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Evaluation of Honey Bee as Entomovector of *Ha*NPV

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ABSTRACT

Investigations were carried out to evaluate the efficiency of honey bee as entomovector of *Ha*NPV at Anand Agricultural University, Anand, Gujarat during 2016-17. The entomovector bee activity (as reflected by their number) in pigeonpea ranged from 1.19 bee/5 min/m^2 to 2.78 bee/5 min/ m^2. The entomovector bee activity was significantly different among the treatments. The maximum activity was recorded at 10 m distance; it was lower at 50 m distance and lowest at 100 m distance from the dispenser. Similar trend was observed in individual applications too. The overall mean *Ha*NPV load carried by bees was records as 4.00×10^5 POB. The mean *Ha*NPV load carried by bees in different applications ranged from 1.70×10^5 to 5.95×10^5 POB. The overall mean *Ha*NPV load on flower was $13.9\times10^3 \pm 53.2\times10^3$ POB. The overall mean *Ha*NPV load on flower at 10m from dispenser was significantly higher than it recorded at 50 m and 100 m distance from dispenser. The *H. armigera* mortality differed among the treatments. The overall mortality of *H. armigera* was similar in both bioassays (laboratory and field). In both bioassays, the *H. armigera* larval mortality decreased with increasing distance from the dispenser. The lowest population of *H. armigera* larvae (2.22 larvae/ 10 twigs) was found at 10 m from dispenser than it was recorded at 2.42 larvae/10 twig at 50 m, 2.63 larvae/10 twig at 100 m distance from dispenser. The *H. armigera* population was not significantly differed among the treatments during pre-application period. During application and post-application period, the *H. armigera* population was significantly differed among the treatments.

Key words *Dispenser, HaNPV, Helicoverpa armigera, Honey bee, Apis mellifera, Pigeonpea, Cajanas cajan L., Entomovector technology*

An entomovector is a pollinating insect used as a vector to spread pathogen used in the bio-control of plant pests and diseases. The primary reasons for introducing entomovector technology as a bio-control strategy were to reduce the application of synthetic pesticides because of concerns of their impacts on human life and the environment and development of resistance against pests. The main assumption was that the entomovector technology perform better than spraying of bio-control agents because of the ability to deliver the bio agents directly on to the target locations *i.e.* the flower (Ngugi and Scherm, 2006). European honeybee, *Apis mellifera* L., in search of nectar and pollen, has established innumerable primary and secondary associations with available flora (McGregor, 1976; Behera *et al*., 2014; Pushpalatha and Hariparasad, 2015). Because of these associations and our easy access to managed population of honey bees whose habitation can be adapted and transported as needed, there is an opportunity to use honey bee as disseminators of insect pathogens against an array of pests affecting agricultural crops. In this study, we intend to examine the honey bee to serve as disseminators of *Helicoverpa* nuclear polyhedrosis virus (*Ha*NPV).

In the crop fields, NPV is usually disseminated mechanically using convectional insecticide spraying pumps. Use of entomovector to disseminate NPV came in light only after 1990s (Smagghe *et al*, 2012). Some experimental studies have been done in USA, Australia and European countries, however no such studies are done in Indian conditions to disseminate NPV or other pathogens using "entomovector technology". The entomovector technology was demonstrated for dissemination of *Ha*NPV on crimson clover flower (Gross *et al*, 1994). This technology has enabled us to disseminate the rightly amount of bio agent at right place, at right time, and minimize the barriers of NPV treatment limitations (Mommaerts and Smagghe, 2011).

Pigeonpea, *Cajanus cajan* (L.) is one of the major pulse crop of the tropics and subtropics. It is the second most important pulse crop of India, after chickpea (Anon., 2015). Among the pigeonpea crop pod borer *Helicoverpa armigera* (Hubner) is one of the most important pests infesting from the bud formation to the maturity of the crop (Patil *et al*., 1990). It is a polyphagous pest occurring on a variety of crops (Chari *et al*., 1990 & Mehrvar *et al*., 2009). All over the world an estimated loss due to this pest is found to be exceeding US $ 300.00 million, forcing several research groups to investigate various strategies to control this pest. It is a matter of concern as it is inflicting 56.22 per cent damage in India alone (Sharma *et al.,* 1991). Damage to pods due to the borer complex was reported to be 20 to 72 percent (Lateef and Reed, 1983). In middle Gujarat, pod damage due to the *H. armigera* was 39.20 percent in BDN-2 variety of pigeonpea (Patel and Patel, 2010).

