

Kairomone activity of okra, *Abelmoschus esculentus* (L.) Moench genotypes on lepidopteran pests and their entomophages[☆]



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ABSTRACT

The infestation level of two destructive lepidopteran pests, [*Earias vittella* (Fab.) and *Helicoverpa armigera* (Hübner)] and the abundance of entomophages, *Trichogramma chilonis* Ishii and *Chrysoperla zastrowi sillemi* (Esbén-Peterson) were studied on 10 germplasm and 16 cultivars/hybrids of *Abelmoschus esculentus* (L.) under field condition. High populations of the host insects on susceptible genotypes of *A. esculentus* increased the foraging activities of natural enemies. Among 26 genotypes, the hybrid No. 55 was noted as highly susceptible to *E. vittella* and *H. armigera*, with mean counts per 10 plants of 16.66 and 15.40 larvae, respectively. In addition 3.00 eggs/grubs of *C. zastrowi sillemi* were recorded and 9.33% field recovery of *T. chilonis*. The resistant germplasm, AE 9 was less hospitable to host insects and their entomophages. Choice test experiments were conducted in the laboratory to consider kairomonic activity of acetone extracts of various parts of highly susceptible (No. 55), susceptible (Arka Anamika) and resistant (AE 9) genotypes of *A. esculentus*. The tests indicated that No. 55 contained kairomone substances which enhanced the percentage parasitization by *T. chilonis* from 6.67 to 55.33% (1% of flower extract of No. 55) and percentage predation by *C. zastrowi sillemi* from 10.67 to 58.22% (1% of flower extract of No. 55) on eggs of *E. vittella*. Similarly, on eggs of *H. armigera*, the percentage of parasitism by *T. chilonis* and percentage of predation by *C. zastrowi sillemi* were enhanced from 10.67 to 65.33 and 10.67 to 68.67%, respectively. The maximum abundance of herbivores and their entomophages in the susceptible genotype (No. 55) of *A. esculentus* might be due to the abundance of secondary metabolites which are favourable to the enhanced foraging activities of entomophages. A systematic isolation, identification and the synthesis of these chemical cues may lead to the development of kairomone formulations that enhance the foraging efficiency of the entomophages in *A. esculentus* for the successful bio-suppression of *E. vittella* and *H. armigera*.

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1. Introduction

Semiochemicals are potential tools for biocontrol of insect herbivores because entomophages are known to orient to plants on which their hosts are found [1]. Plant volatiles help natural enemies identify and forage on host insects [2]. Female locates their potential hosts by performing a series of complex behaviors in

response to physical or chemical stimuli [3]. The stimuli include volatiles emanated from herbivore-infested [4] and uninfested [5] plants. The nature of response by the receiver of the stimuli depends on the producer context, *i. e.*, insect pest, natural enemy, or crop [6].

The efficiency of natural enemy foraging activity for managing host insects varies from crop to crop and within the crop [6–8]. Eggs of *Helicoverpa armigera* (Hübner) deposited on sunflower hybrid MSFH 17 were more preferred by *Trichogramma chilonis* Ishii for parasitization (53.5%) than eggs on KBSH 1 (38.0%) [9]. Chemical compounds such as linalool-L and heptadecane from fruits and heptadecane from leaves of hybrid tomato (Arka Ahuti) are responsible for higher parasitization of *T. chilonis* on eggs of *H. armigera* in the tomato eco-system while other compounds like

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α -phellandrene, α -pinene, *trans*-caryophyllene, (*Z*)- α -farnesene, *trans*- α -ocimene and selinene are known for their synomonal activity [10].

The volatiles, 9, 12, 15 octadecatrienoic acid and 9-octadecenal, detected in extracts of rice cultivars, Kadamba, MTU-1010, KMT 148 and KCP-1, and hexadecane, heptadecane, pentadecane and hexadecanoic acid detected in rice cultivars, CTH-1, MTU 1010 and VTT-5204, were attractive to *T. chilonis* and *T. japonicum* Ashmead for the management of rice yellow stem-borer and leaf-folder eggs, respectively [11]. The present studies were undertaken to generate information on the effect of different genotypes of *Abelmoschus esculentus* (L.) Moench, one of the major vegetable crops in India and many other warm regions of the world [12] on foraging activities of *Trichogramma chilonis* Ishii and *Chrysoperla zastrowi sillemi* (Esben-Peterson) on shoot and fruit borer, *Earias vittella* (Fab.) and fruit borer, *H. armigera*. An objective was to identify genotypes favourable to entomophages of folivores and borers of *A. esculentus* and to assess volatile extracts of various plant parts of different *A. esculentus* genotypes that attract entomophages for suppression of lepidopteran pests of *A. esculentus*.

2. Methods and materials

2.1. Collection, planting and screening of *A. esculentus* genotypes against *E. vittella* and *H. armigera* and their entomophages

Twenty-six genotypes of *A. esculentus* (10 g of seeds each) comprising 10 germplasm collections (obtained from the Department of Olericulture, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India) and 16 cultivars/hybrids (obtained from local Seed Companies, Madurai, Tamil Nadu, India) were assembled at the Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai, Tamil Nadu, India during November 2013. Seeds were treated in 50% salt solution and carbendazim 0.2% (2 g/kg of seeds) separately to remove diseased/ill-filled seeds and protect them from fungi and other soil organisms.

Fungicide treated seeds were sown, separately in red loam soil at Insectary. Each genotype was dibbled @ 2 seeds in a 10 m length of ridges and furrows with spacing of 60 × 30 cm and each was replicated thrice. All agronomic practices were followed as per the Crop Production Guide of Tamil Nadu Agricultural University, Coimbatore, India. The field screening was carried out in unprotected conditions to expose them to *E. vittella* and *H. armigera* and identify cultivars/hybrids/germplasm which were maximally favourable for the attraction of *T. chilonis* and *C. zastrowi sillemi* through production of volatile chemicals in flowers, fruits or leaves. Ten plants were marked at random for each genotype and the population (number of larvae/10 plants) and per cent shoot damage by *E. vittella* (number of bored shoots to healthy shoots in 10 plants) were observed at 10 d interval starting from 30 days up to 70 days after dibbling. Per cent fruit damage by *E. vittella* and *H. armigera* (determined by the ratio of numbers of bored fruits to numbers of healthy fruits) was observed at each harvest starting from 60 days after dibbling. The numbers of eggs/grubs of *C. zastrowi sillemi* present in 10 randomly tagged plants were counted on 40 and 70 d after dibbling. UV irradiated eggs of rice moth, *Corcyra cephalonica* Stainton, pasted on a 7 × 2-cm card (approximately 4000 eggs/card) were tied in plants @ 2 cards/row on days 15, 35 and 55 after dibbling as supplementary food to *T. chilonis*. The field recovery of *T. chilonis* adults was observed on 20, 40 and 60 days after dibbling by observing the parasitized (black) eggs.

