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Insect pest complex of chilli, *Capsicum annum* L. and their natural enemies in Jabalpur

■ P.S. CHINTKUNTLAWAR[1], U.A. PAWAR* AND A.K. SAXSENA[2]

Department of Agricultural Entomology, K.K. Wagh College of Agriculture, Panchavati, NASHIK (M.S.) INDIA
[1]Department of Plant Protection, Institute of Agricultural Sciences, Palli Siksha Bhavana, Visva Bharti, Bolpur, BIRBHUM (W.B.) INDIA
[2]Department of Agricultural Entomology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, JABALPUR (M.P.) INDIA

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ABSTRACT

A field experiment was conducted at experimental field of the Department of Horticulture, Maharajpur, J.N. Krishi Vishwa Vidyalaya, Jabalpur (M.P.) during *winter* season of 2009-10. In chilli, six species of insect pests and two species of cocinellid predator and one braconid parasitoid of aphid were enumerated. At first sucking pests like whitefly, thrips, aphid and jassid were appeared at 7 days after transplanting and remained active till onset of reproductive stage. The Lepidopteron borers *viz*., *Helicoverpa armigera* and *Spodoptera litura* tobacco caterpillar and gram pod borer appeared were the next group of insects appear on the crop during reproductive stage and remained there till the crop matures. All these are the key pests of chilli in the region which caused colossal yield losses. The natural enemies observed were two species of lady bird beetle and a parasitoid of aphid. Parasitoid of aphid was observed when the crop was about 63 and 84 days old, respectively *i.e.* the reproductive stage it is evident that the natural enemies was present on the aphids when which remained active up to the third week of March *i.e.* aphids population were disappeared in the reproductive stage of the crop.

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***Corresponding author:**
Email: udaypawar91@gmail.com

INTRODUCTION

Chilli, *Capsicum annum* L. (2n=24) is one of the important cash crops grown in almost all parts of the country and is widely grown in the tropics and subtropics as well as under glass houses in temperate regions. It is commonly used as condiments, the pungency in chilli is due to a substance "capsaicin" (Kumar *et al*., 2005). India is a major producer, exporter and consumer of chilli. The area of chilli in the country is 794 thousand hectares with an annual production of 1304 metric tonnes and productivity of 1.6 metric tonnes/ha (Anonymous, 2013).

The major states growing chilli in the country are Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh,

Maharashtra, Orissa, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal etc. Andhra Pradesh ranks first both in area and production (Anonymous, 2005). In Madhya Pradesh area under chilli crop is 54.41 thousand hectare, with total production of 93.57 metric tonnes and productivity with 1.71 metric tonnes/ha (Anonymous, 2013). The major producing districts are Khargone, Dhar, Khandwa, Indore, and Betul (Anonymous, 2005).

The area under Madhya Pradesh is high whereas, production and productivity is less significant. Although there are number of factors responsible for depressing the yield of chilli but incidence of various insect pests is one of major bottlenecks of production.

The insect pests which cause significant damage to the crop are comprises of more than 39 genera and 51 species of insects and mite species in the field as well as in the storage (Hosamani *et al*., 2005). Pests are dynamic in nature and successions of pests occur with the nature of the agro-ecosystem and reports are available on the succession of the insect pests of chilli from the different parts of the country. Various insect and non-insect pests infest chilli; of which thrips, *Scirtothrips dorsalis* Hood (Thripidae: Thysanoptera) is considered as most destructive pest leading to 30 to 50 per cent yield loss under severe infestation (Bhede *et al*., 2008). Thrips alone is reported to be a major pest of chilli in south India and in M.P. (Patel and Khatri, 1982) and Gujarat (Patel *et al.*, 1983) causing 25-50 per cent loss of yield. Whereas, Narvaria (2003) reported 60 to 74 per cent loss in yield. Till date 55 pests are reported to infest chilli crop (Jadhav *et al*., 2004).

In light of these facts and changing scenario of pest complex investigations were planned to study insect pests and their natural enemies associated with chilli crop ecosystem.

