larvae. The per cent pupation ranged from 15.00 to 65.00 and 15.00 to 50.00 per cent, when the virus extracted from black soil kept outdoors and indoors, respectively. Whereas, it ranged from 15.00 to 50.00 and 15.00 to 46.65 per cent, when the virus was extracted from the red soil kept outdoors and indoors, respectively. This indicated that Coimbatore isolate of HaNPV persisted for a shorter period compared to Gulbarga isolate. Similar trend in

Table 34. Persistence of Coimbatore isolate of HaNPV in black soil kept outdoors

Period (Months)	Larval mortality (%)	Mean time for mortality (days)	Pupation (%)	Adult emergence (%)
0	85.00	4.00	15.00	11.65
1	83.35	4.33	16.65	13.35
2	81.65	4.67	18.35	15.00
3	76.65	5.33	23.35	18.35
4	73.35	6.00	26.65	23.35
5	73.35	6.67	26.65	25.00
6	70.00	7.00	30.00	28.35
7	66.65	7.33	33.35	30.00
8	58.35	7.33	41.65	38.35
9	58.35	8.00	41.65	40.00
10	51.65	8.67	48.35	45.00
11	43.35	9.33	56.65	50.00
12	35.00	10.33	65.00	58.35

Table 35. Persistence of Coimbatore isolate of HaNPV in black soil kept indoors

Period (Months)	Larval mortality (%)	Mean time for mortality (days)	Pupation (%)	Adult emergence (%)
0	85.00	4.00	15.00	11.65
1	83.35	4.00	16.65	13.35
2	83.35	4.67	16.65	13.35
3	81.65	5.00	18.35	15.00
4	80.00	5.67	20.00	18.35
5	76.65	6.00	23.35	20.00
6	75.00	6.67	25.00	23.35
7	68.35	7.00	31.65	25.00
8	65.00	7.33	35.00	28.35
9	63.35	7.67	36.65	33.35
10	53.35	8.00	46.65	38.35
11	50.00	8.67	50.00	40.00
12	50.00	9.00	50.00	48.35

Table 36. Persistence of Coimbatore isolate of HaNPV in red soil kept outdoors

Period (Months)	Larval mortality (%)	Mean time for mortality (days)	Pupation (%)	Adult emergence (%)
0	85.00	4.00	15.00	11.65
1	83.35	4.33	15.00	13.35
2	83.35	4.33	16.65	13.35
3	80.00	5.00	20.00	15.00
4	76.65	5.33	23.35	18.35
5	76.65	6.00	23.35	20.00
6	73.35	6.67	26.65	23.35
7	70.00	7.00	30.00	25.00
8	66.65	7.00	33.35	28.35
9	60.00	7.33	40.00	30.00
10	60.00	8.00	40.00	35.00
11	58.35	8.33	41.65	38.35
12	50.00	9.00	50.00	41.65

Table 37. Persistence of Coimbatore isolate of HaNPV in red soil kept indoors

Period (Months)	Larval mortality (%)	Mean time for mortality (days)	Pupation (%)	Adult emergence (%)
0	85.00	4.33	15.00	11.65
1	83.35	4.33	16.65	11.65
2	83.35	4.33	16.65	13.35
3	80.00	4.67	20.00	15.00
4	78.35	5.00	21.65	18.35
5	76.65	5.00	23.35	18.35
6	76.65	5.67	23.35	20.00
7	73.35	6.00	26.65	20.00
8	70.00	6.33	30.00	25.00
9	66.65	6.67	33.35	28.35
10	63.65	7.33	36.65	30.00
11	60.00	7.67	40.00	33.35
12	53.35	8.33	46.65	36.65

adult emergence was observed. However, the per cent adult emergence was more compared to Gulbarga isolate.

Under refrigerator condition, similar trend was observed in the persistence of Coimbatore isolate of HaNPV as that of Gulbarga isolate. However, compared to Gulbarga isolate, Coimbatore isolate was less virulent in terms of recording larval mortality, which declined from 85.00 to 79.65 and 85.00 to 82.00 per cent for black and red soil, respectively, when it was stored for 12 months period (Table 38)

The computed half-life of Coimbatore isolate is presented in Table 39. The computed half life was 10.07 and 12.30 months for the HaNPV incorporated black soil kept outdoor and indoor, respectively. Whereas, it was 13.33 and 15.24 months for the HaNPV treated red soil kept outdoor and indoors, respectively. Further, when stored in refrigerator, the half life for the HaNPV in black soil was 78.98 months while it was 109.81 months for the HaNPV treated soil.

4.4 Assessment of quality of HaNPV produced by private firms

An effort was made to assess the quality of HaNPV produced by different private entrepreneurs. This exercise was made for three successive years from 2001 to 2003 in the Referral Laboratory of Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad. The representative samples received from different ADA offices were subjected for the count of active ingredient (POBs).

4.4.1 2001

During the year 2001, totally 17 samples received from 14 ADA offices were analyzed for the quality standards (Table 40). Of the 17 samples received, 8 samples were from Bio Pest Laboratories (BPL), 5 from Pest Control

Table 38. Persistence of Coimbatore HaNPV in soil stored in refrigerator

Period (Months)	Larval mortality (%)	Mean time for mortality (days)	Pupation (%)	Adult emergence (%)
Black soil				
0	85.00	4.00	15.00	14.65
3	84.35	4.35	15.65	15.00
6	83.65	5.00	16.35	15.00
9	81.65	5.65	18.35	16.65
12	79.65	6.00	20.35	17.35
Red soil				
0	85.00	4.00	15.00	13.35
3	84.35	4.00	15.65	14.00
6	84.00	4.35	16.00	14.65
9	83.35	5.00	16.65	15.00
12	82.00	5.65	18.00	15.65

Table 39. Half life of Coimbatore HaNPV in soil kept outdoors and indoors

Sl. No.	Treatments	Regression equation	Half life (Months)
1	HaNPV treated black soil kept outdoor	Y= 89.36-3.90X	10.07
2	HaNPV treated black soil kept indoor	Y= 89.84-3.24X	12.30
3	HaNPV treated red soil kept outdoor	Y= 88.40-2.88X	13.33
4	HaNPV treated red soil kept indoor	Y= 87.80-2.48X	15.24
5	HaNPV treated black soil kept under refrigerator	Y= 85.54-0.45X	78.98
6	HaNPV treated red soil kept under refrigerator	Y= 85.14-0.32X	109.81

Table 40. Assessment of quality standards of HaNPV samples received during 2001

Sample No.	Office of the ADA	Sample received	Source	Date of Sample received	No. of POB's/ml	Quality Standard	Report sent on
2001-1	Harihar	Helinash	BPM	10.08.2001	1.20×10^9	Std. Quality	22.09.2001
2001-2	Jagalur	Helicide	PCI	24.08.2001	1.02×10^9	Std. Quality	22.09.2001
2001-3	Chikkodi	Helicide	PCI	08.09.2001	1.01×10^9	Std. Quality	22.09.2001
2001-4	Gokak	Helicide	PCI	08.09.2001	1.00×10^9	Std. Quality	22.09.2001
2001-5	Hukkeri	Helicide	PCI	08.09.2001	1.00×10^9	Std. Quality	22.09.2001
2001-6	Raibhag	Helicide	PCI	08.09.2001	1.01×10^9	Std. Quality	22.09.2001
2001-7	Siruguppa	Helinash	BPM	15.09.2001	1.30×10^9	Std. Quality	22.09.2001
2001-8	Sandoor	*Helicoverpa* NPV	BPL	04.10.2001	0.90×10^9	Sub Std. Quality	05.11.2001
2001-9	Raichur	Helinash	BPM	06.10.2001	1.20×10^9	Std. Quality	05.11.2001
2001-10	Raichur	Helinash	BPM	06.10.2001	0.90×10^9	Sub Std. Quality	05.11.2001
2001-11	Haliyal	*Helicoverpa* NPV	BPL	22.10.2001	0.60×10^9	Sub Std. Quality	05.11.2001
2001-12	Yellapur	*Helicoverpa* NPV	BPL	27.10.2001	0.50×10^9	Sub Std. Quality	05.11.2001
2001-13	Mundagod	*Helicoverpa* NPV	BPL	15.11.2001	0.75×10^9	Sub Std. Quality	17.11.2001
2001-14	Sirsi	*Helicoverpa* NPV	BPL	15.11.2001	1.00×10^9	Std. Quality	27.11.2001
2001-15	Mundgod	*Helicoverpa* NPV	BPL	14.12.2001	1.03×10^9	Std. Quality	19.12.2001
2001-16	Mundgod	*Helicoverpa* NPV	BPL	14.12.2001	1.07×10^9	Std. Quality	19.12.2001
2001-17	Gulbarga	*Helicoverpa* NPV	BPL	17.12.2001	1.09×10^9	Std. Quality	19.12.2001

BPM- Bio-Pest Management Pvt. Ltd. (BPM), Opp. Jakkur Aerodrome, Jakkur Post, Bangalore-560 064.
PCI- Pest control India Pvt. Ltd. (PCI), P. B. No. 6426, Yelahanka, Bangalore-560 064.
BPL- Bio-Pest Laboratories Pvt. Ltd. (BPL), No. 81, Narasimhalu Layout, 5th main, Nandini Layout, Bangalore-560 096.

India (PCI) and 4 from Bio Pest Management (BPM). Among the three producers, all the samples from PCI met the minimum quality standards ($1X10^9$ POB/LE) and were of standard quality. Of the eight samples of BPL analyzed for the POB counts, four samples (sample number 2001-8, 2001-11, 2001-12 and 2001-13) recorded less than $1X10^9$ POBs/ml and were characterized as substandard quality products, while the other four samples of the same producer met the requirement. Of the four samples of BPM screened for the actual POB counts, one sample (sample no. 2001-10) failed to meet the minimum prescribed quality and remained substandard quality product. Thus, of the total 17 samples received during the year, five samples failed to address the minimum quality standards.

4.4.2 2002

During the said year, totally twelve samples were received from nine ADA offices, comprising of mainly two producers (BPM and BPL) (Table 41). Out of twelve samples, nine samples were from BPM and only three were from BPL. All the received samples met the minimum quality standards and were given standard quality remark.

4.4.3 2003:

Comparatively few numbers of samples were received during 2003 than 2001 and 2002. Totally nine samples were received from seven ADA offices (Table 42). During the year, major share was from BPM, which contributed eight samples and only one sample was received from BPL (sample no. 2003-6). All the samples received met the minimum requirement of POB count and were put under standard quality category.

Totally 38 samples were received during the period of investigation, of which 21 samples were from BPM, twelve from BPL and only five from PCI. The pooled data indicated in Table 43 revealed that, average POB yield ranged

Table 41. Assessment of quality standards of HaNPV samples received during 2002

Sample No.	Office of the ADA	Sample received	Source	Date of Sample received	No. of POB's/ml	Quality Standard	Report sent on
2002-1	Hospet	Helinash	BPM	16.08.2002	1.08×10^9	Std. Quality	13.09.2002
2002-2	Sandur	Helinash	BPM	16.08.2002	1.23×10^9	Std. Quality	13.09.2002
2002-3	Siraguppa	Helinash	BPM	18.09.2002	1.15×10^9	Std. Quality	13.09.2002
2002-4	Bilagi	Helinash	BPM	20.10.2002	1.11×10^9	Std. Quality	11.11.2002
2002-5	Belur	*Helicoverpa* NPV	BPL	20.10.2002	1.24×10^9	Std. Quality	11.11.2002
2002-6	Dharwad	Helinash-240902-HA	BPM	28.10.2002	1.20×10^9	Std. Quality	11.11.2002
2002-7	Dharwad	Helinash-070702-HA	BPM	06.11.2001	1.21×10^9	Std. Quality	11.11.2002
2002-8	Arsikere	Helinash	BPM	16.12.2002	1.27×10^9	Std. Quality	16.01.2003
2002-9	Dharwad	Helinash-240902-HA	BPM	16.12.2002	1.13×10^9	Std. Quality	16.01.2003
2002-10	Dharwad	Helinash-331002-HA	BPM	18.12.2002	1.13×10^9	Std. Quality	16.01.2003
2002-11	Holenarasipura	*Helicoverpa* NPV	BPL	20.12.2002	1.01×10^9	Std. Quality	16.01.2003
2002-12	Hassan	*Helicoverpa* NPV	BPL	20.12.2002	1.00×10^9	Std. Quality	16.01.2003

BPM- Bio-Pest Management Pvt. Ltd. (BPM), Opp. Jakkur Aerodrome, Jakkur Post, Bangalore-560 064.
PCI- Pest control India Pvt. Ltd. (PCI), P. B. No. 6426, Yelahanka, Bangalore-560 064.
BPL- Bio-Pest Laboratories Pvt. Ltd. (BPL), No. 81, Narasimhalu Layout, 5th main, Nandini Layout, Bangalore-560 096.

Table 42. Assessment of quality standards of HaNPV samples received during 2003

Sample No.	Office of the ADA	Sample received	Source	Date of Sample received	No. of POB/ml	Quality Standard	Report sent on
2003-1	Gundlupet	Helinash	BPM	11.09.2003	1.20×10^9	Std. Quality	24.11.2003
2003-2	Bydagi	Helinash	BPM	18.09.2003	1.12×10^9	Std. Quality	24.11.2003
2003-3	Kalaghatagi	Helinash	BPM	23.09.2003	1.04×10^9	Std. Quality	24.11.2003
2003-4	Dharwad	Helinash	BPM	26.09.2003	1.08×10^9	Std. Quality	24.11.2003
2003-5	Yellapur	Helinash	BPM	27.09.2003	1.20×10^9	Std. Quality	24.11.2003
2003-6	Arsikere	*Helicoverpa* NPV	BPL	29.09.2003	1.00×10^9	Std. Quality	24.11.2003
2003-7	Belur	Helinash	BPM	29.09.2003	1.00×10^9	Std. Quality	24.11.2003
2003-8	Kalaghatagi	Helinash	BPM	13.10.2003	1.04×10^9	Std. Quality	24.11.2003
2003-9	Kalaghataki	Helinash	BPM	14.10.2003	1.20×10^9	Std. Quality	24.11.2003

BPM- Bio-Pest Management Pvt. Ltd. (BPM), Opp. Jakkur Aerodrome, Jakkur Post, Bangalore-560 064.
PCI- Pest control India Pvt. Ltd. (PCI), P. B. No. 6426, Yelahanka, Bangalore-560 064.
BPL- Bio-Pest Laboratories Pvt. Ltd. (BPL), No. 81, Narasimhalu Layout, 5th main, Nandini Layout, Bangalore-560 096.