2.2. Maintenance of host insects and entomophages

2.2.1. *E. vittella*

Different instars of *E. vittella* collected locally from *A. esculentus* fields were reared on *A. esculentus* fruits in the Insectary. Pieces of 2.5 cm long, tender, fresh *A. esculentus* fruits were kept in 12 well-cavity trays @ 2 pieces per well. After larvae were placed on the fruit, the tray was covered using a plastic lid and fastened by a rubber band. Fruit pieces were changed every two days. Virgin adults emerged from boat shaped cocoons were confined in an adult emergence cage (30 × 30 × 30 cm), provided with 10% honey solution + multivitamin solution in 5 ml glass vials provided with a cotton plug. After adults began mating, 4–5 tender *A. esculentus* fruits were placed in cages as oviposition substrates. These fruits containing eggs were incubated at 26 ± 1 °C, RH (75 ± 5%) for hatching. The neonates were offered cut pieces of *A. esculentus* fruits. Thus the culture of *E. vittella* was maintained.

2.2.2. *H. armigera*

Eggs of *H. armigera* obtained from National Bureau of Agricultural Insects Resources, Bengaluru, Karnataka State, India were reared separately in multi-cell trays containing chickpea based artificial diet. Two eggs were inoculated per cell and larvae were separated into separate cells on 10th day to avoid cannibalism. Pupae were kept in an adult emergence cage (30 × 30 × 30 cm), and eggs were laid on muslin cloth in a seven liter plastic container in which the female: male ratio was 1:3. Ten per cent honey solution was fed to adults, which were kept at 75% RH. The muslin cloth with eggs was collected from the third day onwards.

2.2.3. *T. chilonis*

Fresh *C. cephalonica* adults obtained from National Bureau of Agricultural Insects Resources, Bengaluru, Karnataka State, India were placed in a cage (21 × 25 cm) and fed with 50% honey to collect eggs [13]. The eggs, cleaned with a scale separator, were sprinkled @ one cc in a plastic trough (45 × 30 × 10 cm) containing broken pearl millet grains @ 2.5 kg + 10 g of yeast and secured with khada cloth. The medium was mixed with 5 g of wettable sulphur 80 WP and streptomycin sulphate 0.5% to protect the growing larvae from storage mite and bacterial diseases. Eggs of *C. cephalonica* were used for rearing *T. chilonis*. The irradiated eggs were pasted on cardboard and kept in polythene bags along with parasitoids @ 6:1 ratio for parasitization. The parasitized cards were used for bioassay after eight days.

2.2.4. *C. zastrowi sillemi*

Grubs of *C. zastrowi sillemi* obtained from National Bureau of Agricultural Insects Resources, Bengaluru, Karnataka State, India were reared on irradiated eggs of *C. cephalonica* placed in a galvanized iron (GI) round trough (28 cm dia) @ 250 grubs per trough, and the troughs were secured with closely woven khada cloth. Five feedings were offered @ 2.5 cc of eggs/feeding on alternate days. The silken cocoons of the predator were kept in one-liter plastic containers with wire mesh windows for emergence and were maintained at 26 ± 2 °C, RH 75 ± 5%. The adults were allowed into a GI trough for mating and egg laying on brown paper sheets wrapped on the wall of the trough. Two small sponges soaked with adult diet (yeast powder, fructose, honey, protinex at 1:1:1:1) were kept on the nylon cloth as adult feed which was used to secure the trough. The brown paper sheets containing stalked eggs were dislodged and used for further culturing.

2.3. Preparation of acetone extracts of *A. esculentus*

Acetone extracts (1%) of different plant parts (flowers, young

and old fruits and young and old leaves) of highly susceptible hybrid (No. 55), susceptible cultivar (Arka Anamika) and resistant germplasm (AE 9) were prepared for bioassays with eggs of *E. vittella* and *H. armigera*. Twenty g of young and old leaves collected on 30 and 65th day after dibbling, flowers on 35th day and young and old fruits on 60th day were chopped, shade dried for 12 h and taken in a 250 ml conical flask. One hundred ml of acetone (HPLC grade) was poured into the individual conical flask containing chopped plant materials. The mouths of the flasks were covered with non-absorbent cotton and the materials were incubated for 72 h in a water bath (Genuine model) at 28 °C for two hours, followed by 20 min at 50 °C. The supernatant was subsequently concentrated in rotary vacuum evaporator at 40 °C (LARK model, Bengaluru) and stored in a deep freezer (REMI model, Bengaluru) at –20 °C. A concentration of one per cent (10000 ppm) of each extract was prepared after dilution with acetone and used throughout the experiment.

2.4. Preparation of egg card

Clean, healthy, 0–24 h old eggs of *E. vittella* and *H. armigera* sterilized by exposure to 4 W UV light for 30 min were washed twice in hexane to remove the traces of scales or natural kairomones and then shade dried. Egg card was prepared by sticking eggs of both insects @ 80 to 100 on cardboard (7 × 2 cm) with gum Arabica. Kairomone extracts (10000 ppm) of different parts of three genotypes of *A. esculentus* were used separately to treat the eggs of host insects (5 µl/card) [14]. Each treatment was replicated eight times. The control egg card was treated with acetone alone.

2.5. Bio-assay-choice test

The treated egg cards were arranged equidistantly in a petridish (150 × 15 mm dia) and *T. chilonis* adults were released in the centre at 6:1 ratio. Egg cards taken in a glass tube (7.5 × 2.5 cm) after one day exposure were incubated at 23 °C and 65% RH. The per cent parasitization was observed on the 3rd, 5th and 7th day after exposure. Choice tests were conducted separately for each extract and each host species. Similarly, one second instar of *C. zastrowi sillemi* was released in a glass tube containing one egg card of *E. vittella* and *H. armigera* (700–750 numbers/card), treated with various extracts. Per cent predation was estimated 24 h after release [15].

2.6. Statistical analysis

Laboratory and field experiments were conducted in a Randomized Block Design. Data on larval counts of *E. vittella*, *H. armigera* and *C. zastrowi sillemi* were subject to square root transformation while per cent shoot damage, parasitization, and predation by *T. chilonis* and *C. zastrowi sillemi* and per cent recovery of *T. chilonis* were subject to arcsine transformation before using Analysis of Variance (IRRSTAT). Means were separated by Tukey's test.

3. Results

3.1. Population and shoot damage of *E. vittella*

Among various variety/hybrid/germplasm of *A. esculentus* screened, the hybrid No. 55 had the greatest susceptibility to *E. vittella* with a mean population of 16.66 ± 1.57 larvae/10 plants, followed by Arka Anamika, Indam 9821, OH 597, Ankur 40, No. 10, BISCOS 902 and US 7136 with a mean population of 8.93 ± 0.57 , 7.86 ± 0.83 , 7.8 ± 0.79 , 7.66 ± 0.48 , 7.66 ± 0.52 , 7.6 ± 0.57 and 7.13 ± 0.97 larvae/10 plants, respectively (Fig. 1A). Shoot damage by

E. vittella was maximum in No. 55 (21.20%), followed by Arka Anamika (17.21%), while AE 9 was resistant with a mean shoot damage of 7.40% (Fig. 2B).