MATERIAL AND METHODS

Succession of insect pests and their natural enemies were studied on chilli genotype MHCP-307. Crop was raised following all recommended agronomic practices. Mention datail of nursery raising. Seedling at 3-4 leaved stage were transplanted at 25 days old seedlings in main plot size of 72 sq. m with spacing 50cm × 45cm. Regular observations were taken immediately after germination in the nursery as well as in the transplanted plot and continued till last picking of fruits. The sequence in which the pests and natural enemies appeared was noted on 25 tagged plants, for sucking pest complex (whitefly, thrips, aphids and jassids) nymphs and adults population were recorded on six leaves (two each from bottom, middle and top) of each plant whereas borer complex were recorded on whole plant (Fig. A). Observations were recroded twice in a standard week. The crop was kept unprotected for this purpose. The sequence in which the pests appeared was also noted. The status of different insect pests recorded was determined on the basis of the damage caused by them.

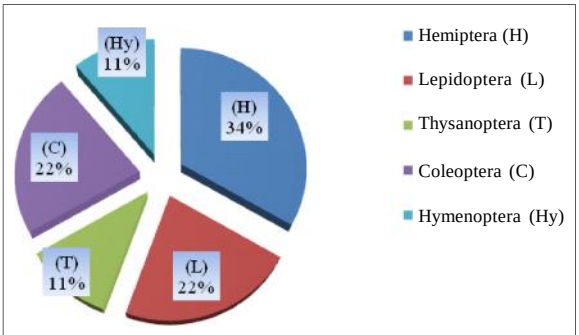

Fig. A : Order wise distribution of insect pests infesting chilli at Jabalpur during 2009–2010

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented in Fig. 1 to 4 and Tables 1 to 4.

Whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) :

Both nymph and adult whitefly were the damaging stages and feed by sucking on the lower side of the leaf. First appearance of whiteflies was observed when the crop age was about 7 days *i.e.* vegetative stage (45th SW) (Table 1 and Fig. 1). From the figure it is evident that the pest was present on the crop during the reproductive stage and remained active upto the first week of April (14th SW) *i.e.* the maturity stage of the crop. However the pest was not observed during 3rd and 4th week of March (11th and 12th SW).

Chilli thrips, *Scirtothrips dorsalis* (Hood) (Thysanoptera: Thripidae):

First appearance of chilli thrips were observed when the crop age was about 21 days *i.e.* vegetative stage (47th SW) (Table 1 and Fig. 1). Nymph and adult thrips

Table 1 : Succession of pest complex and their natural enemies on chilli at Jabalpur during 2009-2010

