

## Original Research Article

# Lifecycle and Feeding Potential of Mycophagous Coccinellid Beetle, *Illeis cincta* Fab. (Coleoptera: Coccinellidae) on Powdery Mildew Fungus, *Erysiphe cichoracearum* DC in Sunflower

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## ABSTRACT

A wide range of agricultural crops are infected by the obligate fungi belongs to family Erysiphaceae causing powdery mildew resulting economic loss. In India, coccinellid beetle, *Illeis cincta* Fab. feeding on the *Erysiphe cichoracearum* DC causing powdery mildew of Sunflower was studied at Gandhi Krishi Vijnana Kendra, University of Agricultural Sciences, Bengaluru. Under laboratory conditions the incubation period of the egg was  $3.59 \pm 0.55$  days, the duration of four consecutive larval instars was  $2.25 \pm 0.32$ ,  $3.85 \pm 0.55$ ,  $4.04 \pm 0.55$  and  $5.23 \pm 0.60$  days, respectively. Pre-pupal and pupal duration lasted for  $1.83 \pm 0.57$  and  $5.02 \pm 0.59$  days. Mean longevity of females and males was  $13.66 \pm 1.30$  and  $9.09 \pm 0.85$  days and their total life cycle occupied 39.47 and 34.90 days, respectively. Feeding by these beetles reduces the spore load on the leaf lamina of sunflower. It was observed that maximum number of spores were consumed by adults (64.30 spores/individual/12 hrs), followed by third instar grub (63.60 spores/individual/12 hrs) and mycelial area cleared by adult beetle was found maximum ( $1.22 \text{ cm}^2$  in 24 hrs) followed by third instar grub ( $1.17 \text{ cm}^2$  in 24 hrs). The mycelial patch of  $4 \text{ cm}^2$  was cleared in 48 hrs by 5 adult beetles and in 36 hrs by five third instar grubs. In another experiment, 5 beetles consumed  $8 \text{ cm}^2$  area of mycelia in 48 hrs and 5 third instar grubs consumed  $8 \text{ cm}^2$  of mycelia in 36 hrs. Thus by considering the feeding potentiality of adults and grubs of *Illeis cincta*, the beetles may be employed effectively in biological control of powdery mildew infecting sunflower.

## Keywords

*Illeis cincta*,  
Powdery  
mildew,  
Mycelia,  
Bionomics,  
*Erysiphe*  
*cichoracearum*

## Introduction

Obligate biotrophic fungi of the family Erysiphaceae (Ascomycota: Erysiphales), commonly known as powdery mildews, are one of the most destructive pathogen infecting wide range of plant species and infect many plant structures (Glawe, 2008). Economic yield losses due to powdery mildew infection have been reported in

various crops within several families including, Asteraceae, Malvaceae, Cucurbitaceae, Verbenaceae, Solanaceae and Leguminosae, besides many cereals and fruit trees (English-Loeb *et al.*, 1999; Khodaparast and Abbasi, 2009). Since many of these host plants are valued as crops, powdery mildew is collectively considered

one of the most important plant pathogens, worldwide.

Among the most important annual crops cultivated in the world for edible oil, sunflower *Helianthus annuus* L. (Asteraceae) is an important one, since it contains 39 to 49 per cent oil in the seed. Its oil is light coloured and a rich source of linolenic acid (64 %) which is good for cardiac patients, and thus considered as a premium oil. Sunflower is susceptible to a large number of economically important diseases which include sunflower necrosis virus disease, *Alternaria* leaf spot, downy mildew and rust. But from the past few years, powdery mildew, caused by *Erysiphe cichoracearum* DC became a major production constraint of sunflower in India. The disease was found throughout the year, reducing the crop yields to considerable extent (Kolte, 1985). It advances senescence of the plant at the flowering or post flowering stages (Zimmer and Hoes, 1978; Gulya *et al.*, 1997) and has the potential to cause 70 – 100 per cent crop loss (Diaz Franco, 1983; Karuna, 2012).