3.2. Population of *H. armigera*

The hybrid, No. 55 was preferred by *H. armigera* with a mean population of 15.4 ± 0.98 larvae/10 plants, followed by Arka Anamika, OH 597, Indam 9821, No. 10, BISCO-S-902, and Ankur with the mean population of 11.26 ± 0.89 , 10.26 ± 0.75 , 10.09 ± 0.97 , 9.93 ± 0.95 , 9.8 ± 0.98 and 9.66 ± 0.87 larvae/10 plants, respectively. AE 9 recorded the lowest mean population of 4.2 ± 0.67 larvae/10 plants (Fig. 1B).

3.3. Fruit damage by *E. vittella* and *H. armigera*

Fruit damage by borers in various entries of *A. esculentus* ranged from 7.00 to 31.67%. The highest fruit damage of 31.67% was noticed in No. 55, followed by Arka Anamika (22.33%). Fruit damage was lowest in AE 9 5.33% (Fig. 2A).

3.4. Population of *C. zastrowi sillemi*

The population of *C. zastrowi sillemi* was 0.3–3.0% per 10 plants in various entries of *A. esculentus*. It was 3.00 ± 0.57 per 10 plants in No. 55, 2.50 ± 0.27 per 10 plants in Arka Anamika and 0.33 per 10 plants in AE 9 (Fig. 3A).

3.5. Field recovery of *T. chilonis* adults

Field recovery of *T. chilonis* was maximum in No. 55 (9.33%), followed by Arka Anamika (6.44%). It was minimum in AE 6 (1.11%) (Fig. 3B).

3.6. *T. chilonis* on extract-treated eggs of *E. vittella*

Acetone extracts of various parts of highly susceptible (No. 55) and susceptible (Arka Anamika) genotypes of *A. esculentus* significantly increased the activity of *T. chilonis* on the eggs of *E. vittella* at 10000 ppm when compared to resistant (AE 9) genotype. The parasitization level by *T. chilonis* on eggs of *E. vittella* treated with acetone extract of flowers of No. 55 was highest (18.33%) during the third day after introduction of parasitoids, and it was significantly greater than on acetone extracts of young fruits (15.33%), young leaves (12.67%) and old fruits of No. 55 (11.67%), while it was only 2.67% in the acetone treated control eggs. On the 5th day after introduction, the parasitization by *T. chilonis* was 47.67%, which was superior to that from acetone extracts of various parts of susceptible and resistant genotypes of *A. esculentus*, as well as the 4.67% in acetone treated control eggs. The level of parasitization by *T. chilonis* on eggs of *E. vittella* treated with acetone extract of flowers of No. 55 was 53.33 during the seventh day after introduction of parasitoids whereas it was 16.67% in acetone treated control eggs (Table 1).

3.7. *C. zastrowi sillemi* on extract-treated eggs of *E. vittella*

Eggs of *E. vittella* treated with acetone extracts of flowers from No. 55 (10,000 ppm) enhanced the predatory activity of *C. zastrowi sillemi*. The predation was 58.83%, 24 h after egg exposure, which was higher than from predation on eggs exposed to acetone extract of various parts of susceptible and resistant genotypes of *A. esculentus*, as compared to acetone treated control eggs (10.17%) (Table 2).

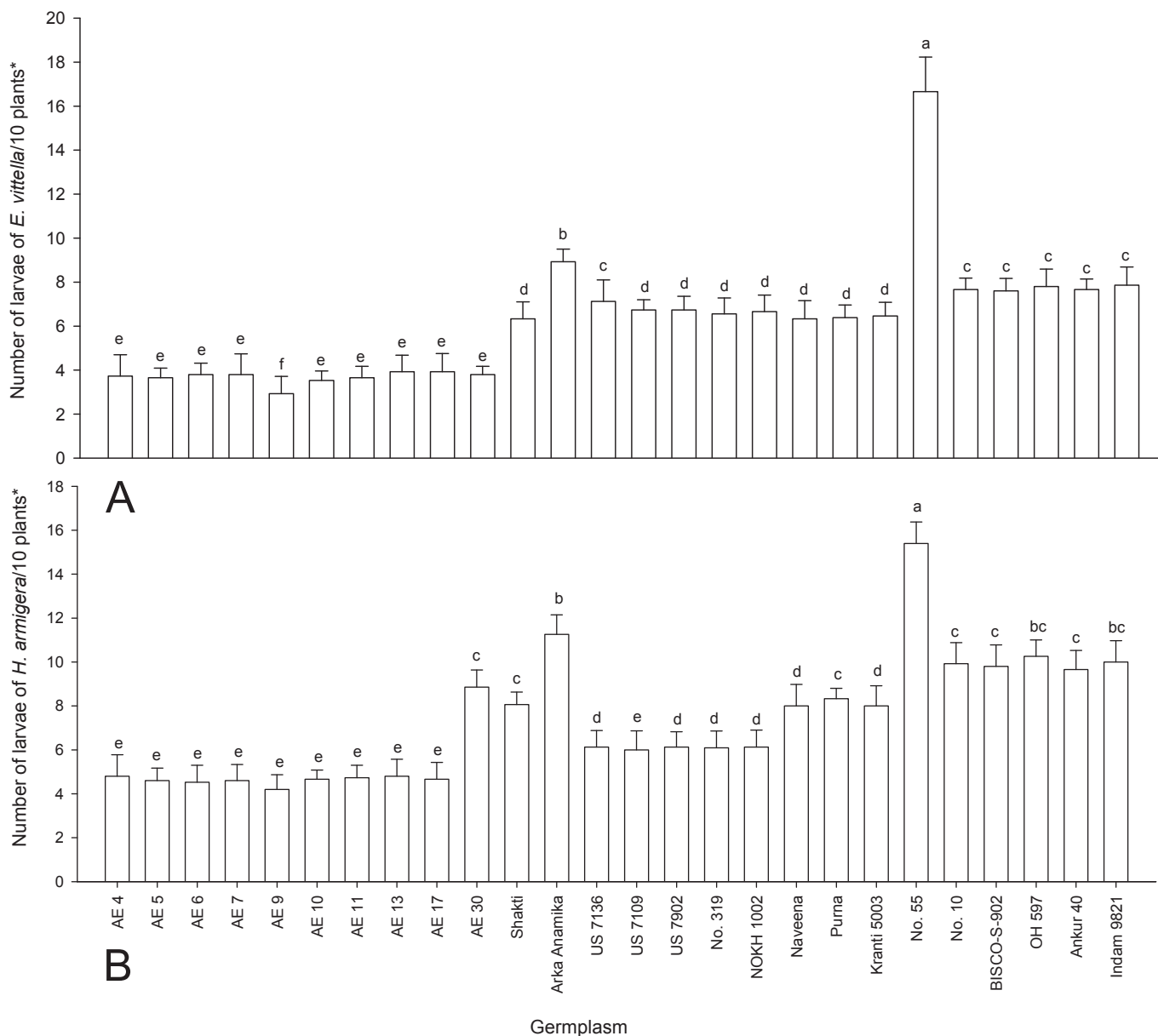


Fig. 1. Population of *E. vittella* (A) and *H. armigera* (B) in germplasm/variety/hybrid of okra. Means (\pm (SE) standard error) followed by the same letters above bars indicate no significant difference ($P < 0.05$) according to a Tukey test.