Date of Obs.	S.W.	Insects				Crop age (DAT)	Crop growth stage
		Common name	Scientific name	Order	Family		
10 Nov., 2009	45	Whitefly	*Bemisia tabaci* (Gennadius)	Hemiptera	Aleyrodidae	7	Vegetative
17 Nov2009	46	Whitefly	*Bemisia tabaci* (Gennadius)	Hemiptera	Aleyrodidae	14	Vegetative
24 Nov2009	47	Whitefly	*Bemisia tabaci* (Gennadius)	Hemiptera	Aleyrodidae	21	Vegetative
		Thrips	*Scirtothrips dorsalis* (Hood)	Thysanoptera	Thripidae		
1 Dec 2009	48	Whitefly	*Bemisia tabaci* (Gennadius)	Hemiptera	Aleyrodidae	28 to	Vegetative and
to	to	Thrip	*Scirtothrips dorsalis* (Hood)	Thysanoptera	Thripidae	56	Reproductive
29 Dec 2009	52	Aphid	*Aphis gossypii* (Glover)	Hemiptera	Aphididae		
1 Jan 2010	1	Whitefly	*Bemisia tabaci* (Gennadius)	Hemiptera	Aleyrodidae	63 to	Reproductive
To	to	Thrips	*Scirtothrips dorsalis* (Hood)	Thysanoptera	Thripidae	77	
19 Jan 2010	3	Aphid	*Aphis gossypii* (Glover)	Hemiptera	Aphididae		
		Lady bird beetle	*Coccinella septempunctata* (Linnaeus)	Coleoptera	Coccinellidae		
			Menochilus sexmaculatus (Fabricius)	Coleoptera	Coccinellidae		
26 Jan 2010	4 to 8	Whitefly	*Bemisia tabaci* (Gennadius)	Hemiptera	Aleyrodidae	84 to	Reproductive
to		Thrips	*Scirtothrips dorsalis* (Hood)	Thysanoptera	Thripidae	112	
23 Feb		Aphid	*Aphis gossypii* (Glover)	Hemiptera	Aphididae		
		Lady bird beetle	*Menochilus sexmaculatus* (Fabricius)	Coleoptera	Coccinellidae		
		Aphid parasitoid	*Aphidius colemani*	Hymenoptera	Braconidae		
2 Mar 2010	9	Whitefly	*Bemisia tabaci* (Gennadius)	Hemiptera	Aleyrodidae	119	Reproductive
		Thrips	*Scirtothrips dorsalis* (Hood)	Thysanoptera	Thripidae		
		Aphid	*Aphis gossypii* (Glover)	Hemiptera	Aphididae		
		Lady bird beetle	*Menochilus sexmaculatus* (Fabricius)	Coleoptera	Coccinellidae		
		Aphid parasitoid	*Aphidius colemani*	Hymenoptera	Braconidae		
		Fruit borer	*Helicoverpa armigera (*Hubner)	Lepidoptera	Noctuidae		
9 Mar 2010	10	Whitefly	*Bemisia tabaci* (Gennadius)	Hemiptera	Aleyrodidae	126	Reproductive
		Thrips	*Scirtothrips dorsalis* (Hood)	Thysanoptera	Thripidae		
		Aphid	*Aphis gossypii* (Glover)	Hemiptera	Aphididae		
		Lady bird beetle	*Menochilus sexmaculatus* (Fabricius)	Coleoptera	Coccinellidae		
		Aphid parasitoid	*Aphidius colemani*	Hymenoptera	Braconidae		
		Fruit borer	*Helicoverpa armigera (*Hubner)	Lepidoptera	Noctuidae		
		Jassid	*Amrasca bigutula* (Ishida)	Hemiptera	Cicadellidae		
16 Mar 2010	11	Whitefly	*Bemisia tabaci* (Gennadius)	Hemiptera	Aleyrodidae	133	Reproductive
		Thrips	*Scirtothrips dorsalis* (Hood)	Thysanoptera	Thripidae		
		Aphid	*Aphis gossypii* (Glover)	Hemiptera	Aphididae		
		Lady bird beetle	*Menochilus sexmaculatus* (Fabricius)	Coleoptera	Coccinellidae		
		Aphid parasitoid	*Aphidius colemani*	Hymenoptera	Braconidae		
		Fruit borer	*Helicoverpa armigera (*Hubner)				
		Jassid	*Spodoptera litura* (Fabricius)	Lepidoptera	Noctuidae		
			Amrasca bigutula (Ishida)	Hemiptera	Cicadellidae		
23 Mar 2010	12	Thrips	*Scirtothrips dorsalis* (Hood)	Thysanoptera	Thripidae	140	Reproductive
		Aphid parasitoid	*Aphidius colemani*	Hymenoptera	Braconidae		
		Fruit borer	*Helicoverpa armigera (*Hubner)	Lepidoptera	Noctuidae		
		Jassid	*Spodoptera litura* (Fabricius)	Lepidoptera	Noctuidae		
			Amrasca bigutula (Ishida)	Hemiptera	Cicadellidae		
30 Mar 2010	13	Thrips	*Scirtothrips dorsalis* (Hood)	Thysanoptera	Thripidae	147	Reproductive
		Jassid	*Amrasca bigutula* (Ishida)	Hemiptera	Cicadellidae		
		Aphid parasitoid	*Aphidius colemani*	Hymenoptera	Braconidae		
6 April 2010	14	Whitefly	*Bemisia tabaci*(Gennadius)	Hemiptera	Aleyrodidae	154	
		Thrips	*Scirtothrips dorsalis* (Hood)	Thysanoptera	Thripidae		Reproductive
		Jassid	*Amrasca bigutula* (Ishida)	Hemiptera	Cicadellidae		

SMW (Standard Meteorological Week) Obs. = Observation SW = Standard week CAD = Crop age in days CGS = Crop growth stage

were the damaging stages and feed by rasping and sucking on the leaf, tender shoot, flower buds and fruits. Under heavy infestations, when buds and flowers are attacked, abortion usually occurs. Thrips attack may also result in premature fruit shed. Thrips feeding causes scarring of flowers and skin blemishes and distortion of fruits (scarring, russeting, fruit cracking or splitting), which affects fruit quality.