Table 43. Assessment of quality standards of HaNPV samples (Pooled data for three years)

Sl. No.	Source	Total Samples	Standard Quality	Samples with Standard quality (%)	Sub-Standard Quality	Average POB/ml
1	Bio Pest management (BPM)	21	20	95.24	1	$1.14X10^9$
2	Bio Pest Laboratories (BPL)	12	8	66.67	4	$0.99X10^9$
3	Pest Control India (PCI)	5	5	100.00	0	$1.00X10^9$
Total		**38**	**33**	-	**5**	-

BPM- Bio-Pest Management Pvt. Ltd. (BPM), Opp. Jakkur Aerodrome, Jakkur Post, Bangalore-560 064.
PCI- Pest control India Pvt. Ltd. (PCI), P. B. No. 6426, Yelahanka, Bangalore-560 064.
BPL- Bio-Pest Laboratories Pvt. Ltd. (BPL), No. 81, Narasimhalu Layout, 5th main, Nandini Layout, Bangalore-560 096.

from $0.93 X 10^9$ POBs to $1.14 X 10^9$ POBs per ml. The samples obtained from BPM recorded highest average POB count ($1.14 X 10^9$ POBs per ml) with 95.24 per cent samples with standard quality, followed by PCI, where the average POB count was $1.00 X 10^9$ POBs per ml with 66.67 per cent samples with standard quality. The average POB count of BPL samples was not satisfactory which registered $0.93 X 10^9$ POBs per ml.

4.5 Evaluation of HaNPV isolates under field condition on chickpea

The promising HaNPV isolates (Gulbarga, Coimbatore and Dharwad), which emerged as virulent isolates from the laboratory studies, HaNPV samples from the prominent private suppliers (BPM Ltd. and PCI Ltd.) including recommended package of practices as a treated standard check and untreated control check were evaluated for their efficacy under field conditions during 2002-03 and 2003-04 (Plate 8 & 9).

4.5.1 First Year (2002-03)

Field evaluation of different HaNPV isolates was carried out at Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad. Totally four sprays were scheduled after 30 days of sowing with an interval of 15 days between the two sprays.

4.5.1.1 After First Spray

The number of larvae before spray in different treatments was uniform as indicated in the Table 44. The data revealed that all the treatments were statistically significant over the untreated check in reducing the larval load. This indicated the inherent and differential efficacy of NPV. Among the HaNPV isolates tested, Gulbarga isolate recorded maximum per cent larval mortality (44.62%), which is followed by Coimbatore (41.15%), Dharwad (33.23%) and BPM isolates (31.11%), which are statistically on par in reducing the larval load

Plate 8: Incidence of *Helicoverpa armigera* on chickpea

Plate 9: General view of the chickpea experimental plot

Table 44. Efficacy of HaNPV isolates in the IPM of *Helicoverpa armigera* on chickpea after first spray (2002-03)

Sl. No.	Treatments	Before spray	Larval mortality (%)		
			5 DAS	10 DAS	15 DAS
1	Dharwad isolate	1.20a	33.23bc (35.19)	48.48bc (44.16)	42.37bc (40.62)
2	Gulbarga isolate	1.30a	44.62b (41.90)	59.96b (50.73)	54.06b (47.34)
3	Coimbatore isolate	1.20a	41.15bc (39.90)	58.70b (50.02)	52.80b (46.63)
4	BPM isolate	1.40a	31.11bc (33.89)	40.56c (39.56)	34.44c (35.89)
5	PCI isolate	1.20a	28.33c (32.14)	40.56c (39.56)	33.89c (35.55)
6	RPP	1.30a	60.29a (50.73)	79.87a (63.44)	71.41a (57.69)
7	Untreated check	1.30a	02.57d (5.37)	5.90d (11.52)	5.90d (11.52)

DAS: Days after spray
BPM- Bio-Pest Management Ltd. Bangalore
PCI- Pest Control India Ltd. Bangalore
RPP- Recommended Package of Practice
Figures in the parentheses are the arc sine transformed values
Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.05)

at 5 DAS. At 10 DAS, Gulbarga (59.96%), Coimbatore (58.70%) and Dharwad (48.48%) isolates recorded highest larval mortality and were found statistically on par with each other. Though, the larval mortality at 15 DAS was reduced, but, similar trend in the efficacy of different isolates in recording the larval mortality was recorded. Gulbarga and Coimbatore isolates registered higher larval mortality (54.06 and 52.08 %), which are statistically on par with Dharwad isolate (42.37 %). The HaNPV isolates obtained from two private firms (BPM and PCI) were found statistically inferior to other treatments (34.44 and 33.89 % mortality) in combating the larval population being on par with each other. However, RPP at all the days of observation recorded highest larval mortality being significantly superior to all other treatments.

4.5.1.2 After Second Spray

The larval mortality recorded at different days after the spray indicated that all the treatments were significantly superior over the untreated check (Table 45). At 5 days after the spray, the chickpea plot sprayed with Gulbarga isolate recorded a maximum of 51.28 per cent larval mortality and was found statistically on par with the recommended package (63.33%). The efficacy of remaining four isolates was same in causing larval mortality, as they were statistical on par with each other at 5 DAS.

Similar trend was observed in the larval mortality among the different treatments at 10 DAS. Coimbatore and Gulbarga isolates were statistically on par with each other by recording 60.51 and 59.96 per cent larval mortality, respectively. PCI and BPM isolates emerged as the least effective treatments in recording larval mortality.

At 15 DAS, Gulbarga (55.56 %) and the Coimbatore isolates (52.78%) excelled over others in recording larval mortality and were on par with the

Table 45. Efficacy of HaNPV isolates in the IPM of *Helicoverpa armigera* on chickpea after second spray (2002-03)

Sl. No.	Treatments	Larval mortality (%)		
		5 DAS	10 DAS	15 DAS
1	Dharwad isolate	42.37bc (40.63)	51.28bc (45.75)	48.48bc (44.14)
2	Gulbarga isolate	51.28ab (46.59)	59.96ab (50.75)	55.56ab (48.19)
3	Coimbatore isolate	47.05bc (43.32)	60.51ab (51.08)	52.78ab (46.59)
4	BPM isolate	34.44c (35.89)	41.11c (39.89)	34.81c (35.78)
5	PCI isolate	34.44c (35.89)	40.56c (39.56)	34.81c (35.89)
6	RPP	63.33a (52.74)	70.00a (56.84)	65.00a (53.76)
7	Untreated check	2.57d (5.37)	6.67d (12.29)	6.67d (12.29)

DAS: Days after spray
BPM- Bio-Pest Management Ltd. Bangalore
PCI- Pest Control India Ltd. Bangalore
RPP- Recommended Package of Practice
Figures in the parentheses are the arc sine transformed values
Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.05)

recommended package of practice (65.00%). BPM (34.81%) and PCI (34.81%) isolates were least effective and were on par with each other.

4.5.1.3 After third spray

The per cent larval mortality recorded at 5 DAS ranged from 6.67 to 57.95 per cent in different treatments (Table 46). Among the different isolates included in the study, Gulbarga and Coimbatore isolates recorded highest per cent larval mortality (52.57 and 51.28 %) and were statistically on par with the RPP (57.95%). Dharwad isolate recorded 40.00 per cent mortality and emerged as the second best isolate. At 10 DAS, the per cent larval mortality varied from 10.00 to 65.00 per cent. All the treatments established superiority over the untreated check in suppressing the pod borer menace. Gulbarga and Coimbatore isolates excelled over other isolates of HaNPV by recording 62.69 and 60.51 per cent larval mortality and were statistically on par with the standard treated check (65.00 %) in reducing the pest load. The superiority of Gulbarga and Coimbatore isolates continued even at 15 days of the spray. The efficacy of all the isolates followed the same trend in reducing the larval population.

4.5.1.4 After fourth spray

The per cent larval mortality recorded after fourth spray revealed that all the isolates of HaNPV included in the study performed similar trend in their efficacy in recording the larval mortality as detailed after second and third spray (Table 47). The per cent larval mortality varied from 10.00 to 65.00, 3.33 to 81.67 and 6.67 to 76.67 at 5, 10 and 15 days after the fourth spray, respectively. Gulbarga and Coimbatore isolates consistently recorded higher kill of the larvae at all the days of observation after the fourth spray.

Table 46. Efficacy of HaNPV isolates in the IPM of *Helicoverpa armigera* on chickpea after third spray (2002-03)

Sl. No.	Treatments	Larval mortality (%)		
		5 DAS	10 DAS	15 DAS
1	Dharwad isolate	40.00bc (39.15)	52.56b (46.49)	43.33ab (41.16)
2	Gulbarga isolate	52.57ab (46.49)	62.69a (52.34)	55.90a (48.42)
3	Coimbatore isolate	51.28ab (45.75)	60.51ab (51.06)	53.33a (46.92)
4	BPM isolate	32.22c (34.56)	40.56c (39.56)	33.33bc (35.25)
5	PCI isolate	31.11c (33.89)	40.00c (39.23)	30.00c (33.21)
6	RPP	57.95a (49.59)	65.00a (53.73)	56.67a (48.84)
7	Untreated check	06.67d (12.29)	10.00d (18.44)	06.67d (12.29)

DAS: Days after spray
BPM- Bio-Pest Management Ltd. Bangalore
PCI- Pest Control India Ltd. Bangalore
RPP- Recommended Package of Practice
Figures in the parentheses are the arc sine transformed values
Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.05)

Table 47. Efficacy of HaNPV isolates in the IPM of *Helicoverpa armigera* on chickpea after fourth spray (2002-03)

Sl. No.	Treatments	Larval mortality (%)		
		5 DAS	10 DAS	15 DAS
1	Dharwad isolate	33.33cd (41.16)	51.67bc (45.96)	45.00c (42.12)
2	Gulbarga isolate	50.00bc (45.00)	63.33b (52.74)	56.67b (48.84)
3	Coimbatore isolate	53.33b (46.91)	65.00b (53.74)	56.67b (48.84)
4	BPM isolate	36.67d (37.26)	46.67c (41.16)	43.33c (41.16)
5	PCI isolate	36.67d (37.92)	43.33c (41.16)	40.00c (39.21)
6	RPP	65.00a (53.76)	81.67a (64.70)	76.67a (61.22)
7	Untreated check	10.00e (18.44)	3.33d (6.15)	6.67d (14.76)

DAS: Days after spray
BPM- Bio-Pest Management Ltd. Bangalore
PCI- Pest Control India Ltd. Bangalore
RPP- Recommended Package of Practice
Figures in the parentheses are the arc sine transformed values
Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.05)

4.5.1.5 Pod damage, Yield and Economics

The pod damage among different treatments ranged between 8.44 and 33.72 per cent (Table 48). Among the different HaNPV isolates screened, Gulbarga isolate registered lower per cent of damaged pods (11.39), which was found superior among the isolates and on par with RPP (8.44). This was followed by Coimbatore isolate with 12.81 per cent pod damage. The isolates collected from PCI and BPM were statistically inferior to other treatments with a pod damage of 22.43 and 20.96 per cent, respectively.

The yield obtained in different treatments revealed that, all the treatments established superiority over control. Gulbarga (7.32 q/ha) and Coimbatore (7.28 q/ha) isolates were equally superior to RPP in registering higher yield and were statistically on par with each other. Dharwad isolate was found to be the next best treatment, recording 6.88 quintal grain yield per hectare. PCI isolate proved ineffective (4.75 q/ha), which was on par with the untreated check (3.21 q/ha). BPM and PCI isolates were equally but least effective among the five different isolates tested.

The Incremental Benefit Cost Ratio (ICBR) was highest in Gulbarga (2.88) followed by Coimbatore (2.85) and Dharwad (2.57). BPM and PCI isolates recorded least IBCR among the various treatments, however, RPP recorded highest IBCR (5.58)

4.5.2 Second year (2003-04)

To confirm the results obtained during the first year, an experiment was also planned during 2003-04 at Main Agricultural Research Station (MARS), University of Agricultural Sciences Dharwad on chickpea crop ecosystem

The three isolates (Gulbarga, Coimbatore and Dharwad) which emerged as virulent isolates from the laboratory studies and the two isolates (BPM and PCI)

Table 48. Effect of HaNPV isolates on pod damage and yield of chickpea (2002-03)

Sl. No.	Treatments	Pod damage (%)	Yield (q/ha)	IBCR
1	Dharwad isolate	18.06bc (25.15)	6.88b	2.57
2	Gulbarga isolate	11.39de (20.08)	7.32ab	2.88
3	Coimbatore isolate	12.81cd (22.68)	7.28ab	2.85
4	BPM isolate	20.96b (27.90)	5.08c	0.32
5	PCI isolate	22.43b (27.91)	4.75cd	0.27
6	RPP	08.44e (16.86)	8.89a	5.58
7	Untreated check	33.72a (35.50)	3.21d	-

BPM- Bio-Pest Management Ltd. Bangalore
PCI- Pest Control India Ltd. Bangalore
RPP- Recommended Package of Practice
IBCR – Incremental Benefit Cost Ratio
Figures in the parentheses are the arc sine transformed values
Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.05)

which have largely being produced and supplied by the private firms were included in the study keeping recommended package of practice as a standard treated check.

4.5.2.1 After first spray

The larval load before the imposition of treatments was uniform in all the treatments. The HaNPV isolates which showed encouraging performance during the first year were also found superior in their efficacy in recording higher mortality of larvae in the second year of the investigation (Table 49).

The per cent mortality at 5, 10 and 15 DAS, ranged from 6.36 to 62.63, 3.33 to 79.55 and 9.70 to 70.72 per cent, respectively. Gulbarga and Coimbatore isolates proved superior in recording higher percentage of larval mortality while, BPM and PCI isolates were inferior in their efficacy. However, RPP recorded highest per cent larval mortality at all the days of observation.

4.5.2.2 After second spray

All the treatments established superiority over the untreated check in reducing the larval population after the second spray (Table 50). The efficacy of Gulbarga and Coimbatore isolate remained same at 5, 10 and 15 days after spraying with 51.22 and 51.52; 61.21 and 58.18 and 57.81 and 54.55 per cent larval mortality, respectively and they were significantly superior to BPM and PCI isolates. However, the efficacy of Gulbarga and Coimbatore isolate was comparable with RPP at all the days of observation in recording higher larval mortality.