3.8. *T. chilonis* on extract-treated eggs of *H. armigera*

Acetone extract of flowers of No. 55 enhanced the percentage parasitization by *T. chilonis* on eggs of *H. armigera* during third day after introduction of parasitoids to 22.63%. The flowers extract elicited a significantly higher parasitization percentage than acetone extract of young fruits (18.67%), old fruits (17.67%) and young leaves of No. 55 (15.67%), as well as the 5.50% in acetone treated control eggs. On fifth day after introduction, the parasitization by *T. chilonis* was 57.00% which was comparatively higher than that resulting from acetone extracts of various parts of susceptible and resistant genotypes of *A. esculentus*, and the 9.33% parasitization of acetone treated control eggs. The percentage of parasitization by *T. chilonis* on eggs of *E. vittella* treated with acetone extract of flowers of No. 55 was 65.33%, during the seventh day after introduction of parasitoids whereas it was 10.67% in acetone treated control eggs of *H. armigera* (Table 3).

3.9. *C. zastrowi sillemi* on extract-treated eggs of *H. armigera*

Eggs of *H. armigera*, treated with acetone extract of flowers of No. 55 enhanced the predatory activity of *C. zastrowi sillemi* compared with control eggs. The predation was 68.67%, 24 h after exposure of eggs to predator which were less in acetone extract of various parts of susceptible and resistant genotypes of *A. esculentus*. The predation was 10.50% in acetone treated control eggs of *H. armigera* (Table 4).

4. Discussion

Visual stimuli, and contact and volatile chemicals play a vital role in the host-selection sequences [16]. Volatiles may emanate from the plant, the host insects and host by-products. The volatile chemicals which guide the parasitoids to locate their hosts are called kairomones or synomones [17].

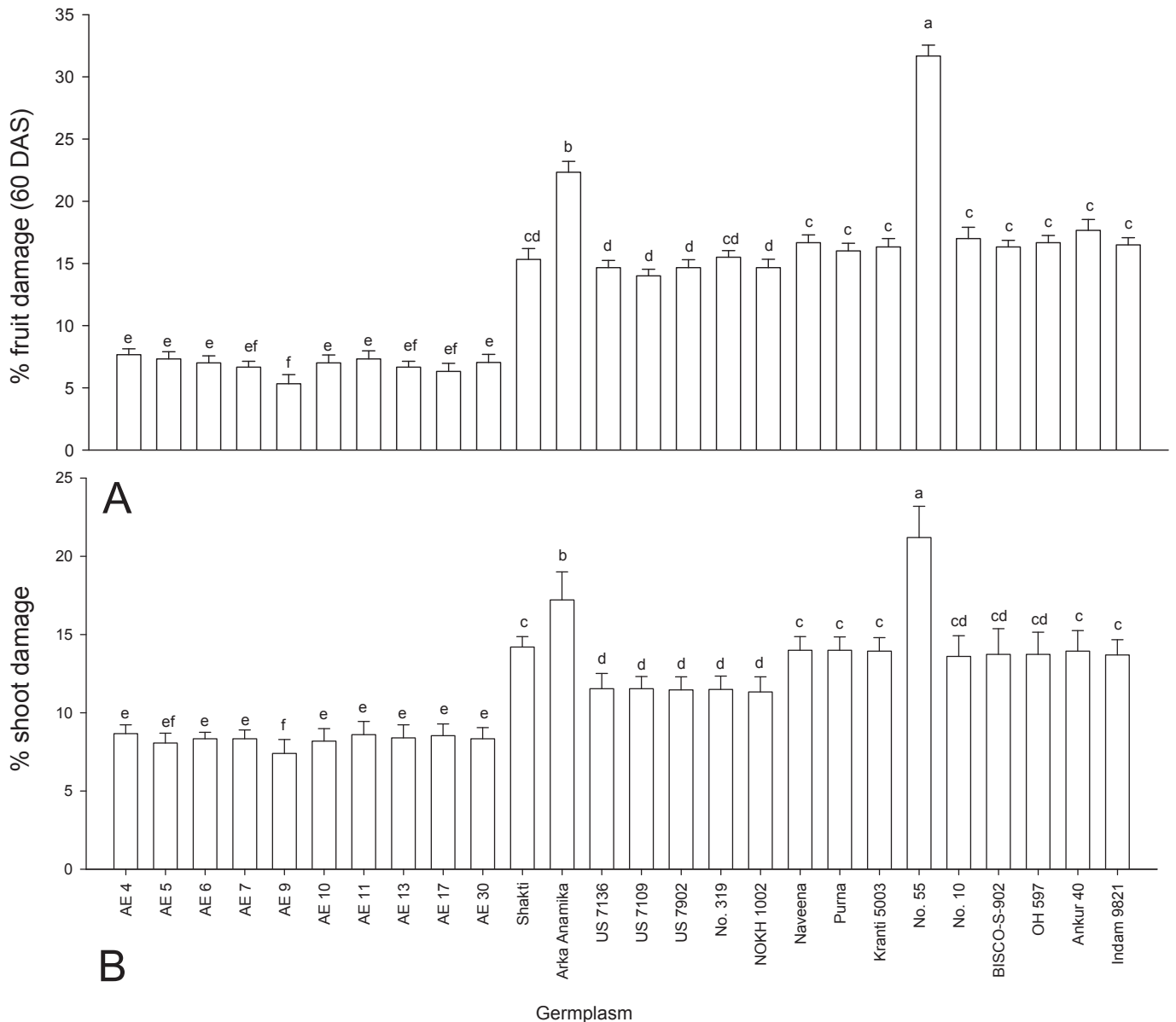


Fig. 2. A. Shoot damage by *E. vittella* in germplasm/variety/hybrid of okra B. Percent fruit damage by *E. vittella* and *H. armigera* in germplasm/variety/hybrid of okra. Means (\pm (SE) standard error) followed by the same letters above bars indicate no significant difference ($P < 0.05$) according to a Tukey test.

Many important tri-trophic interactions of field environments involve the use of plant chemical cues by entomophages to locate insects [18]. In long- or short-range searching for habitats of their insect hosts, natural enemies use host-plant phyto-chemicals to enhance their searching effectiveness. Plants invite entomophages for rescue through releasing volatiles in response to herbivour damage which in turn help the plants to protect from insect herbivores. Many parasitoids occur in specific habitats within which their hosts occur, habitat location forming an important aspect in their host selection process [19].

The present studies observed variation in the foraging activities of *T. chilonis* and *C. zastrowi sillemi* on eggs of *E. vittella* and *H. armigera* exposed to volatiles from different genotypes/germplasm of *A. esculentus*, which may possibly be due to the effects of the morphological and biochemical characteristics of host plant belong to same species, as suggested by Hugar et al. [20]. The high populations of *E. vittella* and *H. armigera* feeding on susceptible

genotypes, No. 55 and Arka Anamika suffered significantly greater to foraging activity by *T. chilonis* and *C. zastrowi sillemi* than other genotypes of *A. esculentus* evaluated under field condition. This result is in agreement with Kaur et al. [7] who reported that the parasitoid activity tends to increase in response to the increase of pest incidence. In the present studies, the increased susceptibility of *E. vittella* and *H. armigera* to natural enemies on susceptible genotypes of *A. esculentus* may also be influenced by the survival of great numbers of host insects on susceptible genotypes, and lack of resistance of the herbivore, as witnessed previously by War et al. [5].