From the figure it is evident that the pest was present on the crop during the vegetative stage, reproductive stage and remained active upto the first week of April (14th SW) *i.e.* the maturity stage of the crop

Aphid, *Aphis gossypii* (Glover) (Hemiptera: Aphididae) :

First appearances of aphids were observed when the crop age was about 28 days *i.e.* vegetative stage (48th SW) which both nymph and adult feed on tender shoot and underside of leaf forming colonies they suck sap and secrete a sticky honey-dew which encouraged a sooty mould growth on shoot and leaves (Table 1 and Fig. 1). From the figure it is evident that the pest was present on the crop during the vegetative stage and remained active upto the first week of March (9th SW) *i.e.* the maturity stage of the crop**.**

Jassids, *Amrasca bigutula* (Ishida) (Hemiptera: Cicadellidae):

First appearance of jassids observed on 26th Feb 2010 at the crop was about 126 days old *i.e.* the maturity stage (9th SW) (Table 1 and Fig. 1). From the figure it is evident that the pest was present on the crop during the

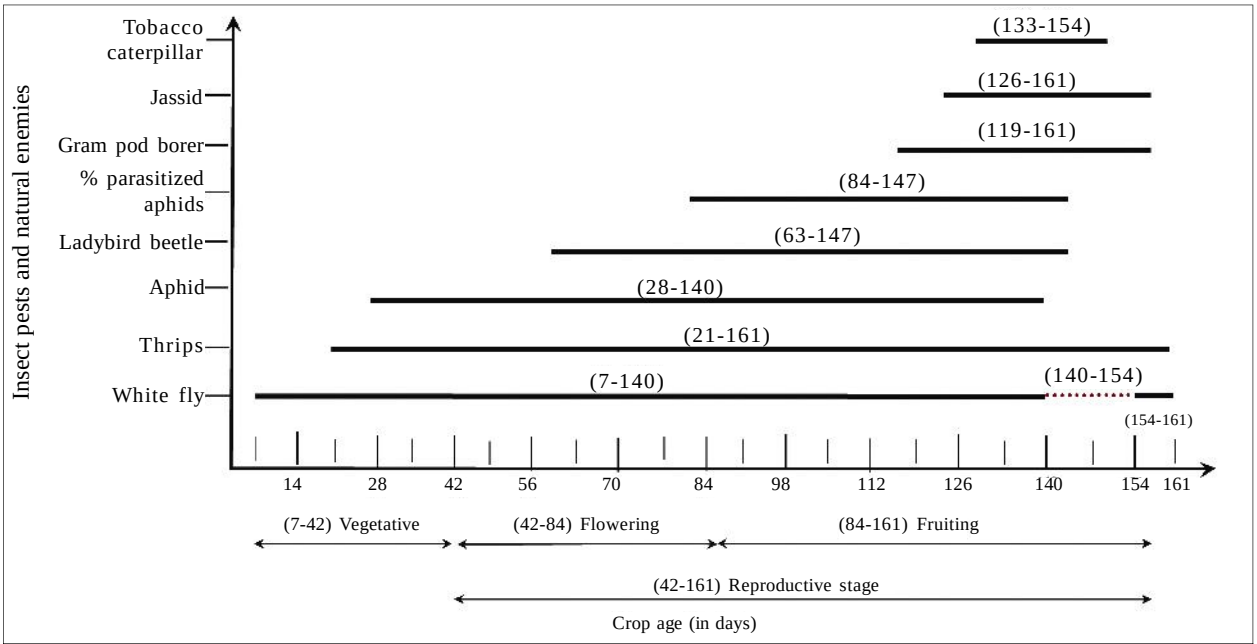

Fig. 1 : Succession of insect pest complex and natural enemies on chilli at Jabalpur during 2009-10

Table 2 : List of insect pests infesting chilli and their natural enemies at Jabalpur during 2009-2010

Common name	Scientific name	Order	Family
Chilli thrips	*Scirtothrips dorsalis* (Hood)	Thysanoptera	Thripidae
White fly	*Bemisia tabaci* (Gennadius)	Hemiptera	Aleyrodidae
Cotton Aphid	*Aphis gossypii* (Glover)		Aphididae
Jassid	*Amrasca bigutula* (Ishida)		Cicadellidae
Gram pod borer	*Helicoverpa armigera* (Hubner)	Lepidoptera	Noctuidae
Tobacco caterpillar	*Spodoptera litura* (Fabricius)		
Ladybird beetle complex	*Menochilus sexmaculatus* (Fabricius)	Coleoptera	Coccinellidae
	Coccinella septempunctata (Linnaeus)		
Aphidus (Parasitized aphid: Mummies)	*Aphidius colemani*	Hymenoptera	Braconidae

reproductive stage and remained active upto the first week of April (14th SW) *i.e.* the maturity stage of the crop**.** Nymphs and adult suck sap from plant attacked plant turn pale colour.