4.5.2.3 After third spray

The results on the larval mortality recorded in different treatments at 5, 10 and 15 days after the third spray (Table 51) revealed that there was increase in the per cent larval mortality from 5 to 10 DAS and declined

Table 49. Efficacy of HaNPV isolates in the IPM of *Helicoverpa armigera* on chickpea after first spray (2003-04)

Sl. No.	Treatments	Before spray	Larval mortality (%)		
			5 DAS	10 DAS	15 DAS
1	Dharwad isolate	1.30a	35.45bc (36.52)	48.48cd (44.14)	44.85c (42.03)
2	Gulbarga isolate	1.20a	45.15b (42.22)	64.55b (53.48)	58.18b (49.71)
3	Coimbatore isolate	1.20a	45.15b (42.22)	61.56bc (51.73)	54.85b (47.80)
4	BPM isolate	1.40a	32.12bc (34.51)	41.82d (40.29)	34.45d (36.52)
5	PCI isolate	1.20a	29.09c (32.64)	38.79d (38.52)	35.45d (36.52)
6	RPP	1.30a	62.63a (52.39)	79.55a (63.16)	70.72a (57.25)
7	Untreated check	1.20a	06.36d (12.00)	03.33e (6.15)	09.70e (18.15)

DAS: Days after spray
BPM- Bio-Pest Management Ltd. Bangalore
PCI- Pest Control India Ltd. Bangalore
RPP- Recommended Package of Practice
Figures in the parentheses are the arc sine transformed values
Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.05)

Table 50. Efficacy of HaNPV isolates in the IPM of *Helicoverpa armigera* on chickpea after second spray (2003-04)

Sl. No.	Treatments	Larval mortality (%)		
		5 DAS	10 DAS	15 DAS
1	Dharwad isolate	44.85bc (42.03)	51.52bc (45.86)	45.15ab (42.22)
2	Gulbarga isolate	51.52ab (45.88)	61.21ab (51.44)	57.81a (49.50)
3	Coimbatore isolate	51.52ab (48.18)	58.18ab (49.71)	54.55a (47.57)
4	BPM isolate	35.45cd (36.52)	45.15cd (42.22)	35.45b (36.52)
5	PCI isolate	32.42d (34.65)	42.12d (40.45)	35.45b (36.52)
6	RPP	64.45a (53.43)	70.46a (57.13)	61.63a (51.71)
7	Untreated check	06.36e (12.00)	03.36e (12.00)	06.67c (12.29)

DAS: Days after spray
BPM- Bio-Pest Management Ltd. Bangalore
PCI- Pest Control India Ltd. Bangalore
RPP- Recommended Package of Practice
Figures in the parentheses are the arc sine transformed values
Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.05)

Table 51. Efficacy of HaNPV isolates in the IPM of *Helicoverpa armigera* on chickpea after third spray (2003-04)

Sl. No.	Treatments	Larval mortality (%)		
		5 DAS	10 DAS	15 DAS
1	Dharwad isolate	45.15bc (42.22)	54.85b (47.80)	51.52a (45.88)
2	Gulbarga isolate	54.85ab (47.80)	64.55ab (53.48)	61.21a (51.48)
3	Coimbatore isolate	51.52ab (45.88)	61.21ab (51.48)	58.18a (49.73)
4	BPM isolate	35.45c (36.52)	41.82c (40.29)	35.45b (36.52)
5	PCI isolate	35.45c (36.52)	40.00c (39.23)	35.45b (36.52)
6	RPP	61.63a (51.72)	67.43a (55.26)	60.00a (50.77)
7	Untreated check	03.36d (12.00)	09.70d (18.15)	10.00c (18.44)

DAS: Days after spray
BPM- Bio-Pest Management Ltd. Bangalore
PCI- Pest Control India Ltd. Bangalore
RPP- Recommended Package of Practice
Figures in the parentheses are the arc sine transformed values
Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.05)

thereafter from 10 DAS to 15 DAS, indicating the loss in the virulence of the treatments after 10 days. The larval mortality ranged from 3.36 to 61.63, 9.70 to 67.43 and 10.00 to 60.00 per cent at 5, 10 and 15 days after the initiation of third spray, respectively. The superiority of Gulbarga and Coimbatore isolates was further confirmed even after the third spray with higher larval mortality (54.85, 64.55 and 61.21 per cent by Gulbarga isolate and 51.52, 61.21 and 58.18 per cent by Coimbatore isolate at 5, 10 and 15 days after the spray, respectively) being on par with RPP. Dharwad isolate was as effective as Gulbarga and Coimbatore isolates at all the days after spraying. BPM and PCI isolates were inferior to Gulbarga and Coimbatore isolates in reducing larval mortality.

4.5.2.4 After fourth spray

The status of different isolates in reducing the larval population presented in table 52 revealed that at 5 DAS, Gulbarga and Coimbatore isolates recorded 54.85 per cent larval mortality and they were on par with RPP (64.65). The efficacy of Dharwad (45.45), BPM (38.78) and PCI (35.45) isolates remained same. Ten days after spraying, Gulbarga and Coimbatore isolates proved inferior to RPP (81.11%), but on par with Dharwad (54.85%) isolate. While, the efficacy of BPM (44.85%) and PCI (40.00%) isolates remained same as they were statistically on par with each other after 15 DAS. Gulbarga (61.21%), Coimbatore (58.18%) and Dharwad (51.52%) isolates were equally effective in recording the larval mortality and they were significantly superior to BPM (38.79%) and PCI (35.45%) isolates. However, RPP recorded highest larval mortality of 71.67 per cent being significantly superior to all the NPV isolates included in the study.

Table 52. Efficacy of HaNPV isolates in the IPM of *Helicoverpa armigera* on chickpea after fourth spray (2003-04)

Sl. No.	Treatments	Larval mortality (%)		
		5 DAS	10 DAS	15 DAS
1	Dharwad isolate	45.45bc (42.22)	54.85bc (47.80)	51.52b (45.88)
2	Gulbarga isolate	54.85ab (47.80)	64.55b (53.48)	61.21b (51.48)
3	Coimbatore isolate	54.85ab (47.80)	61.21b (51.48)	58.18b (49.73)
4	BPM isolate	38.78bc (38.52)	44.85cd (42.03)	38.79c (38.52)
5	PCI isolate	35.45c (36.52)	40.00d (39.23)	35.45c (36.52)
6	RPP	64.65a (53.48)	81.11a (64.25)	71.67a (57.86)
7	Untreated check	06.67d (12.29)	06.67e (12.29)	10.00d (18.44)

DAS: Days after spray
Dharwad isolate BPM- Bio-Pest Management Ltd. Bangalore
PCI- Pest Control India Ltd. Bangalore
RPP- Recommended Package of Practice
Figures in the parentheses are the arc sine transformed values
Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.05)

4.5.2.5 Pod damage, yield and Economics

The pod damage and yield recorded is stated in Table 53. The pod damage in different treatments ranged from 8.07 to 31.78 per cent. Gulbarga and Coimbatore isolates were significantly more effective in recording lower per cent pod damage (10.82 and 10.94, respectively) than other isolates, and these two are on par with each other. Whereas, BPM (20.30) and PCI (21.02) isolates were significantly least effective than other three isolates. However, RPP recorded lowest pod damage of 8.07 per cent being significantly superior to all other treatments.

Gulbarga (7.56q/ha) and Coimbatore (7.45q/ha) isolates were significantly superior to BPM (5.33q/ha) and PCI (5.17 q/ha) isolates but on par with Dharwad (6.32q/ha) isolate and RPP (8.33q/ha) in recording the grain yield. Further, Gulbarga (2.88) and Coimbatore (2.80) isolates recorded higher IBCR followed by Dharwad (2.20) isolate. The performance of PCI (0.33) and BPM (0.30) remained same as observed during the first year. However, RPP remained superior in recording higher IBCR of 4.81 as compared to other treatments.

4.5.3 Pooled analysis of two years

The pooled analysis of both the years revealed the same trend in larval reduction (Table 54 to 57). Among the various isolates included in the study, the performance of Gulbarga and Coimbatore isolates was better in terms of their ability in recording higher larval mortality during all the sprays. BPM and PCI isolates recorded least per cent larval mortality as compared to other isolates.

The data presented on pod damage, yield and economics (Table 58) revealed the superiority of Gulbarga (11.11%) and Coimbatore isolates (11.88%) in recording lower pod damage. BPM (20.63%) and PCI (21.73%)

Table 53. Effect of HaNPV isolates on pod damage and yield of chickpea (2003-04)

Sl. No.	Treatments	Pod damage (%)	Yield (q/ha)	IBCR
1	Dharwad isolate	14.75c (24.17)	6.32ab	2.20
2	Gulbarga isolate	10.82d (16.22)	7.56a	2.88
3	Coimbatore isolate	10.94d (19.31)	7.45a	2.80
4	BPM isolate	20.30b (26.78)	5.33b	0.33
5	PCI isolate	21.02b (27.28)	5.17b	0.30
6	RPP	08.07e (16.52)	8.35a	4.81
7	Untreated check	31.78a (34.32)	3.45c	-

BPM- Bio-Pest Management Ltd. Bangalore
PCI- Pest Control India Ltd. Bangalore
RPP- Recommended Package of Practice
IBCR – Incremental Benefit Cost Ratio
Figures in the parentheses are the arc sine transformed values
Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.05)

Table 54. Efficacy of HaNPV isolates in the IPM of *Helicoverpa armigera* on chickpea after first spray (Pooled data for two years)

Sl. No.	Treatments	Before spray	Larval mortality (%)		
			5 DAS	10 DAS	15 DAS
1	Dharwad isolate	1.25a	34.34c (35.85)	48.48c (44.14)	43.61cd (41.32)
2	Gulbarga isolate	1.25a	44.89b (42.07)	62.26b (52.12)	56.12b (48.50)
3	Coimbatore isolate	1.20a	43.15b (41.15)	60.13b (50.83)	53.83bc (47.18)
4	BPM isolate	1.40a	31.62c (34.20)	41.19c (39.93)	34.45d (35.97)
5	PCI isolate	1.20a	28.71c (32.39)	39.68c (39.06)	34.67d (36.09)
6	RPP	1.30a	61.46a (51.65)	79.71a (63.22)	71.07a (57.48)
7	Untreated check	1.25a	04.47d (12.25)	04.62d (12.39)	7.80e (16.22)

DAS: Days after spray
BPM- Bio-Pest Management Ltd. Bangalore
PCI- Pest Control India Ltd. Bangalore
RPP- Recommended Package of Practice
Figures in the parentheses are the arc sine transformed values
Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.05)

Table 55. Efficacy of HaNPV isolates in the IPM of *Helicoverpa armigera* on chickpea after second spray (Pooled data for two years)

Sl. No.	Treatments	Larval mortality (%)		
		5 DAS	10 DAS	15 DAS
1	Dharwad isolate	43.61bc (41.32)	51.40bc (45.80)	46.82b (43.17)
2	Gulbarga isolate	51.40b (45.80)	60.58b (51.12)	56.69ab (48.85)
3	Coimbatore isolate	49.29b (44.60)	59.35b (50.42)	53.67ab (47.12)
4	BPM isolate	34.95c (36.27)	43.13cd (41.03)	35.13c (36.33)
5	PCI isolate	33.43c (35.30)	41.34d (39.39)	35.13c (36.33)
6	RPP	63.89a (53.07)	70.23a (56.91)	63.32a (52.71)
7	Untreated check	04.47d (12.25)	05.02e (12.92)	06.67d (15.00)

DAS: Days after spray
BPM- Bio-Pest Management Ltd. Bangalore
PCI- Pest Control India Ltd. Bangalore
RPP- Recommended Package of Practice
Figures in the parentheses are the arc sine transformed values
Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.05)

Table 56. Efficacy of HaNPV isolates in the IPM of *Helicoverpa armigera* on chickpea after third spray (Pooled data for two years)

Sl. No.	Treatments	Larval mortality (%)		
		5 DAS	10 DAS	15 DAS
1	Dharwad isolate	42.58bc (40.74)	53.71b (47.12)	47.43a (43.51)
2	Gulbarga isolate	53.71ab (47.12)	63.62a (52.89)	58.56a (49.95)
3	Coimbatore isolate	51.40ab (45.80)	60.86ab (51.30)	55.76a (48.33)
4	BPM isolate	33.84c (35.55)	41.19c (39.93)	34.39b (35.91)
5	PCI isolate	33.28c (35.24)	40.00c (39.23)	32.73b (34.88)
6	RPP	59.79a (50.65)	66.22a (54.45)	58.34a (49.78)
7	Untreated check	05.02d (12.92)	09.85d (18.34)	08.34c (16.74)

DAS: Days after spray
BPM- Bio-Pest Management Ltd. Bangalore
PCI- Pest Control India Ltd. Bangalore
RPP- Recommended Package of Practice
Figures in the parentheses are the arc sine transformed values
Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.05)

Table 57. Efficacy of HaNPV isolates in the IPM of *Helicoverpa armigera* on chickpea after fourth spray (Pooled data for two years)

Sl. No.	Treatments	Larval mortality (%)		
		5 DAS	10 DAS	15 DAS
1	Dharwad isolate	39.39c (38.88)	53.26bc (46.89)	48.26bc (44.03)
2	Gulbarga isolate	52.43b (46.38)	63.94b (53.07)	58.94b (50.13)
3	Coimbatore isolate	54.09b (47.35)	63.11b (52.59)	57.43b (49.23)
4	BPM isolate	37.73c (37.88)	45.76cd (42.59)	41.06c (39.87)
5	PCI isolate	36.06c (36.93)	41.67d (40.22)	37.73c (37.88)
6	RPP	64.83a (53.61)	81.39a (64.45)	74.17a (59.47)
7	Untreated check	08.34d (16.74)	05.00e (12.92)	08.34d (16.74)

DAS: Days after spray
BPM- Bio-Pest Management Ltd. Bangalore
PCI- Pest Control India Ltd. Bangalore
RPP- Recommended Package of Practice
Figures in the parentheses are the arc sine transformed values
Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.05)

Table 58. Effect of HaNPV isolates on pod damage and yield of chickpea (Pooled data for two years)

Sl. No.	Treatments	Pod damage (%)	Yield (q/ha)	IBCR
1	Dharwad isolate	17.40c (23.50)	6.60ab	2.39
2	Gulbarga isolate	11.11d (19.46)	7.44ab	2.88
3	Coimbatore isolate	11.88d (20.18)	7.37ab	2.83
4	BPM isolate	20.63b (26.99)	5.21bc	0.33
5	PCI isolate	21.73b (27.76)	4.90cd	0.29
6	RPP	08.26d (16.74)	8.62a	5.20
7	Untreated check	32.72a (34.94)	3.33d	-

BPM- Bio-Pest Management Ltd. Bangalore
PCI- Pest Control India Ltd. Bangalore
RPP- Recommended Package of Practice
IBCR – Incremental Benefit Cost Ratio
Figures in the parentheses are the arc sine transformed values
Means followed by same alphabet in vertical column do not differ significantly by DMRT (P=0.05)

isolates recorded highest pod damage and on par with each other. However, RPP recorded least per cent pod damage (08.26) as compared to other treatments. Similar trend was found as far the IBCR is concerned. Gulbarga (2.88) and Coimbatore (2.83) recorded higher IBCR followed by Dharwad isolate (2.39). However, the IBCR in the crop treated with RPP was highest (5.20) compared to other isolates.