Favourable volatile chemicals of shoots and fruits of *A. esculentus* (No. 55) may encourage attraction of *E. vittella* and *H. armigera* to lay more eggs on this genotype than on the others in this study, resulting in higher levels of activity of *T. chilonis* and *C. zastrowi sillemi* than when these pests laid eggs on cultivar Arka Anamika and germplasm, AE 9. The preference of *E. vittella* and *H. armigera*

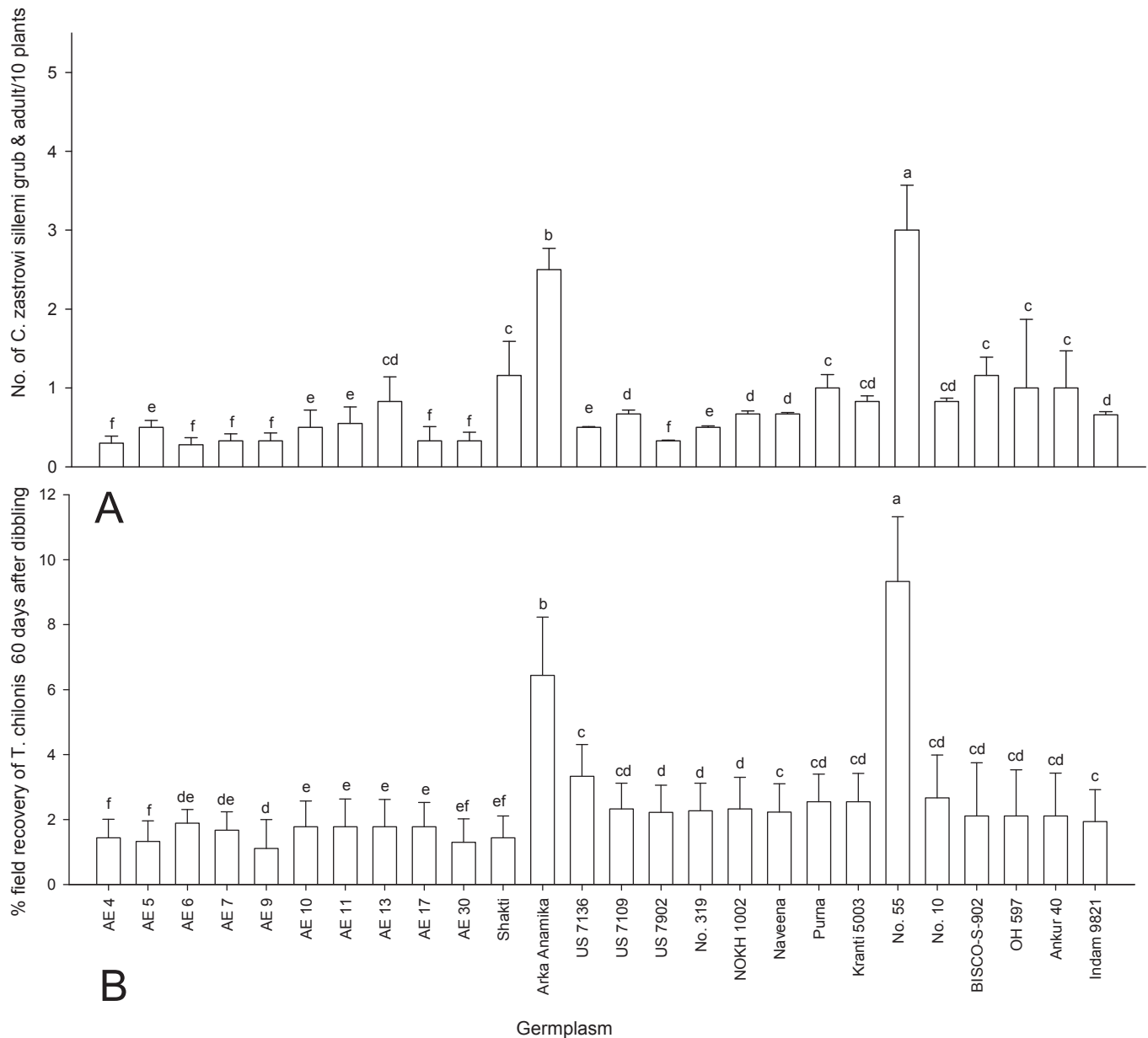


Fig. 3. A. Population of *C. zastrowi sillemi* in germplasm/variety/hybrid of okra B. Per cent field recovery of *T. chilonis* in germplasm/variety/hybrid of okra. Means (\pm (SE) standard error) followed by the same letters above bars indicate no significant difference ($P < 0.05$) according to a Tukey test.

and their entomophages, *T. chilonis* and *C. zastrowi sillemi* was for *A. esculentus* genotype of No. 55, followed in descending order by Arka anamika, Indam 9821, OH 597, Ankur 40, No. 10, BISCO 902, US 7136 and AE 9. This difference in abundance may be due to the host plant phenology and herbivore induced chemical composition of the genotypes of *A. esculentus*. Firake *et al.* [21] found similarly that, although the female, *H. ebeninus* had a preference for host larvae in a particular experimental crop (Brodeur *et al.* [22]), they were able to locate high-preference hosts on other host plants.

The result of high (No. 55) and low (AE 9) abundance of host insects and their entomophages on *A. esculentus* suggests that allelochemicals were ingested by the host insects from the host genotype which directly influenced the foraging activity of entomophages. It is possible also that low abundances resulted from antibiosis effects of allelochemicals and/or poor nutritional quality, present in AE 9 which caused them to be sub-optimal prey [23].

Herbivory-induced changes in biochemical composition of host plants can also influence the growth and survival of herbivores [24–26] which in turn influences the abundance of entomophages [27]. The parasitization rate or preference was reported to be high on eggs of *H. armigera* when deposited on susceptible rather than resistant genotypes of chickpea [28] and also the parasitism level of *C. chloridae* varied across the genotypes of chickpea [7,29]. The wild tomato, *Lycopersicon hirsutum f. glabratum* (accession PI 134417) is another example of this phenomenon, where the parasitic activities of *Camponotus sonorensis* (Cameron) and *Cotesia congregata* (Say) were reduced but there was no effect on parasitism by *Cotesia marginiventris* (Cresson) and *Cardiochiles nigriceps* Viereck [30]. However such effects were not reported in tests with different pigeonpea genotypes. The results of the present studies suggested that the germplasm AE 1 to 10 were not compatible with entomophages of *E. vittella* and *H. armigera*. The genotypes of

Table 1

Per cent parasitism by *T. chilonis* on eggs of *E. vittella*, as influenced by acetone extracts of various parts of genotypes of *A. esculentus* (okra). Means (\pm (SE) standard error) within the sample indicate the same letter has no significant difference ($P \leq 0.05$) according to a Tukey's test.