The management of this disease typically involves regular applications of fungicides. But the use of chemicals coupled with the high rate of asexual sporulation by *E. cichoracearum* has led to documented resistance to benzimidazoles, sterol inhibitors, demethylation inhibitors (DMI) and strobilurins in both laboratory and field experimentation (Gubler *et al.*, 1996; Del Pino *et al.*, 1999; Heaney *et al.*, 2000; McGrath, 2001).

Biological control of powdery mildew may offer solutions to this resistance phenomenon and other pesticide-related issues such as crop residues, effects on non-target organisms, workers health and safety. There were several commercially available

microbial biological control agents, including the spore-forming bacterium *Bacillus subtilis* and the fungal hyperparasite, *Ampelomyces quisqualis* Ces. Little is known about the potential of arthropod agents to manage or reduce disease through feeding on powdery mildew.

Numerous species of coccinellids are predators of hemipteran pests such as aphids, mealybugs and scale insects, as well as thrips and mites in all parts of the world (Majerus, 1994). Although majority of coccinellids are predators of other arthropods, not all are purely entomophagous insects. Phytophagy within the Epilachninae and mycophagy (both facultative and obligative), within the Coccinellinae have evolved from a common coccidophagous ancestor (Giorgi *et al.*, 2009 and Lundgren, 2009). All members of the *Psylloborini* Casey (Coleoptera: Coccinellidae) are obligate consumers of various powdery mildew conidia and hyphae at all mobile life stages. The cosmopolitan distribution of *Psyllobora* and their wide host range (Sutherland and Parrella, 2009; Joshi and Sharma, 2008) may suggest their importance in natural control of the powdery mildews.

Since 2006, severe incidence of powdery mildew disease has been observed in all the sunflower growing areas of the country and a strong positive relation has been established between the ladybird beetle, *Illeis cincta* (Fabricius) (Coleoptera: Coccinellidae) and the powdery mildew infection in sunflower (Jagdish *et al.*, 2006).

Therefore this research work was initiated to study life cycle and feeding potential of *Illeis cincta* Fab. with the aim to utilize it as biocontrol agent against *E. cichoracearum* infecting sunflower.

## **Materials and Methods**

### **Biology of *Illeis cincta* under laboratory conditions**

The adult beetles of *I. cincta* were collected from the field and they were allowed to mate by enclosing them in glass jars and the mated females were kept enclosed in separate glass test tubes and provided with sunflower leaf infected with powdery mildew. The batch of eggs deposited by *I. cincta* was transferred by using fine camel hair brush onto powdery mildew infected sunflower leaf discs taken in petriplates. The petriplates were then kept enclosed in a wooden cage. The freshly hatched first instar larvae were transferred onto separate petriplates containing powdery mildew infected leaf discs placed on moist cotton pad to maintain the leaf freshness. As and when the need arose, fresh leaf bits with excess powdery mildew spores/mycelia were provided to the developing larvae. Observations were made thrice a day, on moulting period (to arrive at duration of each instar), total number of instars and adult longevity. Totally twenty five replications were maintained. The temperature and relative humidity during the study period ranged between 27 °C to 35 °C and 39 to 72 per cent, respectively.

Few individuals of each larval instar were collected and preserved in 70 per cent ethyl alcohol for taking morphometric measurements of their body and also to study other morphological features. The measurements were made by using ocular micrometer after standardizing it with stage micrometer at 40x magnification.

### **Incubation period**

The time that lapsed between the egg deposition and egg hatching was recorded.

This duration was computed as the incubation period of the egg.

### **Instar duration**

After transferring one first instar larvae onto each petriplate containing infected leaf disc, the date and time of moulting of each larva was recorded in each of the 25 replications, in order to arrive at the duration of each larval instar. Moulting was detected based on deposition of the exuviate on the leaf disc. The time lapse between two moults was taken as the instar duration. After every moult the cast skin was removed after making observations. The entire leaf discs were removed and replaced with the fresh leaf disc as and when the need arose. The total number of instars was also recorded.