Gram pod borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae):

First appearance of gram pod borer larvae was observed when the crop was about 119 days old *i.e*. the maturity stage (9th SW) early instars feed on leaves and then turn on fruits which bore hole on part of fruit (Table 1 and Fig. 1). From the figure it is evident that the pest was present on the crop during the maturity stage and remained active up to the first week of April (14th SW) *i.e*. maturity stage of the crop.

Tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae):

First appearance of tobacco caterpillar was observed when the crop was about 113 days old *i.e.* reproductive stage (8th SW) (Table 1 and Fig. 1). From the figure it is evident that the pest was present on the crop during reproductive stage and remained active up to the forth week of March (13th SW) *i.e*. the maturity stage of the crop.

Ladybird beetle complex, *Menochilus sexmaculatus* (Fabricius) and *Coccinella septempunctata* (Linnaeus) (Coleoptera: Coccinellidae):

The natural enemy lady bird beetle grub and adults were the predators, which devour eggs of some lepidopteran insect pests, nymph and adult stages of soft bodied insect species *viz.,* whitefly, aphids, jassids and thrips etc.

First appearance of ladybird beetle complex was observed when the crop was about 63 days old *i.e.* reproductive stage (Table 1 and Fig. 1). From the figure it is evident that the natural enemies were present on the crop during the reproductive stage and remained active up to the third week of March *i.e*. aphids population were disappear.

Aphid parasitoid (Aphidius), *Aphidius colemani* (Hymenoptera: Braconidae) :

The female wasp insert egg into the body of both immature and adult aphids, as well as winged and wingless forms of aphids can be parasitized. The larva remains within the aphid's body feeding on the internal tissues and eventually kills it.

The Aphidius larva pupates within the aphid body and spins a cocoon which, in turn, makes the aphid body wall appear gold or bronze in colour. This is known as

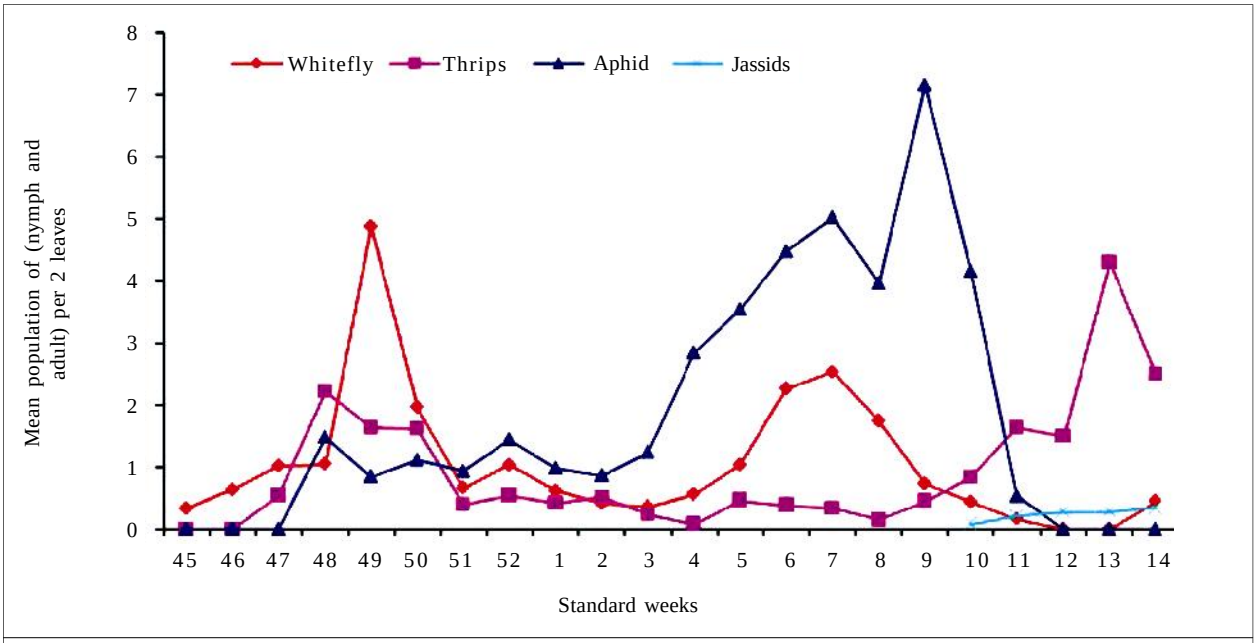

Fig. 2 : SMW (Standard Meteorological Week): Incidence of sucking pest complex on chilli at Jabalpur during 2009-2010