Okra samples	% parasitization by <i>T. chilonis</i> after		
	3 rd day	5 th day	7 th day
Flowers of			
No. 55 (HS)	18.33 \pm 0.0 ^a	47.67 \pm 0.03 ^a	53.33 \pm 0.02 ^a
Arka Anamika (S)	11.67 \pm 0.05 ^d	37.33 \pm 0.01 ^c	46.33 \pm 0.03 ^c
AE 9 (R)	9.67 \pm 0.02 ^f	12.67 \pm 0.04 ⁱ	16.67 \pm 0.01 ⁱ
Young fruits of			
No. 55 (HS)	15.33 \pm 0.03 ^b	43.33 \pm 0.02 ^b	48.67 \pm 0.04 ^b
Arka Anamika (S)	10.33 \pm 0.01 ^e	31.67 \pm 0.01 ^e	40.67 \pm 0.01 ^d
AE 9 (R)	8.33 \pm 0.04 ^f	10.67 \pm 0.04 ^j	13.67 \pm 0.03 ^j
Old fruits of			
No. 55 (HS)	11.67 \pm 0.01 ^d	32.33 \pm 0.05 ^d	35.67 \pm 0.02 ^f
Arka Anamika (S)	5.33 \pm 0.05 ^e	25.67 \pm 0.01 ^h	28.33 \pm 0.01 ^h
AE 9 (R)	5.33 \pm 0.03 ^g	8.67 \pm 0.03 ^k	10.33 \pm 0.03 ^l
Young leaves of			
No. 55 (HS)	12.67 \pm 0.01 ^c	31.33 \pm 0.02 ^e	37.67 \pm 0.06 ^e
Arka Anamika (S)	9.67 \pm 0.02 ^e	30.67 \pm 0.01 ^f	35.33 \pm 0.04 ^f
AE 9 (R)	5.67 \pm 0.04 ^g	7.67 \pm 0.03 ^l	11.33 \pm 0.03 ^k
Old leaves of			
No. 55 (HS)	10.67 \pm 0.01 ^e	29.67 \pm 0.03 ^f	35.67 \pm 0.01 ^f
Arka Anamika (S)	8.33 \pm 0.04 ^f	28.67 \pm 0.04 ^g	32.67 \pm 0.02 ^g
AE 9 (R)	4.33 \pm 0.03 ^h	6.67 \pm 0.02 ^m	10.67 \pm 0.03 ^m
Control	2.67 \pm 0.01 ⁱ	4.67 \pm 0.01 ⁿ	6.67 \pm 0.01 ⁿ

Table 2

Per cent predation by *C. carnea* on eggs of *E. vittella*, as influenced by acetone extracts of various parts of genotypes of *A. esculentus* (okra). Means (\pm (SE) standard error) within the sample indicate the same letter has no significant difference ($P \leq 0.05$) according to a Tukey's test.

Okra samples	% predation by <i>C. zastrowi sillemi</i> after 24 h
Flowers of	
No. 55 (HS)	58.83 \pm 0.01 ^a
Arka anamika (S)	48.83 \pm 0.04 ^b
AE 9 (R)	27.47 \pm 0.02 ⁱ
Young fruits of	
No. 55 (HS)	47.87 \pm 0.02 ^b
Arka anamika (S)	45.17 \pm 0.03 ^c
AE 9 (R)	29.17 \pm 0.05 ^e
Old fruits of	
No. 55 (HS)	40.83 \pm 0.03 ^d
Arka anamika (S)	41.83 \pm 0.01 ^d
AE 9 (R)	25.17 \pm 0.02 ^e
Young leaves of	
No. 55 (HS)	38.13 \pm 0.01 ^d
Arka anamika (S)	23.87 \pm 0.04 ^f
AE 9 (R)	18.17 \pm 0.03 ^m
Old leaves of	
No. 55 (HS)	27.17 \pm 0.04 ^e
Arka anamika (S)	19.83 \pm 0.05 ^g
AE 9 (R)	16.17 \pm 0.03 ^g
Control	10.17 \pm 0.01 ^h

A. esculentus, No. 55 and Arka Anamika, are hospitable to *T. chilonis* and *C. zastrowi sillemi* and are amenable for use in minimizing the damage by *E. vittella* and *H. armigera* in *A. esculentus*.

Floral parts of any crop are an attractive stage for egg laying by many lepidopteran insects; thus, it is not unexpected that natural enemies might be attracted to floral volatiles also. The flower extract of *A. esculentus* genotype, No. 55 of the present study, may contain kairomonal substances responsible for enhancing the percentage parasitization by *T. chilonis* from 6.67% (hexane treated eggs) to 55.33% (1% of flower extract of No. 55) and percentage predation by *C. zastrowi sillemi* from 10.67% (hexane treated eggs) to 58.22% (1% of flower extract of No. 55) on eggs of *E. vittella*. Similarly, on eggs of *H. armigera*, the percentage parasitization of

Table 3

Per cent parasitism by *T. chilonis* on eggs of *H. armigera*, as influenced by acetone extracts of various parts of genotypes of *A. esculentus* (okra). Means (\pm (SE) standard error) within the sample indicate the same letter has no significant difference ($P \leq 0.05$) according to a Tukey's test.

Okra samples	% parasitization by <i>T. chilonis</i> after		
	3 rd day	5 th day	7 th day
Flowers of			
No. 55 (HS)	22.63 \pm 0.03 ^a	57.00 \pm 0.03 ^a	65.33 \pm 0.02 ^a
Arka Anamika (S)	15.67 \pm 0.01 ^{bc}	47.33 \pm 0.01 ^c	56.67 \pm 0.01 ^b
AE 9 (R)	12.67 \pm 0.05 ^c	20.33 \pm 0.02 ^f	25.33 \pm 0.04 ^f
Young fruits of			
No. 55 (HS)	18.67 \pm 0.02 ^b	51.33 \pm 0.03 ^b	58.00 \pm 0.01 ^b
Arka Anamika (S)	15.33 \pm 0.03 ^{bc}	43.67 \pm 0.01 ^d	45.67 \pm 0.03 ^d
AE 9 (R)	10.33 \pm 0.04 ^d	18.33 \pm 0.04 ^{fg}	21.67 \pm 0.01 ^f
Old fruits of			
No. 55 (HS)	17.67 \pm 0.0 ^b	48.33 \pm 0.02 ^{bc}	51.67 \pm 0.01 ^c
Arka Anamika (S)	13.33 \pm 0.02 ^c	40.67 \pm 0.01 ^{de}	42.67 \pm 0.04 ^{de}
AE 9 (R)	10.33 \pm 0.05 ^d	15.67 \pm 0.04 ^f	18.63 \pm 0.02 ^{fg}
Young leaves of			
No. 55 (HS)	15.67 \pm 0.02 ^{bc}	38.67 \pm 0.05 ^e	47.67 \pm 0.03 ^d
Arka Anamika (S)	12.67 \pm 0.03 ^c	35.67 \pm 0.03 ^e	42.33 \pm 0.04 ^d
AE 9 (R)	10.33 \pm 0.01 ^d	13.33 \pm 0.02 ^g	15.67 \pm 0.01 ^m
Old leaves of			
No. 55 (HS)	9.67 \pm 0.04 ^{de}	30.67 \pm 0.04 ^e	40.67 \pm 0.04 ^{de}
Arka Anamika (S)	10.33 \pm 0.05 ^d	29.67 \pm 0.02 ^e	38.67 \pm 0.01 ^e
AE 9 (R)	8.33 \pm 0.01 ^e	12.67 \pm 0.03 ^g	15.67 \pm 0.03 ^g
Control	5.503 \pm 0.03 ^f	9.33 \pm 0.01 ^h	10.67 \pm 0.02 ^h