MATERIALS AND METHODS

Field Experiment

The experiments was conducted at Agronomy farm, B. A. College of Agriculture, Anand Agricultural University, Anand (Gujarat) during *Kharif* 2016-17. BDN-2 variety of pigeonpea sown with plant spacing 90 × 45 cm. The dissemination of *Ha*NPV mainly depends on activity pattern of honey bee. Hence, the measure the effect of disseminated *Ha*NPV on *H. armigera* population. The treatments plots at 10 m, 50 m and 100 m from dispenser and one netted control was created.

The first application was applied at the time of full bloom stage (January 2017). 10g of *Ha*NPV 8.0% dust

Table 1. Entomovector bee (marked) activity in the pigeonpea crop

Treatment	No. of marked bee/5 minutes/m^2 during each application				Pooled
	I	II	III	IV	
T1- 10 m from dispenser	1.64 (2.20)	1.76 (2.60)	1.76 (2.60)	1.81 (2.80)	1.74 (2.54)
T2- 50 m from dispenser	1.51 (1.80)	1.64 (2.20)	1.64 (2.20)	1.37 (1.40)	1.54 (1.88)
T3- 100 m from dispenser	1.30 (1.20)	1.37 (1.40)	1.44 (1.60)	1.23 (1.00)	1.33 (1.27)
S.Em. ±					
T	0.07	0.07	0.07	0.06	0.04
P	-	-	-	-	0.04
T × P	-	-	-	-	0.04
C.D. at 5%					
T	0.21	0.23	0.23	0.19	0.12
P	-	-	-	-	NS
T × P	-	-	-	-	NS
C. V. %	10.13	10.29	10.14	9.21	9.99

Note: Figures in parenthesis () are retransformed value; those outside are square root transformed values.

formulation was disseminated through pathogen applicator devices (PAD) attached with honey bee hives. This practices was carried out during 10:00 to 13:00 hours. The subsequent repetition was performed on 5th, 9th and 15th day from 1st application.

MATERIALS

Honey bee

Honey bee hive (40 × 55 × 30 cm) containing *Apis mellifera* L. was utilized for dissemination of *Ha*NPV. Hive was purchased from market and maintained using standard procedures (Mishra, 1995).

*Ha*NPV Formulation

Liquid formulation of *Ha*NPV 2.0 % AS was purchased from the Bio-control unit, Dept. of Agricultural Entomology, Junagadh Agricultural University, Junagadh. A powder formulation of NPV was made by centrifuging 1 liter aliquot of liquid *Ha*NPV. Centrifuge was done in two phases: 1000 rpm for 1 minute and 5000 rpm for 10 minutes with help of centrifuge machine to produce approximately 2 g of dry material. The dry material was manually crushed and ground using a mortar and pestle. The resulting powder was then mixed with 9 parts industrial talc (200µ particle size) and a trace of pink fluorescent powder as marker to track honey bee in field observation.

Pathogen Applicator Device (PAD)

A pathogen applicator device to disseminate *Ha*NPV using *A. mellifera* was used as designed and developed by Gross *et al*. (1994).

Insect rearing/culture

In order to obtain pure culture of *H. armigera* larvae were collected from pigeonpea and reared in individual plastic vials containing artificial diet, till pupation. After a week, healthy pupae were take outside and transferred separately to the moth emergence cage (33×33×33 cm) for eclosion. The pupae were observed at 12 hours intervals for adult emergence. The freshly emerged male and female moths in 1:1 ratio were released inside empty earthen pots (45×30 cm). The earthen pots were placed in plastic basins surrounded by moist sand to half the height of basin. The sand bed was moistened with sodium hypochlorite (1%) solution to avoid fungal growth. The top portion of pot was covered with black muslin cloth fastened by rubber band for oviposition. Fresh honey solution (10%) enriched with vitamins in cotton wool was provided daily as adult food till death of the moths. Eggs were collected by changing the black cloth daily in morning. The 24 hours old eggs were surface sterilized with sodium hypochlorite (1%) solution. Immediately after hatching of eggs, the neonate larvae were reared in plastic container on artificial