### **Pre-pupal period**

The inactive stage observed when the larvae were about to enter pupation was recorded. This duration was considered as the pre-pupal period.

### **Pupal period**

The time period that lapsed from the formation of cocoon, upto the time of adult emergence was recorded. This duration was considered as the pupal period.

### **Pre-mating and mating period**

Studies on mating and ovipositional behaviour were also carried out on 20 pairs of adult beetles by following the methodology suggested by Chakraborty *et al.*, (1994).

Twenty pairs of adults were released inside separate glasstubes to observe pre-mating and mating duration. Both these parameters were observed and recorded.

### **Pre-ovipositional and ovipositional period**

The mated females were kept enclosed in a glass tube along with the powdery mildew infected leaf bits. The time period that lapsed from the adult emergence upto commencement of egg laying was considered as pre-ovipositional period. Likewise, the duration for which egg laying was observed was considered as ovipositional period of *I. cincta*.

### **Fecundity**

The number of eggs deposited in each day was counted and recorded. The eggs were removed after counting with the help of fine camel hair brush and discarded. Counts of new eggs laid in the subsequent days, were made on a day-to-day basis to compute at the fecundity of *I. cincta*.

### **Adult longevity**

After mating, adult males and females were kept enclosed in separate glass tubes, to know their longevity. Powdery mildew infected leaf bits with sufficient spore load was provided as food for the beetles. The period from adult emergence until death of the adult beetle was considered as adult longevity.

### **Determination of the feeding potentiality of *I. cincta* on powdery mildew fungus under laboratory conditions.**

Two methods were adopted to determine the feeding rates of *I. cincta* viz., (i) spore count method and (ii) powdery mildew area cleared by individual stages

#### **Spore count method**

Powdery mildew infected sunflower leaves with same disease score (preferably 7) were

collected from field in order to avoid errors due to varied powdery growth on leaves (disease severity). These leaves were cut into 3 cm<sup>2</sup> discs and counted for spore load before exposure to feeding by *I. cincta*. To count the number of spores on 3 cm<sup>2</sup> leaf area, the leaf disc was immersed in 10 ml water blank and then spores were counted by using haemocytometer. As and when required, further dilutions were made to make spore counting easy. This procedure was replicated 10 times. At the same time, another set of leaves with same disease score was cut into 3 cm<sup>2</sup> leaf discs and were placed in petriplates containing moist cotton to maintain the leaf freshness.

Onto this leaf discs, the individual larval instars of *I. cincta* were released separately for feeding. Similarly, the adult beetles were released onto the leaf discs placed inside the test tubes stoppered with cotton plug. After 24 hours of feeding by the different life stages of *I. cincta*, the leaf discs were collected and counted for the number of spores remaining on the leaves after feeding, by using a haemocytometer. The number of spores fed by the individual instars was calculated by taking difference in count before and after exposure to feeding by *I. cincta*.

#### **Powdery mildew area cleared by larval and adult stages of *I. cincta***

Egg masses deposited on the same day were collected from the *I. cincta* culture and transferred to a leaf disc taken in a petriplate and kept under laboratory conditions for hatching. The powdery mildew infected sunflower leaves were collected from the culture maintained under glass house conditions. Sunflower leaves having uniform disease score of 7 were selected by following the 0-9 scale as suggested by Anon. (2012)

The sunflower leaves with a disease score of 7 were cut into 5 cm<sup>2</sup> leaf disc and were placed in the petriplate containing moist cotton. Before feeding, the mycelial area of powdery mildew, falling in score 7 category was measured by placing graph paper over the powdery mildew patches on the sunflower leaves.