Table 4

Per cent predation by *C. carnea* on eggs of *H. armigera*, as influenced by acetone extracts of various parts of genotypes of *A. esculentus* (okra). Means (\pm (SE) standard error) within the sample indicate the same letter has no significant difference ($P \leq 0.05$) according to a Tukey's test.

Okra samples	% predation by <i>C. zastrowi sillemi</i> after 24 h
Flowers of	
No. 55 (HS)	68.67 \pm 0.01 ^a
Arka anamika (S)	59.33 \pm 0.04 ^b
AE 9 (R)	37.67 \pm 0.02 ^d
Young fruits of	
No. 55 (HS)	55.33 \pm 0.02 ^b
Arka anamika (S)	41.67 \pm 0.03 ^c
AE 9 (R)	32.67 \pm 0.05 ^d
Old fruits of	
No. 55 (HS)	45.33 \pm 0.03 ^c
Arka anamika (S)	42.67 \pm 0.01 ^c
AE 9 (R)	28.33 \pm 0.02 ^{de}
Young leaves of	
No. 55 (HS)	40.33 \pm 0.01 ^g
Arka anamika (S)	25.67 \pm 0.04 ^e
AE 9 (R)	18.67 \pm 0.03 ⁿ
Old leaves of	
No. 55 (HS)	38.67 \pm 0.04 ^d
Arka anamika (S)	23.33 \pm 0.05 ^e
AE 9 (R)	16.33 \pm 0.03 ^f
Control	10.50 \pm 0.01 ^g

T. chilonis and percentage predation *C. zastrowi sillemi* were enhanced from 10.67 to 65.33% and 10.67 to 68.67%, respectively. A blend of chemicals present in flower extracts of highly susceptible genotype of *A. esculentus* may contribute to attractiveness of No. 55 to *T. chilonis* and *C. zastrowi sillemi*. Identification of chemicals present in the most promising extracts of *A. esculentus* may play vital role in management of other lepidopteran pests [26]. Such an effect was observed in cotton, where extracts from genotypes DHH-543, DHH-11, DLSA-17, jayadhar and LRA- 5166 showed higher EAG responses from *Chrysoperla* in correlation with higher levels of egg laying, which is not unreasonable that the higher titer of the detectable volatile caryophyllene oxide in cotton genotypes may be responsible for the increased attraction. Braconids, ichneumonids

and trichogrammatids are twice as abundant in cotton cultivars with extra-floral nectarines that act as an energy source, with sugars and varying types of amino acids, increasing the longevity of parasitoids like *Trichogramma* which contributed in the control of bud worms and boll worms of cotton [31]. *Trichogramma chilonis* showed high preference on *H. armigera* eggs deposited on flowers and young squares of cotton in the presence of nectar source. *Campoletis sonorensis* and *Microplitis croceipes* lived longer, exhibited higher fecundity and showed increased vigour in nectaried cotton varieties [32]. As *A. esculentus* and cotton are members of the family Malvaceae, the chemical profile detected (Caryophyllene, octadecane, undecane and dodecane in LRA and hexadecenoic acid) in the flowers and squares of cotton genotypes of suvin, TCHB, MCU 7 and MCU 11 might also be present in flower extract of *A. esculentus* and favourable for foraging activity of *T. chilonis* [33].

Higher quantities of octadecanoic acid in yellow stem-borer damaged rice plant play a pivotal role in attracting *Trichogramma* spp. in addition to stimulate oviposition behaviour [26]. The volatiles like 9, 12, 15 octadecatrienoic acid and 9-octadecenal and hexadecane, heptadecane, pentadecane and hexadecanoic acid may have been responsible for the attraction of *T. chilonis* and *T. japonicum*, respectively [11]. A total of 16 compounds including alkanes hydrocarbons, monoterpenes, sesquiterpenes and diterpenes present in tomato fruit volatile and 19 compounds in tomato leaf volatiles were conducive chemical cues for high foraging activity of *T. chilonis* on eggs of *H. armigera* [10].

As noted by several authors on various field crops, genotypes of *A. esculentus* differentially attracted or repelled different herbivores. Such differences can be helpful to the natural enemies for locating the host insects. Plants use the third trophic level as another line of defense against herbivores, besides producing volatile and non-volatile compounds in response of damage of herbivores. It may be better to include susceptible genotypes of crops as one of the components in Integrated Pest Management rather than resistant genotypes alone, although the resistant genotypes are helpful for the success of biological control efforts and reduce pesticides usage. The chemical profiles, which are supportive of natural enemies, may vary from genotype to genotype, and need to be identified to determine how they influence the trophic interaction in a particular eco-system. Volatiles which are favourable to entomophages and the genes controlling the traits need to be identified to incorporate these traits in further breeding programmes.