Discussion

V. DISCUSSION

The principle method of *Helicoverpa armigera* (Hubner) control by use of synthetic chemicals has led to an increasing emphasis on the need for the development of Integrated Pest Management because of control and environmental concerns, *viz.,* development of resistance to insecticides, pest resurgence, ecological backlash, economic and energy conservation concerns and finally to provide sustainability for the management systems. Biological control as a major component in IPM would offset the disadvantages associated with chemical control. Nuclear Polyhedrosis Virus, as a biopesticide has paved the way for an amicable control apart from ecofriendlyness and compatibility with other methods of management practices. In addition, the development of resistance to biopesticides is very meager unlike insecticides because of non-toxic action. For successful exploitation of HaNPV, it should be easily mass produced, less expensive, effective under field conditions and safer to nontarget biota.

The virus is often rapidly inactivated by environmental factors like UV rays, pH of the leaf surface, leaf exudates etc., and for maximum effectivity it must be ingested by the host insect prior to degradation. Further, the time lag between ingestion of lethal dose and death under field conditions is to be reduced to minimum possible time so as to contain the plant damage to minimum. Hence, there is a necessity to investigate on the most efficient viral isolate by screening both under invivo and invitro conditions against *H. armigera.*

During the present investigation, genomic characterization of the different isolates with restriction endonuclease enzymes, characterization interms of virulence against the different geographical populations of the host, effect of different cotton species with Bt cottons on the virulence of HaNPV, persistence of the virus in soil condition under laboratory and field conditions and evaluation of the prominent isolates in the Integrated Pest Management (IPM) of *H. armigera,* were carried out in chickpea under field conditions.

5.1 Characterization of different geographical isolates

5.1.1 Genetic diversity of different HaNPV isolates

The DNA restriction analysis of different HaNPV isolates revealed that there exists a diversity among the various isolates as indicated in Fig 1. All the isolates tested form in to two major groups. The first cluster comprises Dharwad, Kalpavruksha, Coimbatore, BPM, PDBC and BPL isolates with a genetic similarity of around 0.57. Whereas, the second major cluster constituted three isolates *viz.,* Gulbarga, Raichur and Guntur isolates with a similarity co-efficient of 0.54.

The variation in the genome level may be due to the difference in the sequence of nucleotides, insertion of host DNA into viral genome (Shapiro, *et al.,* 1991) and deletion of part of the viral genome (Heldens *et al.,* 1996). Similar genetic diversity in the NPV isolates were reported by Geetha and Rabindra (2000); Somasekar, *et al.* (1993); Manickvasagam (2003) and Arora *et al.* (1997), who concluded the existence of genetic diversity among the HaNPV isolates collected from different regions. It is interesting to note that isolates from Gulbarga, Raichur and Guntur not only grouped in to a single cluster but also possessed highest genetic similarity. Interestingly, all these isolates were from regions, which have almost common cropping pattern, weather parameters in terms of temperature, relative humidity and rainfall. Perhaps, these factors might have strong bearing on their evolution and hence might be sharing more nucleotide similarity when compared to isolates from other locations.

It is clear from the results of virulence data that isolates from Gulbarga and Coimbatore are more virulent compared to others, while also being genetically distinct. Hence, these two isolates form two genetically distinct sources of virulence for field application; suitable in rotation of virus in IPM for delaying development of resistance.

5.1.2 Characterization of Biological activity

An effort was made to evaluate the biological activity of HaNPV isolates obtained from different sources against *H. armigera* population collected from different geographical locations *viz.*, Dharwad, Raichur, Nagpur, Gulbarga and Guntur. Different isolates exhibited variations in their virulence interms of differential LT_{50} and LC_{50} values (Table 3-7). Further, the population collected from five locations responded differently in their susceptibility against the isolates. As entomopathogenic viruses must undergo several cycles of replication within susceptible insects, the lethal incubation period and the lethal concentrations for different HaNPV isolates varied greatly.

In the present study, comparative infectivityness of geographical strains of HaNPV against the populations of *H. armigera* collected from different host plants, showed wide variation in its infectivity interms of LT_{50} and LC_{50} values. It is demonstrated that the heterogeneity among the isolates to populations collected from single location or different locations of *H. armigera* is a common phenomenon and thus developed means to identify the most virulent isolates. The most efficient HaNPV isolates obtained were the isolates collected from Gulbarga and Coimbatore. These two isolates consistently recorded lower LT_{50} and LC_{50} values against *H. armigera* irrespective of the location and host. Similarly, the isolates obtained from the private firms performed indifferently and required higher lethal concentration and lethal time to cause mortality against *H. armigera,* collected irrespective of the location and host.

The variation in the activity of different isolates may be due to different reasons. Inherent genetically controlled factors may logically be an important reasons. The other reasons may be that the different isolates had different number of passages in the host, either under natural conditions or in the laboratory. It will be worthwhile to look at the biological activity of these isolates after a fairly large but fixed number of passages in *H. armigera* under laboratory conditions. In the earlier studies, the variations in the biological activity of different isolates was very much confirmed. HaNPV from Ooty isolate recorded lowest LC_{50} value (Anonymous, 1990). Somasekar *et al.* (1993) confirmed that Ooty isolate had the lowest LC_{50} values with Coimbatore isolate recording almost similar LC_{50} values. It is established fact that geographical races of the same NPV may vary in their virulence (Chauthani *et al.,* 1968, Abdul-Nasar *et al.,* 1980, Shapiro and Roberston, 1991) and among the 34 isolates of *H. armigera* NPV studied by Shapiro and Ignoffo (1970), the LC_{50} values ranged from 0.70 to 39.00 POB per mm^2 of the surface treated diet.

The present findings are in conformity with the results of Gopali (1998), who concluded the virulence of Gulbarga and Coimbatore isolates of HaNPV interms of lower LC_{50} and LT_{50} values against *H. armigera.* He concluded that the strainal variations may be due to climatic conditions prevailing in these areas. Gulbarga and Coimbatore regions experience continuous selection of viruses to higher temperature and lower relative humidity prevailing in these locations, which might have led to evolution of stronger or virulent isolates. However this hypothesis lack published evidences.

The inferiority of the HaNPV produced by private firms is in conformity with the results of Arora *et al.* (1997) from Ludhiana (Punjab) who screened HaNPV isolates of Punjab for their virulence in comparison with the isolates collected from the private firms from Bangalore (Karnataka) and Godhra (Gujarat). The three local isolates were collected from PAU (isolate maintained at Punjab Agricultural University), Mudki and Karaiwala. The LC_{50} values of all the isolates ranged between 0.35 to 1.12 LE/l. The native isolates were comparatively virulent against the pest, which is indicated by their lower LC_{50} values than the two commercial formulations obtained from Bangalore and Godhra. Among the five isolates, PAU isolate was most virulent than the others. The Godhra isolate was least virulent which recorded 5-6 times more LC_{50} value. The LT_{50} values also confirmed the virulence of the native isolates of Ludhiana, which recorded shorter LT_{50} values at all the concentrations tested.

Hughes *et al.* (1983) compared the time mortality response of *H. zea* to 14 isolates of HzNPV and identified six activity classes based on median survival time. Manickvasagam (2003) also detected the variation in the virulence of two geographical isolates of *Spilosoma obliqua* (Walker) NPV. He concluded that Punjab isolate of SoNPV was 287 times more virulent than Maharasthra isolate, though there was no difference in the DNA banding pattern.

Since, *H. armigera* has vast host range, there exists a wide variation among the different isolates, which is mainly attributed to the greater selection pressure between the host and the pathogen. Continuous feeding of *H. armigera* on different host plants has created the selection pressure between *H. armigera* and HaNPV, which must have resulted in the variation in the susceptibility to different populations. Shivaprakasam and Rabindra (1997) opined that absence of variation in the susceptibility of *Bombyx mori* to BmNPV, where they mainly attributed to the poor selection pressure between the host and the pathogen. Continuous rearing of silkworm and domestication over years under controlled environmental conditions on a single host plant i.e.

mulberry might have eliminated the selection pressure between the silkworm and BmNPV, which resulted in the absence of variation in NPV susceptibility. Somasekar (1991) also reported the absence of variation among the isolates of BmNPV in their virulence.

The present investigations have revealed the existence of several geographical isolates of HaNPV assembled through limited efforts. Apart from the significant difference of HaNPV of Gulbarga and Coimbatore interms of LT_{50} and LC_{50} values, there were also marked variation between the other isolates considered under the study.

H. armigera is polyphagous, occurring on a variety of crops (Chari *et al.*, 1990) with diverse biochemical makeup. *H. armigera* occurs right from Himachal Pradesh to Kanya Kumari in different agro-ecosystems and there is every possibility that there will be a wide variety of these isolates occurring in nature with many useful genes. Even though, the development of resistance to NPV is not a major problem, there are reports of lepidopteran insect populations exhibiting varied levels of susceptibility. The populations of *Pieris brassicae* (Linnaeus), *Epiphyas postvittana* (Walker) and *Pthorimaea operculella* (Zeller) surviving natural epizootics of GV, NPV and GV respectively are known to have developed resistance to virus (Briese and Podgwaite, 1985). This suggests that repeated inundative application of same virus in an eco-system may lead to selection of a population tolerant to virus. In such a contest, the availability of alternate isolates of different genetic makeup but of equal potency is of relevance. However, more intensive studies are necessary to find out the possible existence of cross-resistance.

In general, population collected from the same place, where the isolates were collected, showed less susceptibility to the local population as compared to the other populations. To substantiate this statement, Gulbarga isolate recorded higher LT_{50} and LC_{50} (107.67 h and $2.94X10^4$ POB/ml) values against the population from Gulbarga compared to its higher infectivity against the populations from Dharwad (105.86 h and $1.89X10^4$ POB/ml), Raichur (98.09 h and $1.59X10^4$ POB/ml), Nagpur (103.43 h and $1.83X10^4$ POB/ ml) and Guntur (98.04 h and $1.86X10^4$ POB/ml). Same observation was made in the virulence of different isolates against the population of the same area. This clearly indicates that the HaNPV from other locations can be more effective against the local population. The superiority of Gulbarga and Coimbatore isolates was consistent even against *H. armigera* collected from various hosts. It indicates that these isolates were equally effective against the *H. armigera* on cotton (Guntur, Raichur and Nagpur), pigeonpea (Gulbarga) and chickpea (Dharwad). Further, the HaNPV isolates were comparatively more effective against the *H. armigera* collected from Gulbarga, which represents pigeonpea ecosystem. The average LT_{50} and LC_{50} values for Gulbarga population was comparatively less irrespective of the isolates tested. The *H. armigera* collected from Gulbarga in pigeonpea ecosystem, characterized by indiscriminate use of pesticides for its management must have developed resistance to insecticides and rendered susceptibility towards the biopesticides like NPV. The susceptibility of *H. armigera* NPV to the insecticides resistant population of *H. armigera* has been documented by Basavana Goud *et al.* (1997) in cotton against cotton bollworm, where the LC_{50} values were less for resistant populations than in susceptible strains. The practical utility of the present finding on this aspect is in conformity with the results of Listov and Nestervo (1976), Rud and Bellonick (1984) and Rabindra and Jayaraj (1985), who reported that entomopathogens could break insecticide resistance, when properly introduced in to the population. Based on the relative efficacy of HaNPV isolates against different populations, it may be concluded that NPV, apparently becomes an obvious choice and hence the usage of virulent isolate can become viable proposition in mitigating the pesticide resistance problem in *H. armigera*.

From the present findings it can be concluded that Gulbarga and Coimbatore isolates could be utilized for further mass multiplication from time to time and for management of the pest effectively.

5.1.3 POB yielding capacity of different isolates

Since NPV can be produced only in living larvae, the cost of production can be quite high as *H. armigera* requires isolated rearing. The yield of virus from a single caterpillar can therefore be an important factor in the cost of NPV production. If a viral isolate is capable of producing a larger number of bigger polyhedra with an increased number of virion per POB per cell and thereby per larvae, which is a possible expression of genotypic variations (McIntosh *et al.*, 1987), the total number of larvae required to produce a certain amount of virus would be fewer, enabling a reduction in production cost. An important investigation would therefore be of the polyhedra yield in different viral isolates. Hence, an intensive search was made to yield a viral strain with more number of POBs. All the HaNPV isolates were screened for their ability to yield the progeny virus in the second, third, fourth and fifth instar larvae of *H. armigera* (Table 10 to13).

The NPV can be multiplied at all the stages of host under consideration but recovery varied with the stage. Irrespective of the isolate, the total viral yield produced from 2nd and 3rd instar was less compared to fourth instar larvae. Further, fourth instar larvae registered highest larval mortality and higher quantity of total viral yield compared to other instars. Though the viral yield per larvae was maximum in fifth instar larvae and the number of larvae required to yield one LE was less. But there was lesser larval mortality, which contributed less viral yield to the total production. As the larvae become aged the chemical constituents in the body vary resulting in increased haemoral defence mechanism to the microbial infection (Boman, 1981).