Table 2. *Ha*NPV load carried by honey bee per visit

Repetition	Application-1	Application-2	Application-3	Application-4	Overall
***Ha*NPV (POB) carried/visit**	$1.70 \times 10^5 \pm 1.66 \times 10^5$	$5.95 \times 10^5 \pm 4.52 \times 10^5$	$4.55 \times 10^5 \pm 2.63 \times 10^5$	$3.80 \times 10^5 \pm 2.82 \times 10^5$	$4.00 \times 10^5 \pm 3.88 \times 10^5$

Note: All the data are represented in Mean±S.D.

Table 3. *Ha*NPV load deposited on the pigeonpea flower by honey bees

Treatment	Amount of *Ha*NPV load on flower (POB)				
	Application-1	Application-2	Application-3	Application-4	Overall
T1- 10m from dispenser	$10.0 \times 10^3 \pm 7.4 \times 10^3$	$32.5 \times 10^3 \pm 8.2 \times 10^3$	$55.0 \times 10^3 \pm 15.6 \times 10^3$	$25.0 \times 10^3 \pm 4.0 \times 10^3$	$30.6 \times 10^3 \pm 8.7 \times 10^3$
T2- 50m from dispenser	$5.00 \times 10^3 \pm 1.5 \times 10^3$	$5.00 \times 10^3 \pm 1.5 \times 10^3$	$12.5 \times 10^3 \pm 2.2 \times 10^3$	$7.50 \times 10^3 \pm 6.8 \times 10^3$	$7.50 \times 10^3 \pm 1.51 \times 10^3$
T3- 100m from dispenser	$2.50 \times 10^3 \pm 1.25 \times 10^3$	$2.50 \times 10^3 \pm 1.25 \times 10^3$	$2.50 \times 10^3 \pm 1.90 \times 10^3$	$7.50 \times 10^3 \pm 6.8 \times 10^3$	$3.75 \times 10^3 \pm 1.06 \times 10^3$
Overall	$5.83 \times 10^3 \pm 2.6 \times 10^3$	$13.3 \times 10^3 \pm 6.2 \times 10^3$	$23.3 \times 10^3 \pm 11.0 \times 10^3$	$13.3 \times 10^3 \pm 9.1 \times 10^3$	$13.9 \times 10^3 \pm 3.2 \times 10^3$

Note: All the data are represented in Mean±S.D.

diet and utilizes for bioassay according to its need (Gopali, 1998).

METHODS

Flower visitation

The entomovector bees visiting pigeonpea flowers were recorded between 10:00 to 13:00 hours. The observations were recorded in randomly five spots of one meter section area from each treatment for five minutes. The count of bee was made simultaneously in three treatments using three independent observers. Presence of fluorescent material on appendages of the bees was confirmed and proportion of bees with and without fluorescent material was worked out.

*Ha*NPV load on honey bee

To estimate the amount of *Ha*NPV carried out by bees, five bees exiting from the PAD, were collected in separate sampling tubes (50 ml). 25 ml sterilized distilled water was added into the glass tubes and transported to the laboratory. The bees were removed after through washing. The polyhedral from the virus suspension (25 ml) was counted with the help of haemocytometer. For counting of polyhedral, one millilitre diluted virus suspension ($1/1000^{th}$ serial dilution) was drawn into pipette and put in the groove of the haemocytometer and then standard cover slip was placed over the slides. After allowing polyhedral to settle down for two minutes, the polyhedral count was taken in 80 squares of the 1/400 square millimetre area at random using stereomicroscope. Concentration of stock solution was expressed as POBs/ml which was calculated by using the following formula. Number of POBs/ml = $(D \times N)/(X \times K)$, Where, D = Dilution factor, N = No. of square counted X = total no. of POBs counted, K = Constant (2.5×10^{-7}).