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References

- [1] C.M. De Moraes, W.J. Lewis, J.H. Tumlinson, Examining plant-parasitoid interactions in tritrophic systems, *An. Soc. Entomol. Brasil* 29 (2000) 189–203.
- [2] J.H.R. Gross, W.J. Lewis, M. Beevers, D.A. Nordlund, *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae): effects of augmented densities and distributions of *Heliothis zea* (Lepidoptera: Noctuidae) host eggs and kairomones on field performance, *Environ. Entomol.* 13 (1984) 981–985.
- [3] J.H. Tumlinson, T.C.J. Turlings, W.J. Lewis, Semiochemically mediated foraging behaviour in beneficial parasitic insects, *Arch. Insect Biochem. Physiol.* 22 (1993) 385–391.
- [4] T.C.J. Turlings, J.H. Tumlinson, Systemic release of chemical signals by herbivore injured corn, *Proc. Natl. Acad. Sci. U. S. A.* 89 (1992) 8399–8402.
- [5] A.R. War, H.C. Sharma, M.G. Paulraj, M.Y. War, S. Ignacimuthu, Herbivore induced plant volatiles—their role in plant defense for pest management, *Pl. Sig. Behav.* 6 (2011) 1973–1978.
- [6] S. Senthil-Nathan, K. Kalaivani, R.W. Mankin, K. Murugan, Effects of millet, wheat, rice, and sorghum diets on development of *Corcyra cephalonica* (Stainton) (Lepidoptera: Galleriidae) and its suitability as a host for *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae), *Environ. Entomol.* 35 (3) (2006) 784–788.
- [7] E.S. Edwin, P. Vasanth-Srinivasan, A. Ponsankar, A. Thanigaivel, S. Selin-Rani, R.W. Mankin, S. Senthil-Nathan, et al., Effects of temperature and nonionizing ultraviolet radiation treatments of eggs of five host insects on production of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) for biological control applications, *J. Asia-Pacific Entomol.* 19 (4) (2016) 1139–1144.
- [8] M.K. Dhillon, H.C. Sharma, Survival and development of *Campoletis chloridae* on various insect and crop hosts: implications for Bt-transgenic crops, *J. Appl. Entomol.* 131 (2007) 179–185.
- [9] P.L. Tandon, N. Bakthavatsalam, Influence of sunflower genotypes on parasitization efficiency of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) on *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), *J. Biol. Control* 18 (2004) 252–261.
- [10] P.L. Tandon, N. Bakthavatsalam, Lant volatile diversity in different tomato genotypes and its influence on parasitization efficiency of *Trichogramma chilonis* Ishii on *Helicoverpa armigera* (Hübner), *J. Biol. Control* 21 (2007) 271–281.
- [11] D. Bhagat, N. Bakthavatsalam, Influence of rice cultivars on the parasitization efficiency of *Trichogramma chilonis* Ishii and *Trichogramma japonicum* Ashmead, *J. Biol. Control* 26 (2012) 424–431.
- [12] NRC. *Lost Crops of Africa, II. National Academies Press, Washington, DC.*
- [13] A.V.N. Paul, M. Srivastava, P. Dureja, A.K. Singh, Semiochemicals produced by tomato varieties and their role in parasitism of *Corcyra cephalonica* (Lepidoptera: Pyralidae) by the egg parasitoid *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae), *Int. J. Trop. Insect Sci.* 28 (2008) 108–116.
- [14] S. Singh, A.V.N. Paul, A.K. Singh, Host effect on the quality of *Trichogramma brasiliensis* and *T. exiguum*, *Indian J. Entomol.* 60 (1998) 379–384.
- [15] A. Kumar, A. Zayem, S. Kanameni, Synomonal effect of cole crops on individual and associative learning behavior of *Cotesia plutellae*, *Int. J. Biol. Pharm. Allied Sci.* 3 (2012) 285–298.
- [16] T.H. Yong, S. Pitcher, J. Gardner, M.P. Hoffmann, Odor specificity testing in the assessment of efficacy and non-target risk for *Trichogramma ostriniae* Hymenoptera: Trichogrammatidae, *Biocontrol Sci. Technol.* 17 (2007) 135–153.
- [17] D.A. Nordlund, W.J. Lewis, R.L. Jones, H.R. Gross, Kairomones and their use for management of entomophagous insects. IV Effects of kairomones on productivity and longevity of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae), *J. Chem. Ecol.* 2 (1976) 67–72.
- [18] T.P. Craig, *Parasitoid Community Ecology*, Oxford University Press, Oxford, 1994, pp. 205–227.
- [19] C.S. Pawar, V.S. Bhatnagar, D.R. Jadhav, *Heliothis* species and their natural enemies, with their potential for biological control, *Proc. Indian Acad. Sci. Anim. Sci.* 95 (1986) 695–703.
- [20] S.V. Hugar, H.C. Sharma, K.B. Goud, Pigeonpea genotypes influence parasitization preference and survival and development of the *Helicoverpa armigera* larval parasitoid, *Campoletis Chloridae*, *Springer Plus* 3 (2014) 378–385.
- [21] D.M. Firake, Lytan Damitre, G.T. Behere, N.S. Azad Thakur, Host plants alter the reproductive behavior of *Pieris brassicae* (Lepidoptera: Pieridae) and its solitary larval endo-parasitoid, *Hyposoter ebenius* (Hymenoptera: Ichneumonidae) in a cruciferous ecosystem, *Fla. Entomol.* 95 (2012) 905–913.
- [22] J. Brodeur, J.B.F. Geervliet, L.E.M. Vet, The role of host species, age and defensive behaviour on ovipositional decisions in a solitary specialist and gregarious generalist parasitoid (*Cotesia* species), *Entomol. Exp. Appl.* 81 (1996) 125–132.
- [23] T. Norin, Semiochemicals for insect pest management, *Pure Appl. Chem.* 79 (2007) 2129–2136.
- [24] A.C. Gange, V.K. Brown, Effects of root herbivory by an insect on a foliar feeding species, mediated through changes in the host plant, *Oecologia* 81 (1989) 38–42.
- [25] T.G. Whitman, J. Maschinski, K.C. Larson, K.N. Paige, Plant response to herbivory: the continuum from negative to positive and underlying physiological mechanisms, in: P.W. Price, T.M. Lewinsohn, G.W. Fernandes, W.W. Benson (Eds.), *Plant Animal Interactions Evolutionary Ecology in Tropical and Temperate Regions*, Wiley and Sons, New York, 1991, pp. 227–256.
- [26] U. Rani, S. Kurra, Specificity of systemically released rice stem volatiles on egg parasitoid, *Trichogramma japonicum* Ashmead behavior, *J. Appl. Entomol.* 136 (2012) 749–760.
- [27] K.A. Bloem, S.S. Duffey, Effect of protein type and quantity on growth and development of larval *Heliothis zea* and *Spodoptera exigua* and the endoparasitoid *Hyposoter exigua*, *Entomol. Exp. Applic.* 54 (1990) 141–148.
- [28] S. Sithanatham, V.R. Rao, W. Reed, The influence of host plant resistance in chickpea on parasitism of *Helicoverpa armigera* Hubner larvae, *Inter. Chickpea Newslett.* 6 (1982) 21–22.
- [29] R.K. Gupta, D. Raj, Natural parasitism by *Campoletis chloridae* Uchida, a

- promising parasitoid of *Helicoverpa armigera* (Hubner) on chickpea, J. Biol. Control 17 (2003) 9–12.
- [30] R.R. Farrar Jr., J.D. Barbour, G.G. Kennedy, Field evaluation of insect resistance in a wild tomato and its effects on insect parasitoids, Entomol. Exp. Applic. 71 (1994) 211–226.
- [31] L. Hanumantharaya, K. Basavana Goud, L. Krishna Nauk, K.A. Kulkarni, Electroantennogram responses of *Chrysoperla carnea* (Stephens) and *Helicoverpa armigera* (Hubner) to volatiles of different cultivars of cotton, Karnataka J. Agric. Sci. 23 (2010) 123–126.
- [32] G.W. Elizen, H.J. Williams, H.B. Vinson, mResponses by the parasitoid *Camponotus sonorensis* (Hymenoptera: Ichneumonidae) to chemicals (Synonomes) in plants: implications for host habitat location, mEnviron. Entomol. 12 (1983) 1973, 1877.
- [33] N. Bakthavatsalam, Tandon, P. L., Patil, S. B., Huga Hosamani, A. Kairomone Formulations as Reinforcing Agents for Increasing Abundance of *Chrysoperla Carnea* (Stephens) in Cotton Ecosystem, J. Biol. Control 21 (1), 1–8.