Among the various isolates tested for their ability to produce POB yield, Gulbarga and Coimbatore isolates yielded maximum number of virus in all the instars of *H. armigera.* Dharwad isolate was the next potent isolate to yield viral yield in all the instars. These isolates recorded maximum mortality due to the virus, which contributed to the final viral yield. On the contrary, the isolates which failed to induce mortality, also failed to contribute to the total viral yield. The isolate obtained from Bio Pest Laboratories and Kalpavruksha Biosystem recorded least per cent larval mortality and yielded minimum quantity of virus. The average yield of all the instars is presented in Table 14. Highest yield was registered by Gulbarga and Coimbatore isolates (8.91 and $7.51X10^{10}$ POBs, respectively). Further these two isolates recorded higher LE and more number of POB per larva. The total number of larvae required to yield one LE is also minimum in these isolates. It is thus concluded that of the ten isolates, Gulbarga and Coimbatore isolates caused higher larval mortality and produced maximum amount of progeny virus. These findings are inconfirmity with the results obtained in the present study under first objective, where Gulbarga and Coimbatore isolates recorded lesser LT_{50} and LC_{50} values and proved to be virulent isolates. Gopali (1998) harvested higher quantity of viral yield per larvae ($2.81X10^9$ POB) in fourth instar larvae, which is in accordance with the present investigation. Hussain *et al.* (2002) opined that the old larvae (5^{th} and 6^{th} instar) of *Spodoptera litura* could yield $3.5X10^9$ POBs per larva and the young larvae (3^{rd} and 4^{th} instar) yield $7.95X10^8$ POBs per larva, suggesting that the dead cadavers have enough inoculum for further spread of disease. Therefore, fourth instar larvae are optimum for maximization of viral yield (Narayanan, 1985 and Jayaraj and Sathiah, 1993). Further, Gulbarga and

Coimbatore isolates could be used to produce higher quantum of viral yield for commercial production.

5.2 Influence of cotton species on the efficacy of HaNPV isolates

Helicoverpa armigera a major pest on cotton, causing extensive yield loss, has been successfully controlled on cotton with the nuclear polyhedrosis virus (NPV) (Dhandapani *et al.,* 1987). Not much information is available on the interaction of host plant varieties and NPV control. In India, *H. armigera* occurs on all the three cultivated species of cotton *viz., Gossypium barbedense, G. hirsutum* and *G. arboreum* and also on all the Bt cotton hybrids. The present investigations were targeted to study the influence of cotton species on the NPV mortality of *H. armigera.*

Decrease in the activity of *H. armigera* NPV on cotton foliage has been well documented (Bullock, 1967). Monochromatic-ultraviolet rays have been shown to inactivate HaNPV. Early work done with dew from cotton leaf surface showed that dew from cotton was alkaline to litmus and phenolphthalein and contain calcium, magnesium and carbon dioxide along with lesser concentrations of sulphur, iron, aluminium and silica (Smith, 1923).

Since persistence studies showed that some viral activity was lost on cotton plant, different cotton species including the Bt cotton hybrids were evaluated for their influence on the infectivity of HaNPV isolates.

The total larval mortality registered on MECH-184 Bt was 100 per cent, of that 50 per cent larvae showed the typical viral symptoms (Table 16). Whereas, the same Bt hybrid recorded 65 per cent larval mortality (Table 15) without HaNPV spray at 10 DAS. The larval mortality due to Bt toxin on NPV sprayed plant was reduced. This may be due to the fact that the effect of Bt on NPV sprayed plants must have masked by the NPV symptoms and the larvae may have died due to NPV. The other explanation is that initially Bt toxin must have rendered the insect to be more susceptible to NPV infection and ultimately the larvae must have died displaying NPV symptoms. However, the total mortality was considerably higher on HaNPV sprayed plants compared to the unsprayed plants. This indicates that the total mortality in the NPV sprayed plants was due to the additive effect of Bt toxin and the NPV infection.

Among the various species of cotton plants, the HaNPV sprayed on Bt cottons recorded highest larval mortality due to the virus at all the intervals of observation. Among the Bt cotton hybrids the HaNPV sprayed on MECH-184Bt recorded highest number of virosed larvae (50 % larval mortality) at 10 days after the spray followed by MECH-162 Bt, MECH-12 Bt, RCH-20 Bt and RCH-144Bt, which harboured more number of larvae succumbed to viral infection and remained statistically on par with each other.

All the three cotton species, one intraspecific hybrid and the non-Bt versions of Bt hybrids could not exhibit larval mortality on the HaNPV unsprayed plants (Table-15). When these plants were sprayed with HaNPV isolates, the total larval mortality observed was mainly due to the NPV infection, since all the dead larvae recorded presence of viral particles in their midgut.

Among the three species, and one intraspecific hybrid, B-82 registered highest larval mortality followed by DHH-11 irrespective of the HaNPV isolates tested.

In general, among the ten isolates, the plants sprayed with Gulbarga and Coimbatore isolates of HaNPV recorded highest total larval mortality and mortality due to the virus. The total larval mortality on the plants sprayed with Gulbarga and Coimbatore isolates ranged from 03.33 to 100.00 per cent (Table 17 & 20) where, the larval mortality due to virus ranged from 03.33 to 50.00 per cent in both the cases at 10 DAS. This indicates the superiority of these soil was kept outdoors. Further the larval mortality due to *H. armigera* NPV declined from 88.35 to 60.00 per cent and 90.00 to 63.35 per cent, when the HaNPV treated red soil was kept outdoors and indoors, respectively. The virulence of Gulbarga isolate of HaNPV is not much affected when stored under refrigerator, where the larval mortality due to virus suspension declined from 88.35 to 83.35 and 90.00 to 86.00 per cent, when the NPV treated black and red soil was stored in refrigerator. The Gulbarga HaNPV treated black soil kept indoors retained its 50 per cent activity even up to 12.92 months as compared to only 10.57 months when the same soil was kept under field conditions. Whereas, the NPV treated red soil retained its 50 per cent activity even up to 17.13 months as compared to 15.70 months when the same soil was kept outdoors. The computed half-life for HaNPV stored at refrigerator was 96.17 and 124.40 months for black and red soils, respectively. The results revealed that the soil kept outdoor can loose its infectivity at a much faster rate than the soil kept indoors.

The results obtained on the larval mortality due to the NPV infection of Coimbatore isolate of HaNPV kept outdoors, indoors and in refrigerated conditions remained same. However, compared to Gulbarga isolate, the larval mortality obtained due to the Coimbatore NPV infection when the soil was kept outdoors, indoors and in refrigerator conditions declined very fast. This clearly reveals that the retention of virulence of Gulbarga isolate is quite longer than that of Coimbatore isolate.

The larval mortality due to the infection of Coimbatore NPV treated black soil kept outdoors and indoors declined from 85.00 to 35.00 per cent and 85.00 to 50.00 per cent, respectively. While, it was from 85.00 to 50.00 per cent and 85.00 to 53.35 per cent, when Coimbatore NPV treated red soil was kept outdoors and indoors, respectively. Further, the computed half-life of

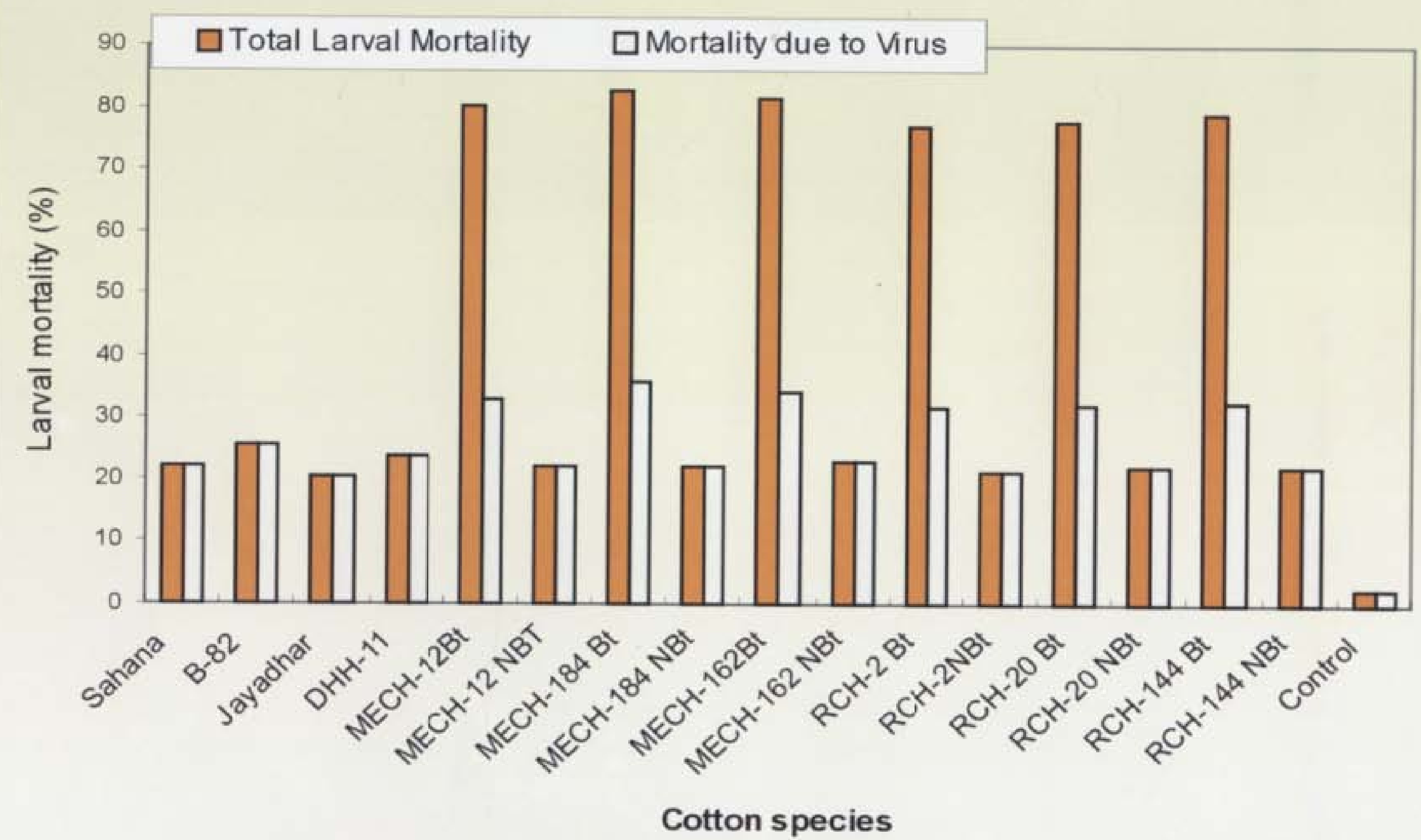

Fig. 2: Influene of cotton species on the efficacy of HaNPV isolates

the isolates, the virus sprayed on Bt cotton plants recorded higher number of virosed larvae. This indicates that the larvae fed on NPV sprayed Bt cottons were more prone to viral infection than that of the larvae fed on other plants. This reveals that Bt toxin might have increased the efficacy of viral infection.

No research work has been carried out in this direction to compare the information gathered during the present investigation. However, the studies carried out by Dhandapani *et al.* (1993) confirmed the results of present study where, they concluded that among the three species of cotton, the mortality of *H. armigera* larvae to NPV was significantly higher on *G. barbadense* than on *G. hirsutum* and *G. arboreum.* They opined that a higher leaf consumption by *H. armigera* larvae on *G. barbadense* than other cultivars would have increased the virus acquisition leading to increased kill at a shorter time. Hence, there is a better scope for NPV application on *G. barbadense* than on other species. In the present investigation among the three species, *G. barbadense* (B-82) recorded higher percent larval mortality of larvae than Sahana (*G. arboreum)* and Jayadhar (*G. hirsutum).* Similarly, Nathagopal (1987) reported that *G. barbadense* was more preferred by *H. armigera* than *G. hirsutum* and *G. arboreum.* Santharam and Jayaraj (1987) observed a higher mortality of *Spodoptera litura* due to NPV on *G. barbadense* than *G. hirsutum.*

There are no reviews available from the literature scan to indicate the influence of different Bt cotton hybrids on the efficacy of HaNPV isolates. However, Udikeri *et al.* (2003) reported the superiority of MECH-184Bt followed by MECH-162Bt and MECH-12Bt in recording significantly less number of *H. armigera* on cotton, which partly confirm the present findings. It is documented earlier that alkaline dew on cotton leaf surface was responsible for the inactivation of the virus. The leaf glands were responsible for high pH and cation concentration (Elleman and Entwistle, 1982) and the inactivation

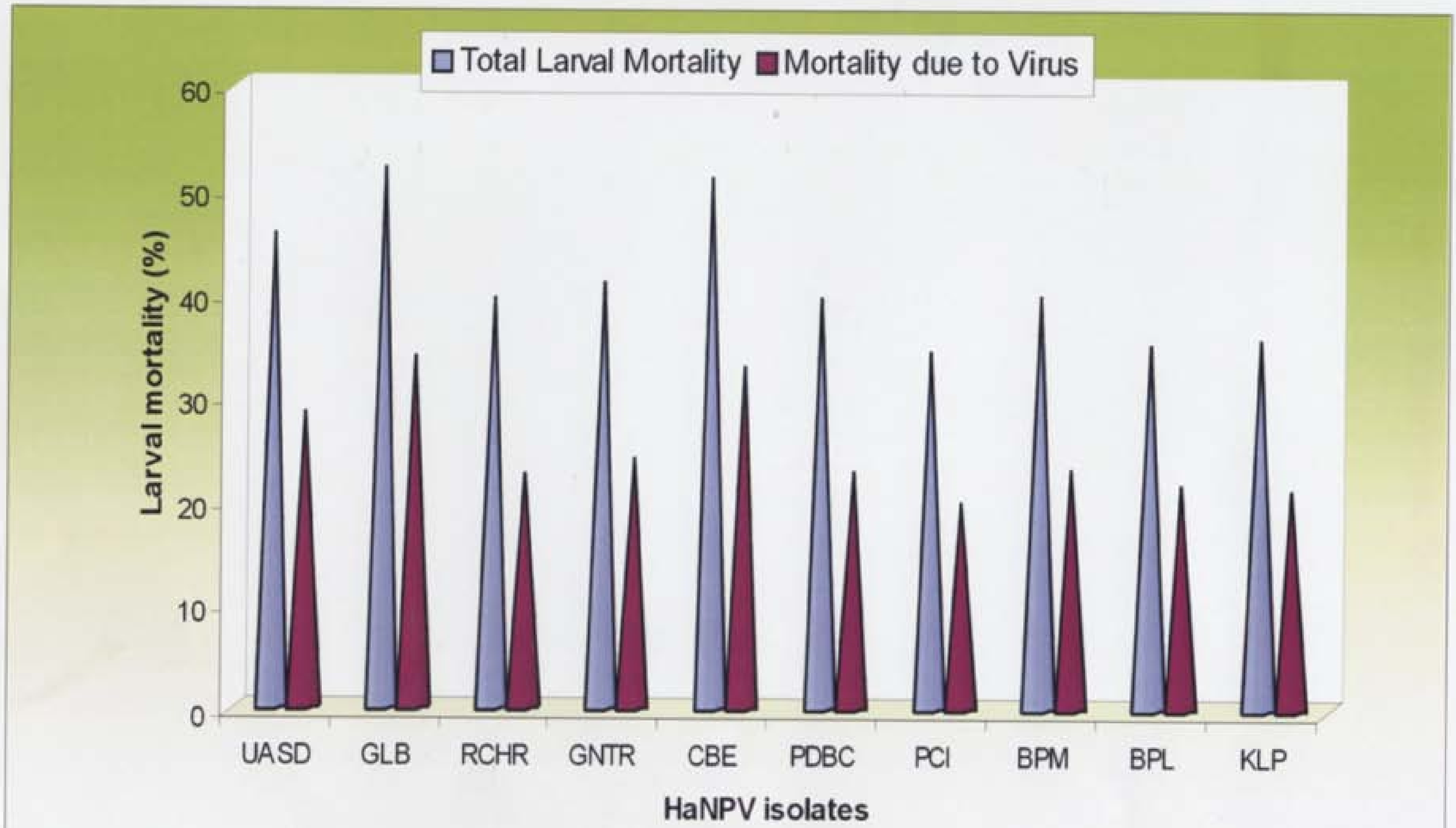

Fig. 3: Influence of cotton species on the efficacy of HaNPV isolates

might be due to presence of magnesium and calcium ions (Elleman and Entwistle, 1985a and b) on the cotton leaf. Further, the expression of Bt toxin was less in floral and fruiting structures compared to terminal foliage (Greenplate, 1999). In this background, the utilization of virulent HaNPV isolates on Bt hybrids has a better scope in reducing the larval population of *H. armigera* on Bt cotton.