an aphid "mummy". The adult wasp will chew a neat circular hole in the abdomen of the mummy and emerge. This hole is visible once the Aphidius has emerged. First appearance of a parasitoid on aphid was observed is visible once the Aphidius has emerged when the crop was about 84 days old *i.e.* the reproductive stage (Table

Table 3 : Incidence of sucking pest complex on chilli at Jabalpur during 2009-2010

S.W.	Mean population of (adult and nymph) of sucking pest complex / 2 leaves / plant															
	Whitefly (Mean of the observations/S.W.)				Thrips (Mean of two observations/S.W.)				Aphid (Mean of two observations/S.W.)				Jassid (Mean of two observations/S.W.)			
	U	M	L	Mean	U	M	L	Mean	U	M	L	Mean	U	M	L	Mean
45	0.40	0.25	0.35	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
46	0.75	0.60	0.55	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
47	0.75	1.55	0.75	1.02	0.55	0.80	0.30	0.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
48	1.35	1.05	0.75	1.05	2.90	2.30	1.45	2.22	1.30	1.40	1.75	1.48	0.00	0.00	0.00	0.00
49	1.25	0.90	12.5	4.88	2.10	1.45	1.35	1.63	1.05	0.60	0.90	0.85	0.00	0.00	0.00	0.00
50	2.55	1.65	1.70	1.97	1.75	1.75	1.35	1.62	1.00	0.95	1.40	1.12	0.00	0.00	0.00	0.00
51	0.80	0.95	0.25	0.67	0.15	0.80	0.25	0.40	1.10	1.05	0.65	0.93	0.00	0.00	0.00	0.00
52	1.00	1.25	0.85	1.03	0.65	0.40	0.60	0.55	1.15	1.55	1.65	1.45	0.00	0.00	0.00	0.00
1	0.50	0.45	0.90	0.62	0.60	0.55	0.10	0.42	0.80	1.65	0.50	0.98	0.00	0.00	0.00	0.00
2	0.60	0.45	0.20	0.42	0.70	0.60	0.25	0.52	1.75	0.60	0.25	0.87	0.00	0.00	0.00	0.00
3	0.40	0.50	0.20	0.37	0.35	0.30	0.05	0.23	1.55	1.65	0.55	1.25	0.00	0.00	0.00	0.00
4	0.40	0.60	0.70	0.57	0.10	0.05	0.10	0.08	4.60	2.25	1.70	2.85	0.00	0.00	0.00	0.00
5	1.10	0.95	1.05	1.03	0.85	0.50	0.05	0.47	2.75	5.40	2.50	3.55	0.00	0.00	0.00	0.00
6	2.70	2.20	1.90	2.27	0.35	0.70	0.10	0.38	3.85	5.70	3.90	4.48	0.00	0.00	0.00	0.00
7	2.40	3.00	2.20	2.53	0.20	0.65	0.15	0.33	6.65	6.20	2.25	5.03	0.00	0.00	0.00	0.00
8	1.85	1.70	1.70	1.75	0.00	0.30	0.15	0.15	3.30	3.55	5.05	3.97	0.00	0.00	0.00	0.00
9	1.40	0.45	0.35	0.73	0.40	0.50	0.45	0.45	6.35	7.25	7.90	7.17	0.00	0.00	0.00	0.00
10	0.45	0.65	0.20	0.43	0.80	1.00	0.70	0.83	3.40	4.70	4.40	4.17	0.15	0.05	0.05	0.08
11	0.15	0.20	0.15	0.17	1.65	1.75	1.50	1.63	0.35	0.90	0.35	0.53	0.10	0.40	0.15	0.22
12	0.00	0.00	0.00	0.00	1.40	1.80	1.30	1.50	0.00	0.00	0.00	0.00	0.35	0.45	0.05	0.28
13	0.00	0.00	0.00	0.00	4.50	4.50	3.90	4.30	0.00	0.00	0.00	0.00	0.10	0.40	0.35	0.28
14	0.45	0.65	0.25	0.45	2.75	2.95	1.80	2.50	0.00	0.00	0.00	0.00	0.55	0.45	0.05	0.35