Tab1e 4a. Laboratory bioassay of *Ha*NPV disseminated by honey bee on pigeonpea flower

Treatment	Mortality (%) of *H. armigera*
T1- 10m from dispenser	29.89 (24.83)
T2- 50m from dispenser	26.56 (20.00)
T3- 100m from dispenser	18.43 (10.00)
T4- Control	0.90 (0.00)
S.Em. ±	0.96
C.D. at 5%	2.96
C.V. %	10.13

Note: Figures in parenthesis () are retransformed value; those outside are arc sin transformed values

*Ha*NPV load on flower

After releasing of *Ha*NPV, on following days, 10 samples of pigeonpea flowers were collect randomly from each treatment plots and kept in individual 50-ml glass tubes for analysis of amount of *Ha*NPV loaded onto the flowers. The next procedures was follow as given above for counting *Ha*NPV load on bees.

Effect of *Ha*NPV on *H. armigera*

Bioassy

Bioassay was carried out in two ways; Laboratory bioassay and filed bioassay. Laboratory bioassay was carried out by rearing of *H. armigera* in laboratory at ambient temperature. Twenty larvae of 2^{nd} instar were used for bioassay in each treatment. For first three days (72 hours) larvae were fed with the flowers brought from respective treatment. On 4^{th} day, the larvae were switch over to artificial diet. Every day, the larvae were closely examined for any disease symptoms. Dead larvae were subjected to microscopic examination for presence of NPV polyhedra. Whereas filed bioassay was carried out by collecting ten larvae from each of treatment after 3 days (72 hrs.) of application and were placed on artificial diet at normal environmental conditions. Larvae were observed daily and mortality due to *Ha*NPV and the other causes were recorded.

Population index

The observations on population of *H. armigera* larvae were recorded by counting the number of larvae per 10 cm twigs of pigeonpea crop. Fifty twigs from each treatment were observed at weekly interval, starting from flowering

Table 4b. Field bioassay of *Ha*NPV disseminated by honey bee on pigeonpea flower

Treatment	Mortality (%) of *H. armigera*
T1- 10m from dispenser	26.56 (20.00)
T2- 50m from dispenser	22.50 (14.64)
T3- 100m from dispenser	18.44 (10.01)
T4- Control	0.90 (0.00)
S.Em. ±	1.17
C.D. at 5%	3.62
C.V. %	13.72

Note: Figures in parenthesis () are retransformed value; those outside are arc sin transformed values

initiation period (December 2016) till completion of the experiment (February 2017).

Statistical Analysis

Statistical analysis was carried out by following standard procedures (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Entomovector bee activity

The entomovector bee activity in pigeonpea ranged from 1.19 bee/5 min/m^2 to 2.78 bee/5 min/ m^2 (Table 1). The entomovector bee activity significantly differed among the treatments ($F=23.48$, $P < 0.05$, $df=11$). The mean number of entomovector bee was significantly higher at 10 m from dispenser (2.54 bees/m^2). Subsequently, the bee count decreased with increasing distances from dispenser, (1.88 bees/m^2 at 50 m and 1.16 bee/ m^2 at 100 m from dispenser).

Similar trend in bee activity was observed in each repetition too. Where, the entomovector bee activity significantly differed among the treatment ($P < 0.05$, $df=11$). The bee activity was observed significantly higher at 10 m distance from dispenser during all the applications. However, it was at par with bee activity observed at 50 m from dispenser during 1st and 2nd application. The bee activity observed at 50 m from dispenser was at par with bee activity observed at 100 m from dispenser in each repeatition. The non-entomovector bees (77.42 %) were maximum at 100 m from dispenser, than it was recorded 56.00 % at 50 m and 50.00 % at 10 m from the dispenser (Fig. 1). The entomovector bee proportion in field decreased with increasing distances from dispenser.

Study, the entomovector bee activity in pigeonpea ranged from 1.19 bee/5 minute/m^2 to 2.78 bee/5 minute/m^2 and the foraging activity decreased with increasing distances from hive in present study. Dillon (2002) reported that bee activity in cotton field ranged from 0.0 bees/10 min/2 m^2 to 24 bees/10 min/2 m^2 and foraging activity decreased with increasing distance from hives. The foraging distance for bee colonies varies among place to place, and it is affected by colony strength, food sources, month or the time of the day in same region (Abou-Shaara, 2014). In present study, the entomovector bee activity was observed lower in comparison to it was observed by Dillon (2002). It is mainly due to colony strength, it was 5000 bees in present study whereas Dillon (2002) used 50,000 bees per ten hives.