Under challenging situation of growing cotton sustainabally with successful strategies for insect pest management, Bt transgenics with promising isolates of HaNPV offer a good opportunity. Development of specific protection packages involving HaNPV for Bt cotton hybrids for different localities and pest situations seem to be essential for better utilization of transgenic technology with viral biopesticides.

5.3 Persistence of HaNPV isolates in soils

There are number of studies on the persistence of nuclear polyhedrosis virus (NPV) and granulosis viruses (GV) in soil, but little is known about how long the virus can persist in different soils. Jaques (1967) believed that the NPV of the cabbage looper, *Trichoplusia ni*, persisted in the soil as polyhedra rather than as free naked virus rods because the virus occluded in polyhedra was more resistant to inactivation by physical and chemical agents. The polyhedra are also highly resistant to decomposition by microbial action (Jaques and Huston, 1969). In the present investigation, the HaNPV isolates obtained from Gulbarga and Coimbatore, which were proved to be effective interms of their biological activity, were further screened for their persistence in two soil types kept under field and laboratory conditions.

The per cent larval mortality due to Gulbarga virus infection declined from 88.35 to 38.35 per cent in 12 months when the virus treated black soil was kept outdoor, while it declined from 90.00 to 51.65 per cent when the same soil was kept outdoors. Further the larval mortality due to *H. armigera* NPV declined from 88.35 to 60.00 per cent and 90.00 to 63.35 per cent, when the HaNPV treated red soil was kept outdoors and indoors respectively. The virulence of Gulbarga isolate of HaNPV is not much affected when stored under refrigerator, where the larval mortality due to virus suspension declined from 88.35 to 83.35 and 90.00 to 86.00 per cent, when the NPV treated black and red soil was stored in refrigerator. The Gulbarga HaNPV treated black soil kept indoors retained its 50 per cent activity even up to 12.92 months as compared to only 10.57 months when the same soil was kept under field conditions. Whereas, the NPV treated red soil retained its 50 per cent activity even up to 17.13 months as compared to 15.70 months when the same soil was kept outdoors. The computed half-life for HaNPV stored at refrigerator was 96.17 and 124.40 months for black and red soils respectively. The results revealed that the soil kept outdoor can loose its infectivity at a much faster rate than the soil kept indoors.

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The larval mortality due to the infection of Coimbatore NPV treated black soil kept outdoors and indoors declined from 85.00 to 35.00 per cent and 85.00 to 50.00 per cent respectively. While, it was from 85.00 to 50.00 per cent and 85.00 to 53.35 per cent, when Coimbatore NPV treated red soil was kept outdoors and indoors respectively. Further, the computed half-life of

Coimbatore isolate in black soil kept outdoors and indoors was 10.07 and 12.30 months, respectively whereas, it was 13.33 and 15.24 months when the same virus was incorporated in red soil kept outdoors and indoors. Under refrigerated conditions, the calculated 50 per cent activity prolonged to be 78.98 and 109.81 months, when the Coimbatore NPV treated black and red soils were kept in refrigerator.

Overall, the HaNPV from Gulbarga persisted for a longer time compared to Coimbatore isolate irrespective of the soil in which they were incorporated. Between the two soils, red soil retained the activity for a longer time compared to the black soil (Fig. 4).

Adsorption of HaNPV polyhedra in two soil types was studied by Narayanan *et al.* (1987). It was found that the adsorption rate was higher in alfisols than vertisols. Similar reports on the persistence of *Spodoptera mauritia* (Biosduval) NPV in paddy soils was made by Nair and Jacob (1988) from Kerala and present results mostly agree with these studies.

The high amount of persistence in red soil may be because of the fact that the adsorption rate of polyhedra was higher in red soil than in black soil. Similar reports have been made by Narayanan *et al.* (1987) opined that adsorption rate of HaNPV was higher in alfisol than in vertisol.

The remarkable stability of baculoviruses in soil has been reported by several workers (Thomas *et al.*, 1972; Thompson and Scot, 1979; Evans and Harrap, 1982). The present studies proved the significant resistance of polyhedra to decomposition in a chemically and microbiologically complex environment like soil. Jaques and Huston (1969) found that polyhedral protein was highly resistant to microbial putrification. However, there are some proteolytic bacteria and fungi prevailing in the soil can degrade the polyhedra and make them inactive (Jaques and Huston, 1969). It is also concluded that

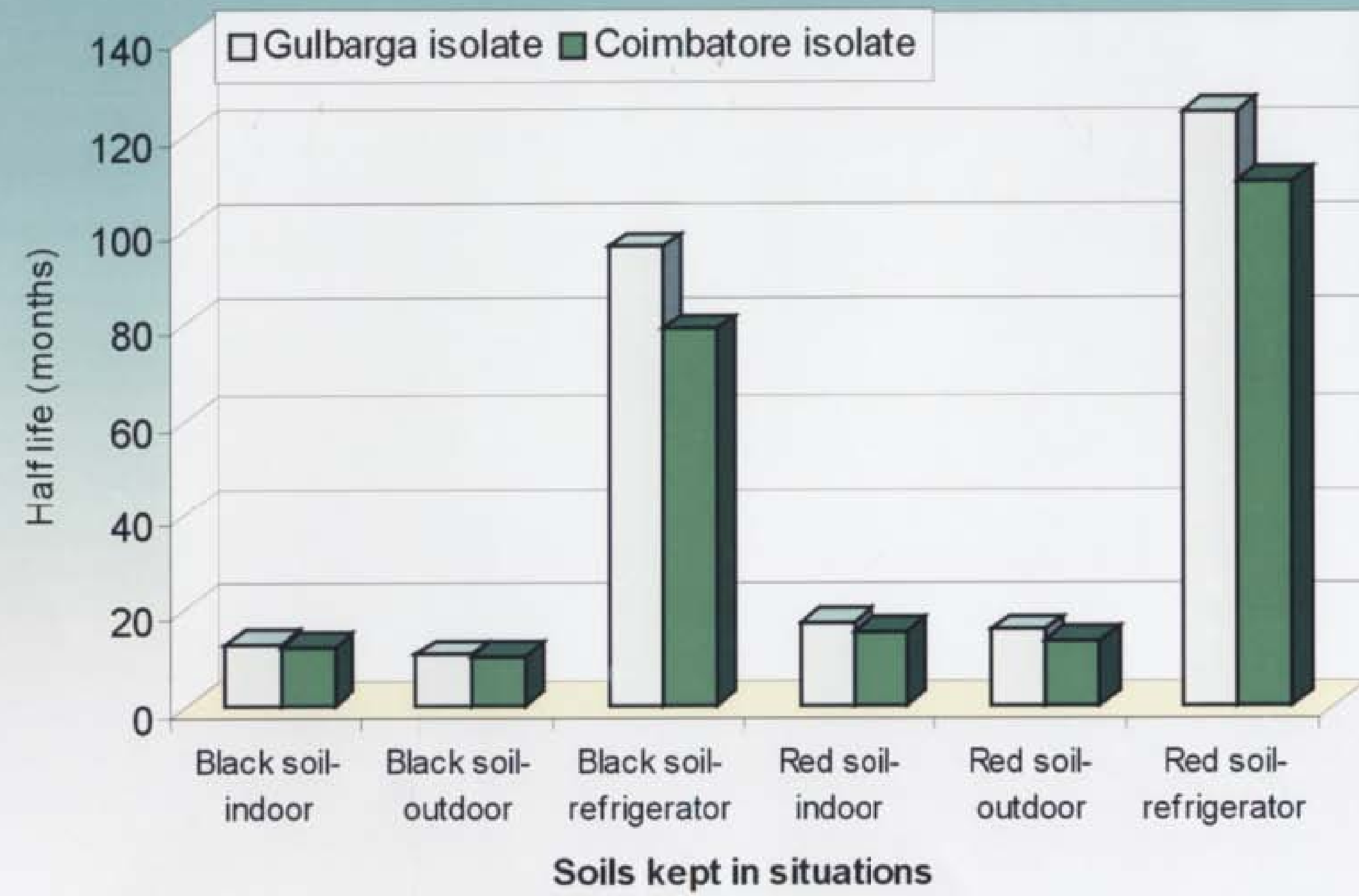

Fig. 4: Persistence of HaNPV isolates in soils

since these POBs are negatively charged they will be more adsorbed to the soil particles, which have more positively charged sites. It is also possible that the high silicon content of polyhedra (Estes and Faust, 1966) might contribute to its resistance to breakdown.

The estimated half-life of the virus in soil kept outdoors was less than the life of virus in soil kept indoors. This may be expected, since under field conditions, the soil is subjected to more direct action of environment factors like sunlight. Further, soil temperature under field conditions may rise to 40^{0}C and above in summer months and exposure to such a high soil temperature for prolonged periods can speed up the inactivation of the virus. Inactivation of baculoviruses was attributed to high field temperature and solar radiation particularly of UV spectrum (Gudauskas and Channerday, 1968; Ignoffo, 1968; Ignoffo and Hostetter, 1977). Hugar (1993) also conducted similar studies to investigate the persistence of *Mythimna separata* (Walker) NPV in red and black soil situations. He concluded that the virus treated black soil kept indoors could retain its 50 per cent activity even up to 12 months whereas, it was 9.00 months for the same soil when kept outdoors. The computed half-life of MsNPV was 13.20 months when the red soil treated NPV was kept indoor as compared to 13.00 months when the same soil was kept outdoors.

It is evident from these results that NPV of *H. armigera* can remain stable in soil without much loss of infectivity for a longer period even though it is exposed to the environment. The half life of 10.57 and 15.70 months for Gulbarga and 10.07 and 13.33 months for Coimbatore isolates in black and red soils under open field conditions is very useful for the field crops like cotton, pigeonpea, chickpea and other hosts of *H. armigera*, where the interval between successive crops rarely exceed 6 months. The virus deposited in soil from spray drift or the inoculum from the virosed larvae remains in the soil and serves as a source of inoculum for initiating early epizootics in subsequent seasons. The long self-life of the virus was also evident from the present results.

When the NPV isolate of Gulbarga and Coimbatore were stored in refrigerated condition for a varied length of periods, the rate of larval mortality was reduced from 88.35 to only 83.35 per cent for Gulbarga isolate in black and red soil whereas, it declined from 85.00 to 79.65 per cent and 85.00 to 82.00 per cent for Coimbatore isolate in black and red soils respectively. This shows that under refrigerator, the virulence of HaNPV can be retained over a longer period. However, it is assumed that over a period, when the pH of the medium is converted in to alkaline due to microbial decomposition of insect debries left over after centrifugation, there will be a loss in the infectivity of virus due to the breakdown of POBs in the alkaline medium.

5. 4 Assessment of Quality standards of HaNPV produced by private firms

In India, there has been a rapid expansion in the production and use of biocontrol products and particularly nuclear polyhedrosis viruses (NPVs) in the last decade. The Indian Government has supported a policy of promoting biocontrol agents as part of IPM programmes, particularly against *H. armigera.* There was also a rapid expansion in the number of products being produced in the early 1990s, but this was accompanied by reports of problems in product quality.

In the present investigation the HaNPV samples received from different sources were analyzed for the presence of number of polyhedral occlusion bodies under laboratory condition. During the first year of investigation 17 samples were received and during subsequent years 12 and 9 samples were received. The number of samples received declined from first year to third year of investigation (Table 40 to 42). The severe drought experienced during these years might have reduced the production and supply of HaNPV and intern there was reduction in the number of samples being received for quality analysis. During the first and second year, the samples were received during August month as compared to the delayed receipt during the second year.

During the period of three years, totally 38 samples were received (Table-43) and the same were analyzed for the quality. Of the 38 samples received, 33 samples met the minimum quality requirement ($1X10^9$ POB/ml) and the remaining 5 samples failed to meet the minimum requirement. During 2001, of the 17 samples, 5 samples emerged as sub standard quality samples as compared to 2002 and 2003 where, all the samples received were of standard quality. The pooled analysis showed that of the 8 samples received from Bio Pest Laboratories (BPL), four samples were found to be of substandard quality. Further, of the 20 samples obtained from Bio Pest Management (BPM), only one sample was found to be inferior. Though Pest Control India (PCI) supplied samples during the first year, but all the samples were of desired quality. From this it can be inferred that the samples received from BPM have highest average number of POB count ($1.14X10^9$) followed by PCI ($1.00X10^9$) (Table 43). The samples received from BPL have recorded least average number of POB count ($0.93X10^9$).

In a survey of NPV being either sold or distributed to farmers in 1994, none of the 17 samples examined showed the declared level of NPV. In five of the samples tested, no NPV Occlusion Bodies (OB) were observed. Of those containing NPV none of them contained more than 10 per cent of the expected NPV levels and most had less than 1 per cent. In samples of HaNPV having a nominal 1×10^9 OB/ml the mean OB count was $2x10^4$ OB /ml while, for SlNPV the mean count was 4×10^8 OB/ml (Kennedy *et al.,* 1999).