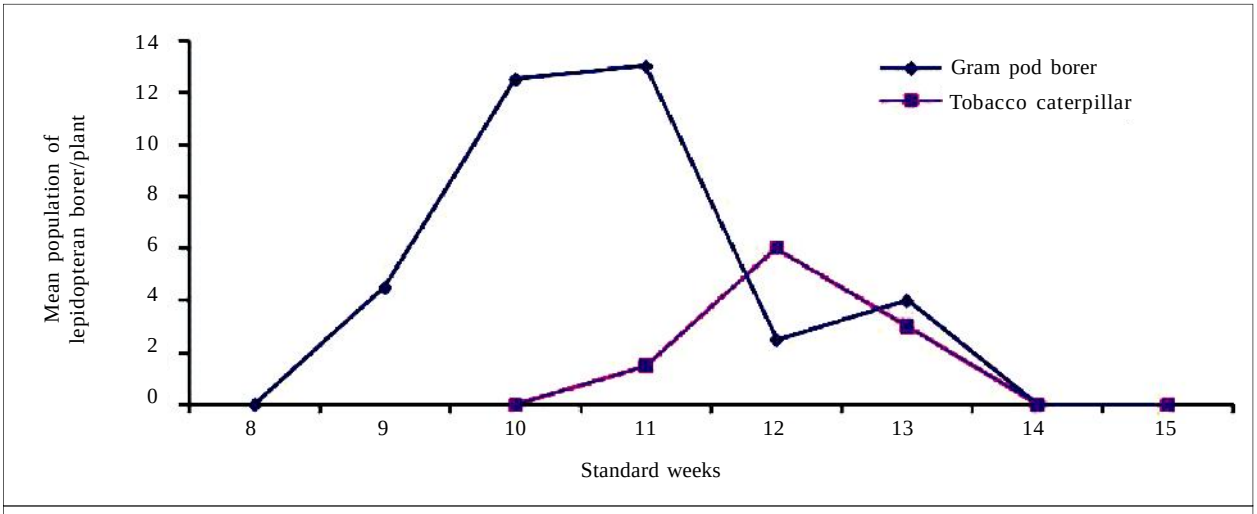

Fig. 3 : Incidence of Lepidopteran borer complex on chilli at Jabalpur during 2009-10

1 and Fig. 1). From the figure it is evident that the parasitoid was present on the aphid when crop in reproductive stage and remained active up to the third week of March.

Compilation of the information on insect pest succession on chilli revealed that 6 species of insect pests appeared at different stages of crop growth which constituted 3 species of Hemiptera (34%), 2 species of

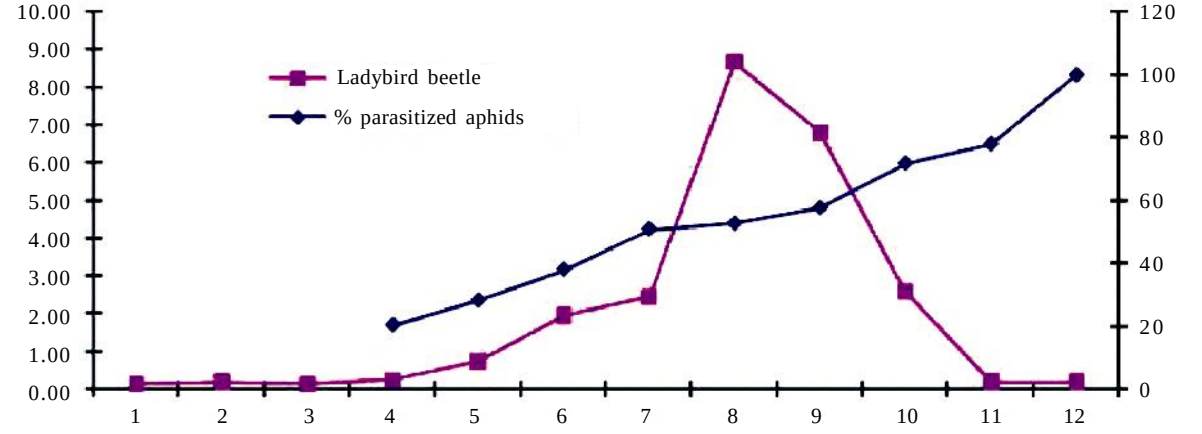

Fig. 4 : Occurance of natural enemies on chilli at Jabalpur during 2009 - 10

Table 4 : Incidence of Pest complex and natural enemies on chilli at Jabalpur during 2009-10