*Ha*NPV load on bees and flower

The overall mean *Ha*NPV load carried by bees was recorded as 4.00×10^5 POB. The mean *Ha*NPV load carried by bees in different applications ranged from 1.70×10^5 to 5.95×10^5 POB (Table 2). There was no significant fluctuation in *Ha*NPV load carried by bees during different application (Fig 2a). The overall mean *Ha*NPV load on flower at 10m from dispenser was found significantly higher than it recorded at 50 m and 100 m from dispenser. *Ha*NPV load on flower was significantly reduced with increasing distance from dispenser. The similar trend of *Ha*NPV load on flower was observed during all application (Fig 2b). The *Ha*NPV load on flower in all the applications decreased with increasing distance from dispenser. The *Ha*NPV load in various applications on flower ranged from $5.83 \times 10^3 \pm 12.6 \times 10^3$ to $23.3 \times 10^3 \pm 91.1 \times 10^3$ POB/flower (Table 3). The *Ha*NPV load on flower was recorded lowest during 1st application. The almost similar *Ha*NPV load was observed over remaining 2nd, 3rd, and 4th applications.

In present study, overall mean *Ha*NPV load carried by bees was 4.00×10^5 POB per flight. According to Shipp *et al*. (2012) the bee transmitted *B. bassiana* in the tomato and pepper and mean CFU load on bees ranged from 9.7×10^5 to 1.8×10^6 in tomato and 1.3×10^6 to 3.4×10^6 CFU/bees in pepper. Smagghe *et al.* (2013) studied on entomovectoring with bumble bees using *Metarhizium anisopliae*. The bumble bee carried $9.3 \pm 1 \times 10^6$ spores per bees. The overall *Ha*NPV load on flower was $13.9 \times 10^3 \pm 53.2 \times 10^3$ POB. According to Saffir *et al*. (2006), average bee disseminated *T. harzianum* load on flower was $2.2 \times 10^4 \pm 4.8 \times 10^3$ CFU per flower. According to Shipp *et al.* (2012), bumble bee transmitted *B. bassiana* load on tomato and pepper flower ranged from 7.6×10^3 to 1.2×10^4 CFU for tomato and, 5.6×10^3 to 1.7×10^4 CFU per flower for pepper.

Bioassay

The evaluation of effectiveness of disseminated *Ha*NPV under lab condition was done by bioassay of *H. armigera* larvae on pigeonpea flowers collected from treatment plots. The *H. armigera* mortality differed among the treatments ($F=92.65$, $P<0.05$, $df=15$) (Table 4a). The maximum mortality (24.83 %) of *H. armigera* larvae was found at 10 m distance from dispenser followed by 20.00 % at 50 m and 10.00% at 100 m from dispenser. *H. armigera* mortality decreased with increasing distance from dispenser. The overall *H. armigera* larval mortality in field condition was significantly differed among the treatments ($F=182.39$, $P<0.05$, $df=15$)(Table 4b). The *H. armigera* larval mortality was recorded maximum at 10 m from dispenser (20.0 %) followed by 50 m (14.64%) and 100 m (10.01%) at distance from dispenser. The overall mortality of *H. armigera* was similar in both bioassays. In both bioassays, the *H. armigera* larval mortality decreased with increasing distance from dispenser.

Present study on effectiveness of honey bee disseminated *Ha*NPV showed that maximum *H. armigera* larval mortality (in laboratory) was 24.83 %. Gross *et al.* (1993) studied honey bee disseminated *Ha*NPV mortality