The causes of the problem of quality lay as much in deficiencies in production techniques and poor quality control procedures in the absence of clearly-understood standards for NPV production (Anonymous, 1993). At the time, India lacked any regulatory standard for baculoviral control agents as they were outside the perview of the Pesticides Act. Contributing to this problem was the widespread use of the Larval Equivalent (LE) as the accepted standard measure of NPV content in place of actual OB counts. A larval equivalent is described as the average amount of NPV/GV produced by a single infected larva. However, the number of OBs produced in a larva is dependent upon a variety of factors, including the quality of the inoculum, rearing condition, size and instar of the larvae as well as the exact production system. Even if healthy larvae are fed with NPV, the amount of progeny NPV produced can vary depending upon the age and size of the larvae at inoculation, rearing conditions and inoculum dose. Another factor governing quality was that many producers (about 70%) use naturally collected insects, many of which were either the wrong (non-susceptible) species or at inappropriate stages for effective NPV propagation.

In some of the products, the NPV content was accompanied by high counts of microsporidian spores, bacterial spores and insect debries. These products could have produced from the correct number of insect larvae, but the incocula and/or insects used for BV production were highly contaminated. Also, in some cases, quality control staff were unable to identify or, count OBs precisely.

Various problems encountered in the production of nuclear polyhedrosis viruses in India are mainly due to the crude methods of processing. If contaminated water is used for processing, problems of microbial contamination are common. The quality of water, which is used in preparing the formulations, has to be maintained. Presence of chlorine (generally used to purify water) can make NPV ineffective (Singh *et al.,* 2001). Hence, for large scale production, it would be useful to utilize distilled water. When field collected host culture is used for producing NPV, the occurrence of microsporidia is more. The crude processing will also lead to increasing bacterial load.

The present findings are partly in line with the findings of Anonymous (2000), who reported that among the 45 samples of both HaNPV and SlNPV received from different sources in Karnataka, all the 45 samples have been characterized as standard samples as they meet the required quality standard of $1x10^9$ POB per ml. The main producers of NPV in Karnataka include, Bio-pest management (BPM), Biocontrol Research Laboratories (BCRL), Biocontrol Laboratories (BCL), Biotech international Limited (BIL), Kalpavruksha Bio-systems (KBS) and Multiplex India Limited (MIL). Among the six important producers, it is the BPM, which is the main supplier of NPV to different departments in Karnataka.

5.5 Efficacy of HaNPV isolates against *Helicoverpa armigera* under field conditions

Helicoverpa armigera commonly known as chickpea pod borer is a serious pest of pulses particularly on chickpea. The nuclear polyhedrosis virus (NPV) isolated from this pest has been extensively studied by Pawar *et al.* (1987) and Pawar and Dhawan (1983) as a biocide against *H. armigera*. The efficacy of different HaNPV isolates against *H. armigera* on chickpea is reported here in comparison with recommended package of practices for two years.

Among the HaNPV isolates tested for their field efficacy in chickpea, Gulbarga and Coimbatore isolates performed well and are statistically on par with each other. Dharwad isolate was the next best after Gulbarga and

Coimbatore. The performance of these isolates was consistent throughout the four spray schedule period, which exhibited same trend in their virulence. However, the crop imposed with recommended chemicals (RPP) excelled over others in recording higher larval mortality during both the years of investigations (Fig. 5). During the second year of experimentation, the isolates, which showed encouraging performance during the first year, excelled over others (Fig. 6). In the data presented on field performance of isolates, it indicated that in all the treatments, the reduction in the larval mortality was increased up to 10 days after the spray and it declined slowly thereafter at 15 DAS. This indicated that all the isolates retained their virulence up to 10 days. However in RPP, there was high reduction in the larval population at 10 DAS and thereafter it declined slowly. Among the isolates the HaNPV samples obtained from two private firms were least virulent in reducing the pest population (Fig. 7).

Maximum reduction in the pod damage was noticed in the plots receiving recommended package of practice. These plots also recorded maximum yield compared to other plots. Among the isolates, the plots imposed with Gulbarga and Coimbatore HaNPV isolates were comparatively free from damaged pods. The plots which have recorded lesser number of larval population yielded more number of healthy pods and higher yield. The incremental Benefit Cost Ratio (IBCR) also revealed the superiority of RPP with highest IBCR of 5.58 and 4.81 during 2002-03 and 2003-04, respectively, with a pooled average of 5.20. The IBCR for Gulbarga, Coimbatore and Dharwad isolates was 2.88, 2.85 and 2.57 during 2002-03 and 2.88, 2.80 and 2.20 during 2003-04, respectively. Though these three isolates performed better over other isolates in recording higher yield and IBCR among the isolates, RPP has consistently maintained its superiority and proved to be highly cost effective. In the contest of associated

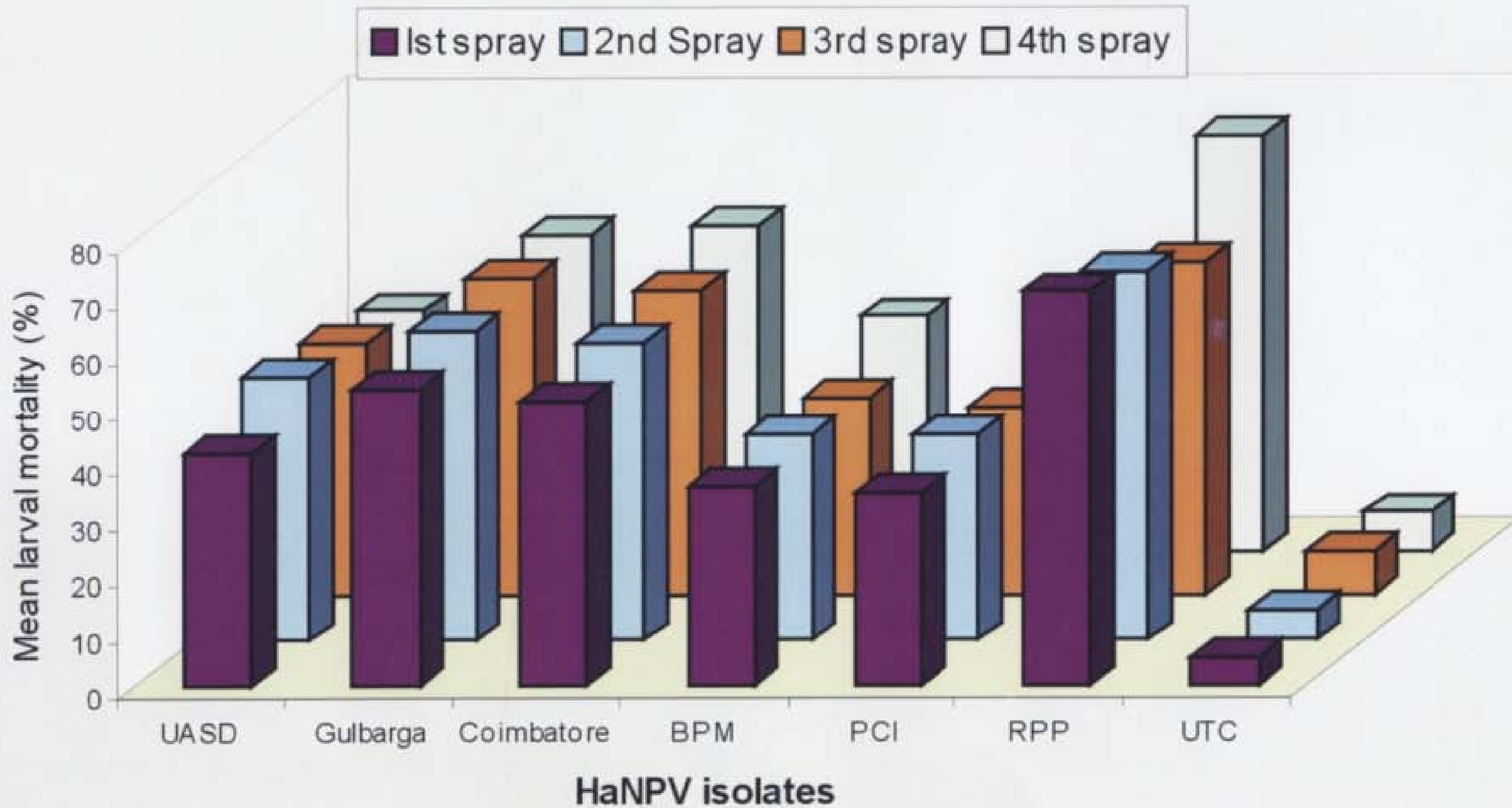

Fig. 5: Field eficacy of HaNPV isolates (I year)

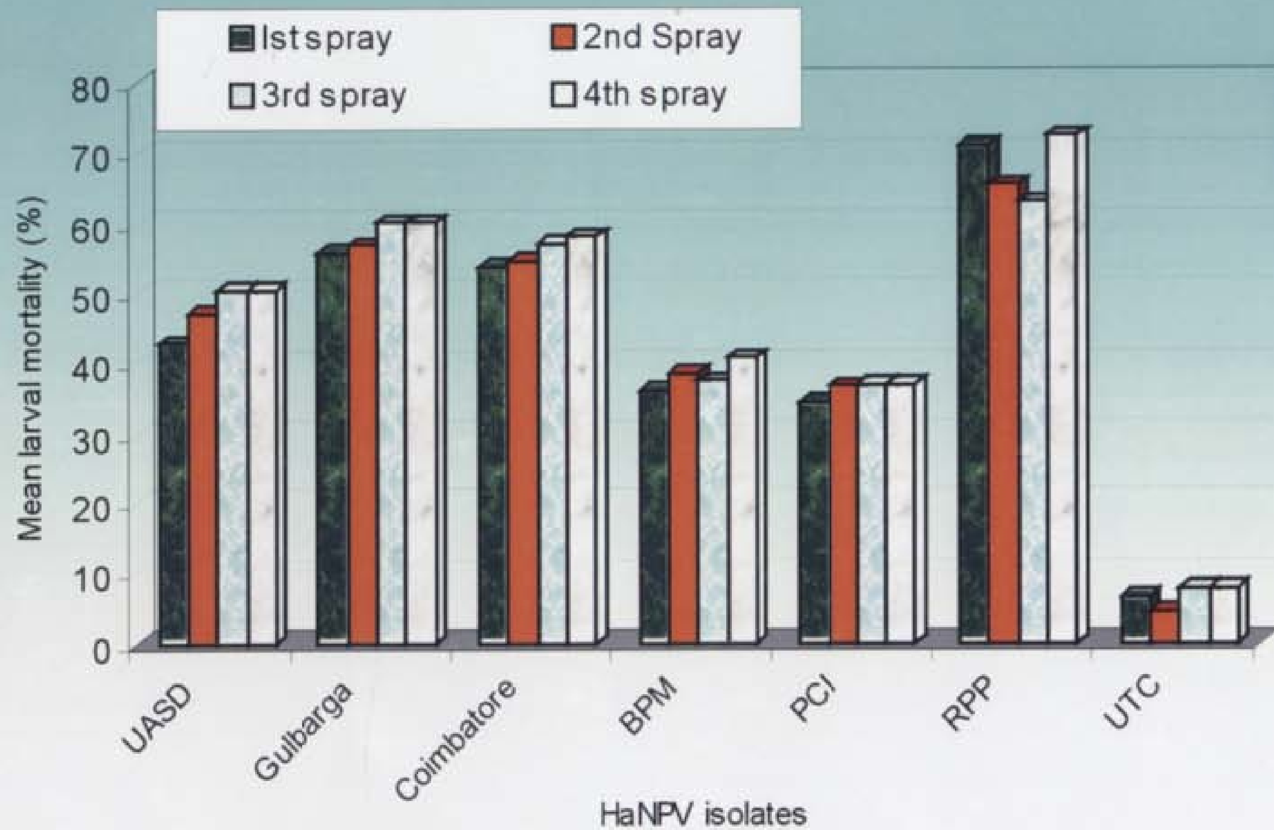

Fig. 6: Field efficacy of HaNPV isolates (II year)

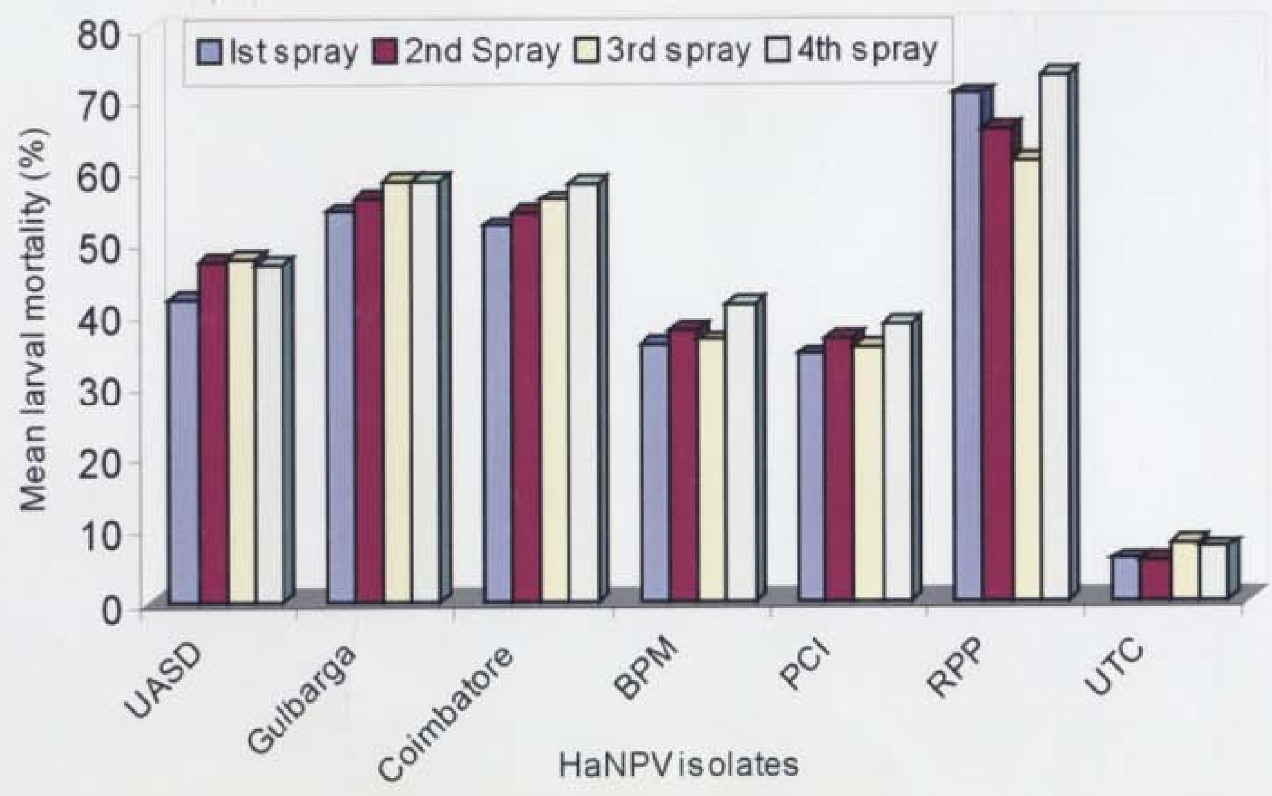

Fig. 7: Field efficacy of HaNPV isolates (Pooled)

hazards with chemical toxicants, the higher benefit risk ratio sublimes the control advantage. The BPM and PCI isolates recorded more numbers of damaged pods and registered lesser grain yield and were statistically inferior to other isolates. Between the two isolates produced by private firms, screened for field efficacy, the performance of BPM isolate was comparatively better over PCI isolate interms of pod damage and yield. The pooled data on the same parameters revealed the same trend. The IBCR for BPM and PCI recorded negative trend with 0.33 and 0.30 for the first year of investigation (Table 48) and 0.32 and 0.27 during the second year of the study (Table 53). The NPV dosage used in the present investigation was 250 LE per ha with an active ingradient of $6X10^9$ POB's per ml, and the POB count in the NPV produced by the private firms is nearly 4 to 6 times (1.00 to $1.50X10^9$ POB's/ml) lesser than that used in the present study. Further, the rate of NPV produced by these firms is Rs. 200 (At an discount of 50 per cent of the original cost Rs. 400) per bottle of 100 LE (1 LE=$1X10^9$ POB's/ml). This might have increased the expenditure over the benefit.