Standard weeks	Insect pests and Natural enemies							
	Mean population of (adult and nymph) of sucking pest complex / 2 leaves / plant				Mean population of larvae/ plant		Mean population of grub and adult / plant	% parasitized aphids by unknown parasitoid
	Whitefly	Thrips	Aphids	Jassids	*H. armigera*	*S. litura*	Ladybird beetle (*C. septempunctata And M. sexmaculatus*)	
45	0.33	0.00	0.00	0.00	-	-	-	-
46	0.63	0.00	0.00	0.00	-	-	-	--
47	1.02	0.55	0.00	0.00	-	-	-	-
48	1.05	2.22	1.48	0.00	-	-	-	-
49	4.88	1.63	0.85	0.00	-	-	-	-
50	1.97	1.62	1.12	0.00	-	-	-	-
51	0.67	0.40	0.93	0.00	-	-	-	-
52	1.03	0.55	1.45	0.00	-	-	-	-
1	0.62	0.42	0.98	0.00	0.00	0.00	1.50	0.00
2	0.42	0.52	0.87	0.00	0.00	0.00	2.00	0.00
3	0.37	0.23	1.25	0.00	0.00	0.00	1.50	0.00
4	0.57	0.08	2.85	0.00	0.00	000	2.50	20.47
5	1.03	0.47	3.55	0.00	0.00	0.00	7.50	28.28
6	2.27	0.38	4.48	0.00	0.00	0.00	19.50	38.16
7	2.53	0.33	5.03	0.00	0.00	0.00	24.50	50.97
8	1.75	0.15	3.97	0.00	0.00	0.00	86.50	52.78
9	0.73	0.45	7.17	0.00	4.50	0.00	68.00	57.76
10	0.43	0.83	4.17	0.08	12.50	0.00	26.00	71.91
11	0.17	1.63	0.53	0.22	13.00	1.50	2.00	78.08
12	0.00	1.50	0.00	0.28	2.50	6.00	2.00	100.00
13	0.00	4.30	0.00	0.28	4.00	3.00	0.00	0.00
14	0.45	2.50	0.00	0.35	0.00	0.00	0.00	0.00

Lepidoptera (22%), 2 species of Coleoptera (22%), 1 species of Thysanoptera (11%) and 1 species of Hymenoptera (11%), respectively (Table 2 and Fig. 4).

Preference of sucking pest complex on different level of plant canopy :

Whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae):

Comparison of mean whitefly population (Table 3) on different levels of plant canopy recorded that maximum population was recorded in lower (12.5 whitefly/2 leaves) followed by upper (9.65 whitefly/2 leaves) and middle (9.09 whitefly/2 leaves) but all upper, middle and lower combinations *i.e*. U/M, M/L and U/L which was show statistically non significant.

Chilli thrips, *Scirtothrips dorsalis* (Hood.) (Thysanoptera: Thripidae):

Comparison of mean thrips population (Table 3) on different levels of plant canopy recorded that maximum population was recorded in middle (10.75 thrips/2 leaves) followed by upper (10.34 thrips/2 leaves) and lower (7.22 thrips/2 leaves) but all upper, middle and lower combinations *i.e*. U/M and U/L which were show statistically non significant and M/L which was show statistically significant and mean value is 3.87 and observed value of t (=5.63).

Aphid, *Aphis gossypii* (Glover) (Hemiptera: Aphididae):

Comparison of mean aphids population (Table 4) on different levels of plant canopy recorded that maximum population was recorded in middle (28.37 aphids/2 leaves) followed by upper (25.56 aphids/2 leaves) and lower (22.31 aphids/2 leaves) but all upper, middle and lower combinations *i.e*. U/M, M/L and U/L which was show statistically non-significant.

Jassids, *Amrasca bigutula* (Ishida)(Hemiptera: Cicadellidae):

Comparison of mean jassids population (Table 3) on different levels of plant canopy recorded that maximum population was recorded in middle (3.5 jassids/ 2 leaves) followed by upper (2.5 jassids/2 leaves) and lower (1.3 jassids/2 leaves) but all upper, middle and lower combinations *i.e*. U/M, M/L and U/L which was show statistically non-significant.

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