Table 5. Population of *H. armigera* larvae in pigeonpea field

Treatment	Larvae/10 twigs												
	Pre-application period				Application period			Post-application period					
Period	1st SMW	2nd SMW	3rd SMW	Pooled	4th SMW	5th SMW	Pooled	6th SMW	7th SMW	8th SMW	9th SMW	Pooled	Overall pooled
T1- 10m from dispenser	2.05 (3.70)	2.22 (4.43)	2.11 (4.21)	2.13 (4.40)	1.69 (2.36)	1.42 (1.52)	1.55 (1.90)	1.44 (1.57)	1.44 (1.57)	1.22 (0.92)	1.22 (0.99)	1.33 (1.27)	1.65 (2.22)
T2-50m from dispenser	2.00 (3.50)	2.25 (4.56)	2.00 (3.50)	2.09 (3.87)	1.81 (2.78)	1.70 (2.39)	1.75 (2.56)	1.50 (1.75)	1.50 (1.57)	1.30 (1.09)	1.30 (1.19)	1.40 (1.46)	1.71 (2.42)
T3-100m from dispenser	2.11 (3.95)	2.16 (4.17)	2.06 (3.74)	2.11 (3.95)	1.84 (2.89)	1.69 (2.36)	1.77 (2.63)	1.50 (1.75)	1.63 (2.16)	1.51 (1.72)	1.44 (1.57)	1.52 (1.81)	1.77 (2.63)
T4- Control	1.89 (3.05)	2.11 (3.95)	2.21 (4.38)	2.07 (3.78)	2.07 (3.78)	1.91 (3.15)	1.99 (3.78)	1.86 (2.96)	1.91 (3.15)	1.75 (2.56)	1.70 (2.39)	1.81 (2.78)	1.94 (3.26)
S.Em. ±													
T	0.15	0.17	0.11	0.09	0.09	0.11	0.08	0.11	0.11	0.30	0.07	0.05	0.02
P	-	-	-	0.07	-	-	0.04	-	-	-	-	0.04	0.05
T × P	-	-	-	0.15	-	-	0.09	-	-	-	-	0.09	0.11
C.D. at 5%													
T	NS	NS	NS	NS	0.26	0.33	0.22	0.32	0.32	0.40	0.20	0.13	0.07
P	-	-	-	NS	-	-	0.14	-	-	-	-	0.12	0.16
T × P	-	-	-	NS	-	-	NS	-	-	-	-	NS	NS
C. V. %	16.18	17.86	11.68	15.51	10.34	14.66	12.49	15.10	14.98	9.52	10.53	13.06	14.67

Note: Figures in parenthesis () are retransformed value; those outside are square root transformed values

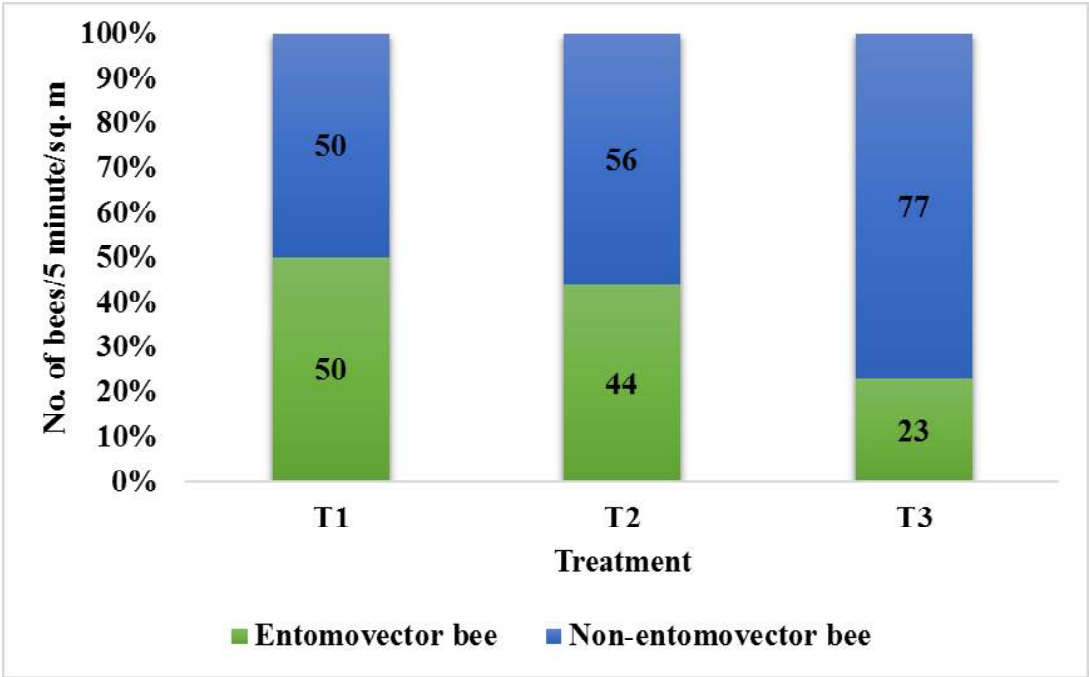


Fig. 1. Entomovector bee and non-entomovector bee activity in the pigeonpea field