During the two years of evaluation, it was observed that the crop stand was poor and there was high temperature during the crop growth period. This high temperature coupled with sparse crop canopy might have reduced the efficacy of these isolates. The virulence of Gulbarga isolate in reducing the larval population of *H. armigera* is in conformity with laboratories studies carried out during the same study, where both the isolates have recorded least LC_{50} and LT_{50} values. The poor performance of isolates obtained from private firms under field conditions is also in conformity with the laboratory findings. There are no studies carried out to know the bio-efficacy of HaNPV isolates in chickpea to compare the present findings. However, the results obtained by Gopali (1998) on the superiority of Gulbarga isolate in reducing the population of *H. armigera* but in pigeonpea ecosystem are in accordance with the present findings.

The efficacy of HaNPV in chickpea has been on record (Santharam and Balasubramanian, 1982; Supare *et al.,* 1991; Rabindra *et al.,* 1994b; Jagadeeshbabu *et al.,* 1995; Kumar *et al.,* 1998; Kumawat and Jheeba, 1999; Loganathan *et al.,* 2000; Bhagwat and Wightman, 2001; Rawat and Shukla, 2001). There are many studies that have documented the variation in the virulence of isolates (Jayaraj *et al.,* 1989; Rabindra, 1993; Odak *et al.,* 1984). Among the various factors responsible for these variations, selective forces that shape the genomic constituent of individual stains assumes utmost importance which might often favour better performance of the isolates.

Thus, it can be inferred from the present investigations that Gulbarga, Coimbatore and Dharwad isolates can well be utilized in the IPM schedule of *H. armigera* in chickpea ecosystem.

Summary

VI. SUMMARY

Studies were conducted on HaNPV isolates and their assessment in the IPM of *Helicoverpa armigera* (Hubner) during 2002-03 and 2003-04 at the Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad and Main Agricultural Research Station, Dharwad. The principle objectives of the study included: i) Characterization of HaNPV isolates collected from different geographical locations; ii) Influence of different cotton species on HaNPV isolates under laboratory condition; iii) Persistence of promising isolates in different soil types under laboratory, field and refrigerator condition; iv) Assessment of quality of HaNPV produced by different private firms in Karnataka; and v) Assessment of promising isolates in the IPM of *H. armigera* on chickpea under field condition.

The DNA restriction analysis of different HaNPV isolates revealed the existence of genetic diversity among the geographical isolates. All the isolates tested formed in to two major clusters. The first cluster comprises Dharwad, Kalpavruksha, Coimbatore, BPM, PDBC and BPL isolates with a genetic similarity of around 0.57. Whereas, the second major cluster constituted three isolates *viz.,* Gulbarga, Raichur and Guntur isolates with a similarity co-efficient of 0.54.

The invitro infectivity of different HaNPV isolates against the second instar larvae of *H. armigera* collected from different locations revealed the superiority of Coimbatore and Gulbarga isolates followed by Dharwad isolate. These isolates recorded lower LC_{50} and LT_{50} values against the different populations of *H. armigera* collected from five locations representing chickpea, pigeonpea and cotton ecosystem. The pooled LT_{50} values for Coimbatore, Gulbarga and Dharwad isolates were 101.62, 102.62 and 137.67 h, respectively while, the pooled LC_{50} values were recorded at 1.98, 2.02 and

$5.18X10^4$ POB's per ml, respectively. Among the four isolates collected from private firms, Bio Pest Management (BPM) isolate excelled over others by recording higher values for LT_{50} (155.43 h) and LC_{50} ($8.86X10^4$ POB's per ml).

Combination of virulence and genetic diversity analysis indicated Gulbarga and Coimbatore isolate as two genetically distinct sources of the most virulent isolates.

Fourth instar larvae was found ideal for the large scale production of virus as the grown up larvae could yield higher quantity of virus. The Gulbarga isolate recorded highest larval mortality and maximum viral yield in all tested instars, followed by Coimbatore and Dharwad isolates.

The average POB yield in all tested instars (2nd to 5th) was maximum when the larvae were fed with Gulbarga ($8.91X10^{10}$ POB) isolate and Coimbatore ($7.51X10^{10}$ POB) followed by Dharwad ($5.10X10^{10}$ POB) isolate. Similarly, the average number of POB's per larvae were highest in Gulbarga ($2.11X10^9$), Coimbatore ($1.87X10^9$) and Dharwad ($1.69\ X10^9$) isolate with less number of larvae to yield one LE virus yield.

Comparatively higher larval mortality was observed when the second instar larvae of *H. armigera* were fed on MECH-184Bt and MECH-162Bt without HaNPV spray. When different HaNPV isolates were sprayed on different cotton species including Bt with their non-Bt versions, there was an additive effect of Bt and NPV resulting in higher larval mortality on Bt plants, irrespective of the isolates used. Among the Bt cotton, the Mahyco Bt cotton hybrids registered higher mortality as compared to Rasi Bt hybrids. Again, among the Mahyco Bt hybrids, MECH-184Bt proved superior followed by MECH-162Bt and MECH-12Bt. Among the Rasi Bt hybrids, RCH-144Bt recorded higher per cent larval mortality followed by RCH-20Bt and RCH-2Bt.

The number of larvae succumbed to viral infection was relatively more in case of Bt cotton hybrids. Among the Bt cotton, the larvae fed on the floral parts of MECH-184Bt encountered higher viral infection followed by MECH-162, MECH-12 and RCH-144Bt. Of the three species of cotton, *G. barbedense* var. B-82 harboured more number of virosed larvae.

Irrespective of the cotton species used, Gulbarga, Coimbatore and Dharwad isolate recorded higher per cent of virosed larvae as compared to the other isolates. The isolates obtained from private firms were less effective with least larval mortality.

The persistence studies carriedout in both red and black soils revealed that, irrespective of the isolates, the half life of the virus was more in NPV treated red soil as compared to black soil. Further, irrespective of the isolate and soil type, the NPV treated soil kept indoors recorded higher persistence as compared to NPV treated soil kept outdoors. The NPV treated soil stored in refrigerator recorded maximum half life with a slight loss in the virulence over a period of time.

Among the two isolates screened for their persistence in soil, Gulbarga isolate persisted for a longer period as compared to Coimbatore isolate with an half life of 15.70, 17.13 and 14.40 months, when the NPV treated red soil was kept outdoors, indoors and refrigerator conditions, respectively. Whereas, the half was 10.57, 12.92 and 96.17 months, when the same isolate treated black soil was kept outdoors, indoors and refrigerator conditions, respectively.

The HaNPV samples received from Bio Pest Management (BPM) met the minimum required concentration of POBs with an average of $1.14X10^9$ POB's per ml followed by Pest Control India (PCI) with $1.00X10^9$ POB's per ml. The average POB content of Bio Pest Laboratories (BPL) was $0.99X10^9$ POB's per ml, which was considered as substandard quality. All the samples received from PCI exhibited minimum quality standards followed by BPM (95.24%), whereas, only 66.67 per cent of samples received from BPL could prove the quality standards.

The filed evaluation of different isolates on chickpea revealed the superiority of Gulbarga, Coimbatore and Dharwad isolates. The pooled data on grain yield and IBCR were maximum for Gulbarga isolate (7.44q/ha and 2.88, respectively) followed by Coimbatore (7.37q/ha and 2.83) and Dharwad isolates (6.60q/ha and 2.39, respectively). Whereas, the virus collected from BPM and PCI recorded higher larval load, pod damage and lower grain yield and IBCR. However, the treatment, which received recommended insecticidal spray (RPP) registered lesser pod damage (8.26%), higher grain yield (8.62q/ha) and IBCR (5.20) as compared to all other treatments.

FUTURE LINE OF WORK

1. Evaluation of HaNPV isolates against *H. armigera* collected on wide hosts from various geographical locations.
2. Quantification of POB yield in the larvae fed on different Bt cotton hybrids
3. Quantification of virus in the soil over a period of time
4. Relationship between soil properties and decomposition of POB
5. Field evaluation of HaNPV isolates against *H. armigera* on various host crops.

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Appendices

Appendix I. Monthly meteorological data for experimental year of Main Agricultural Research Station, University of Agricultural Sciences, Dharwad

Month	Rainfall (mm)		Mean Temperature (°C)				Mean relative humidity (%)	
	2002-03	2003-04	2002-03		2003-04		2002-03	2003-04
			Maximum	Minimum	Maximum	Minimum		
April	67.00	54.4	37.00	21.70	36.5	21.1	53.00	57
May	57.90	0.0	34.90	23.00	36.6	21.7	62.00	57
June	60.50	32.3	29.60	21.90	30.2	21.4	80.00	75
July	15.00	15.3	28.40	21.30	27.7	21.3	79.00	82
August	48.10	8.66	26.60	20.50	27.2	20.2	82.00	81
September	6.60	16.1	29.80	20.00	27.5	19.9	73.00	79
October	103.60	48.7	30.70	20.40	30.8	19.8	67.00	67
November	7.00	1.9	30.50	17.10	30.6	16.8	62.00	60
December	0.00	0.0	30.20	14.40	29.9	14.3	47.00	51
January	0.00	0.0	30.30	16.20	29.6	14.7	52.00	54
February	0.00	0.0	33.90	18.00	32.5	16.4	51.00	53
March	0.70	0.0	35.20	20.20	36.5	19.6	53.00	49

Appendix II. Composition of artificial diet

Sl No.	Ingradients	Qty.(g)
1.	Agar shreds	15.00
2.	Yeast granules	40.00
3.	Bengal gram powder	100.00
4.	Methyl hydroxy benzene	2.40
5.	Sorbic acid	1.20
6.	Streptomycin sulphate	0.25
7.	Ascorbic acid	6.00
8.	Multimineral and Multivitamin	1.00
9.	Cholrine chloride	1.50
10.	Wesson's salt mixture	8.00
11.	Water (1000 ml)	-

INVESTIGATIONS ON HaNPV ISOLATES AND THEIR ASSESSMENT IN THE IPM OF *Helicoverpa armigera* (Hubner)

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ABSTRACT

Investigations were carriedout on HaNPV isolates and their assessment in the IPM of *Helicoverpa armigera* (Hubner) during 2002-03 and 2003-04 at Department of Agricultural Entomology and Main Agricultural Research Station, University of Agricultural Sciences, Dharwad

All the tested isolates grouped into two major clusters in phenetic analysis based RFLP. The first cluster comprised of Dharwad, Kalpavruksha, Coimbatore, BPM, PDBC and BPL isolates with a genetic similarity coefficient of 0.57. Whereas, the second major cluster constituted three isolates *viz.,* Gulbarga, Raichur and Guntur with a similarity co-efficient of 0.54. The virulence of isolates against *H. armigera* varied significantly. The Coimbatore, Gulbarga and Dharwad isolates recorded lower LC_{50} and LT_{50} values against *H. armigera* collected from five geographical locations. The pooled LT_{50} values for Coimbatore, Gulbarga and Dharwad isolates were 101.62, 102.62 and 137.67 h, respectively, while the pooled LC_{50} values were at 1.98, 2.02 and $5.18X10^4$ POB's per ml, respectively.

Fourth instar larvae have found to be ideal for the large scale production of virus The average POB yield in all the tested instars was maximum when the larvae were fed with Gulbarga ($8.91X10^{10}$ POB) and Coimbatore ($7.51X10^{10}$ POB) isolates followed by Dharwad ($5.10X10^{10}$ POB) isolate.

There was an additive effect of Bt and NPV resulting in higher larval mortality on Bt cotton plants. Among Bt cotton, the Mahyco Bt hybrids in general registered higher mortality as compared to Rasi Bt hybrids. Among the Bt hybrids, MECH-184Bt proved superior over MECH-162Bt and MECH-12Bt. The larvae fed on the floral parts of MECH-184Bt encountered higher viral infection followed by MECH-162, MECH-12 and RCH-144Bt. Of the three species of cotton, *G. barbedense* var. B-82 harboured more number of virosed larvae. Irrespective of the cotton species used, Gulbarga, Coimbatore and Dharwad isolates recorded higher per cent of virosed larvae.

The HaNPV samples received from Bio Pest Management (BPM) met the minimum required concentration of POBs with an average of $1.14 X 10^9$ POB's per ml followed by Pest Control India (PCI) with $1.00 X 10^9$ POB's per ml.

The Gulbarga isolate persisted for a longer period as compared to Coimbatore isolate with an half life of 15.70, 17.13 and 14.40 months, when the NPV treated red soil was kept outdoors, indoors and refrigerator conditions, respectively. Whereas, the half life was 10.57, 12.92 and 96.17 months, when the same isolate treated black soil was kept outdoors, indoors and refrigerator conditions, respectively.

The pooled data on grain yield and IBCR were maximum for Gulbarga isolate (7.44 q/ha and 2.88, respectively) followed by Coimbatore (7.37 q/ha and 2.83) and Dharwad (6.60 q/ha and 2.39, respectively) isolates.