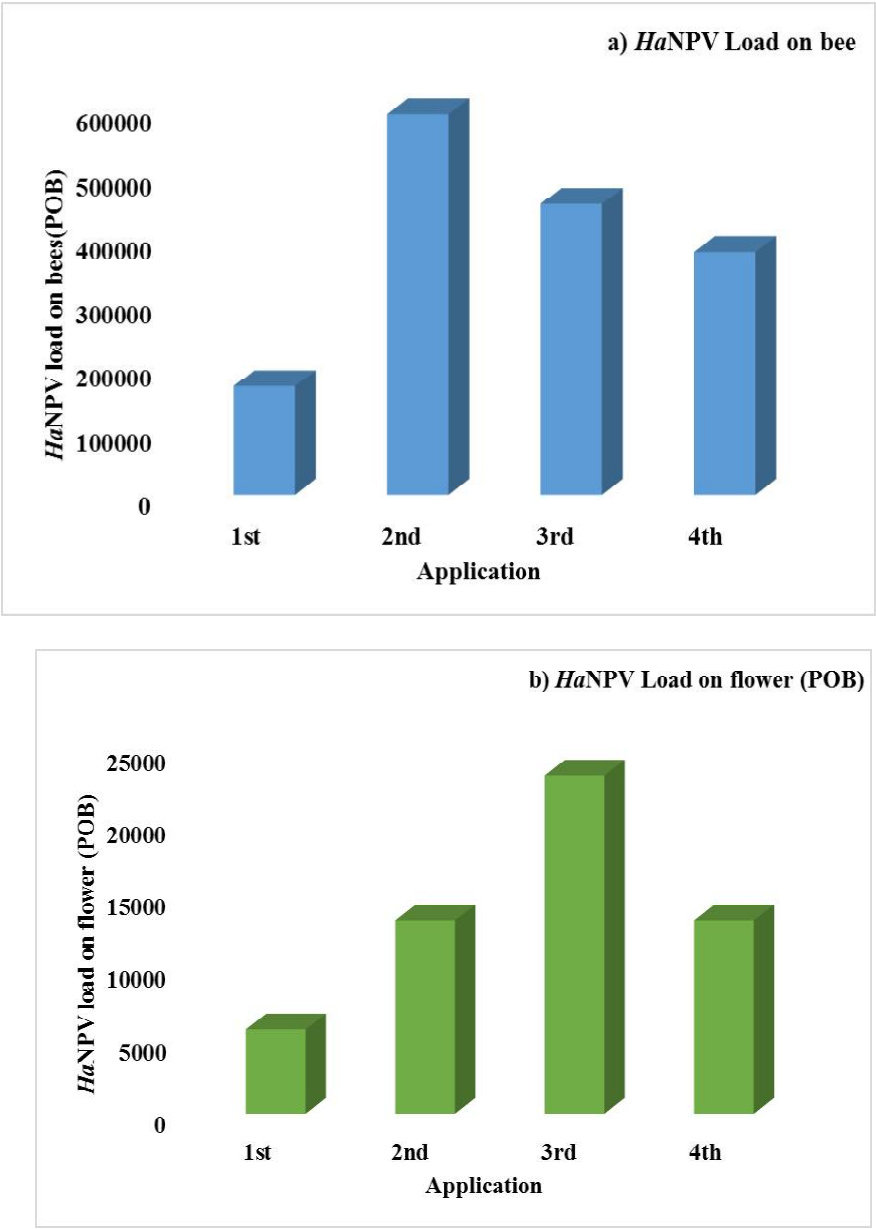


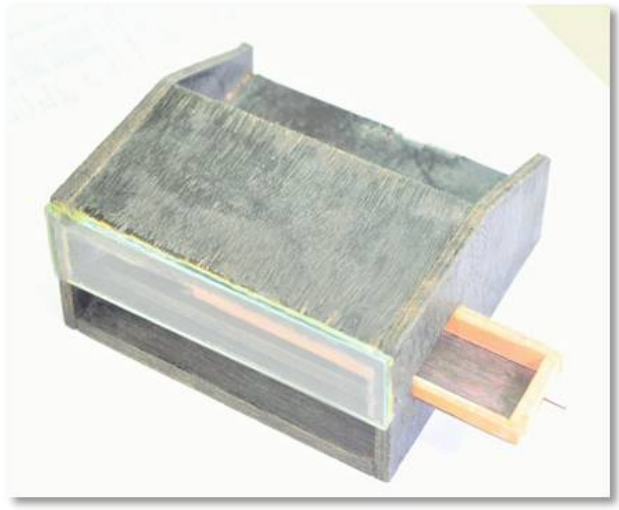
Fig. 2. *Ha*NPV load, a) on bee per visit, and b) Deposited on pigeonpea flower

of *H. armigera* mortality to the tune of 87.00% (Location-1) and 73.90% (Location-2) on crimson clover flowers. Dillon (2002) studied honey bee disseminated *Ha*NPV mortality of *H. armigera* larvae in cotton crop and recorded 64.70% mortality. The *H. armigera* mortality in present study was low which might be due to weaker strength of colonies of entomovector bee in the hive (1 hive=5000 bees) compared to 50,000 bees (Dillon, M. 2002) and 25,000 bees (Gross, *et al*. 1993) used elsewhere.

Effect on *H. armigera* population

The *H. armigera* population was monitored to determine the effect of honey bee, disseminated *Ha*NPV in various treatments. The weekly data was grouped into pre-application, application and post-application period. The overall difference among the treatments was found highly significant ($F=26.99$, $P<0.05$, $df=19$)(Table 5). All the treatments were significantly differed from each other. The number of *H. armigera* larvae before application ranged 3.78 to 4.40 larvae/10 twig and after application of *Ha*NPV it was 1.27 to 2.78 larvae/10 twig. The lowest population of *H. armigera* larvae (2.22 larvae/10 twigs) was found at 10 m from dispenser than it was recorded at 2.42 larvae/10 twig at 50 m, 2.63 larvae/10 twig at 100 m distance from dispenser and 3.26 larvae/10 twig at netted control. The population of *H. armigera* was found to be increased with increasing distance from dispenser. But the *H. armigera* population was not significantly differed among the treatments during pre-application period. During application and post-application period, the *H. armigera* population was significantly differed among the treatments.

Study on population of *H. armigera* larvae before application ranged from (3.78 to 4.40 larvae/10 twig); and after application of *Ha*NPV it was ranged from (1.27 to 2.78 larvae/10 twig). Yogesh and Kumar (2014) studied bio efficiency of *Ha*NPV against *H. armigera* in pigeonpea and found that before application population of *H. armigera*



Pathogen Applicator Device (PAD)

Bee-hive with Pathogen Applicator Device (PAD)

*Ha*NPV load on pigeonpea flower

*Ha*NPV loan on honey bee

was (2.90/5 plant) and after application it was found to be (1.20 larvae/5 plant). Byrappa *et al.* (2012) also studied bio efficiency of *Ha*NPV against *H. armigera* in field bean crop and found that population of *H. armigera* during pre-application period was 20.26 larvae/m^2 area and post application period 11.69 larvae/m^2. The reduction in *H. armigera* population was 27.76 % in present study, while the *H. armigera* population reduction was 42.30 % case the studied made by Byrappa *et al*. (2012). The difference in mortality/control of *H. armigera* achieved in above studies may be due to variation in application method and population of entomovector bee as well as *H. armigera* during application in pigeonpea crop.

This is the first study of its kind involves honey bees *A. mellifera* as the vector of *Ha*NPV to control *H. armigera* in pigeon pea open field. The success of entomovector technology in suppression of pest population depends on dispenser design, selection of vector, control agent and environment safety. Here, care has been taken in selecting the various components for employing entomovectoring, right from selection of dispenser to managing the vector. The mean 5.83×10^3 to 23.3×10^3 POB/flower *Ha*NPV load on pigeon pea through entomovectoring reported here is sufficient to suppress the *H. armigera* population. Moreover, based on obtained results it can be concluded that vectoring of *Ha*NPV by *A. mellifera* bees suppressed *H. armigera* population in pigeon pea efficiently. Further, suppression of *H. armigera* of honey can be enhanced by employing more colonies of honey bee for entomovectoring and its management and initiating application at low pest population pressure.

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