Then these leaves were exposed to feeding by individual larval instars on petriplates and adult beetles in plastic jars separately.

After 24 hours of exposure to feeding by the beetles, the leaves were collected and measured for area of powdery mildew cleared by each individual. Five replications were maintained for each treatment, along with untreated control (having no beetle) in order to study the natural increase of powdery mildew patches.

The individual larval instars or stages of *I. cincta*, which were found to clear maximum leaf mycelial area in this study was again selected for further studies, wherein the third instar larvae and adult stage showed maximum potential to clear the mycelial area on the leaves. Therefore, feeding potential of both adult beetles and third instar grubs were studied for comparison.

#### **Consumption of powdery mildew by variable number of 3<sup>rd</sup> instar grubs and adults of *I. cincta* on powdery mildew leaf discs with same area**

The sunflower leaves with uniform powdery mildew severity score of 7 were selected and exposed to feeding by *I. cincta* grubs (III instar), the area of lesion of powdery mildew falling in score 7 was measured by placing graph paper over the powdery mildew patches on the sunflower leaves. The numbers of squares of graph paper covering the 4 cm<sup>2</sup> area of mycelia were selected for

the study. Different numbers *i.e.*, 2, 3, 4 and 5 *I. cincta* grubs were allowed to feed separately on the leaves in separate petriplates for 3<sup>rd</sup> instar larvae. Eight replications were maintained for each treatment, along with untreated control (having no beetle) in order to study the natural increase of powdery mildew patches. The treatments were maintained as follows:

T1: Leaf lamina having 4 cm<sup>2</sup> of powdery mildew patch enclosed with 2 beetles/grubs

T2: Leaf lamina having 4 cm<sup>2</sup> of powdery mildew patch enclosed with 3 beetles/grubs

T3: Leaf lamina having 4 cm<sup>2</sup> of powdery mildew patch enclosed with 4 beetles/grubs

T4: Leaf lamina having 4 cm<sup>2</sup> of powdery mildew patch enclosed with 5 beetles/grubs

T5: Leaf lamina having 4 cm<sup>2</sup> of powdery mildew patch enclosed with no beetles/grubs

Another set of same treatments were maintained, for estimation of feeding potential of adults by using eight replications. Observations were recorded after every 12 hours with the help of graph sheets to know the area of consumption of powdery mildew by the beetles and grubs upto 72 hrs.

#### **Consumption of powdery mildew by constant number of 3<sup>rd</sup> instar larvae and adults of *I. cincta* on powdery mildew infected leaf discs of variable area**

Powdery mildew infected leaf discs of variable area *i. e.*, 2, 4, 6 and 8 cm<sup>2</sup> were kept enclosed with same number of grubs and adults of *I. cincta* separately. The sunflower leaf discs were selected as per the above procedure and the treatments were imposed as detailed below:

T1: Leaf lamina having 2 cm<sup>2</sup> area infected with powdery mildew and enclosed with 5 beetles/grubs

T2: Leaf lamina having 4 cm<sup>2</sup> area infected with powdery mildew and enclosed with 5 beetles/grubs

T3: Leaf lamina having 6 cm<sup>2</sup> area infected with powdery mildew and enclosed with 5 beetles/grubs

T4: Leaf lamina having 8 cm<sup>2</sup> area infected with powdery mildew and enclosed with 5 beetles/grubs

Similarly, another set of same treatments were maintained for estimating the feeding potential of adults, with eight replications.

Observations were made at 12 hours interval upto 72 hours and results were tabulated. The rate of consumption/ increase in the mycelial patches was computed by employing the following formula as suggested by Illahi *et al.*, (2011).

$$\% \text{ Consumption/increase} = \frac{\text{Mycelial area present}}{\text{Mycelial area at 0 hour}} \times 100$$

## Results and Discussion

### Biology of *Illeis cincta*

The biology of *Illeis cincta* Fab. was studied on sunflower leaves infected with powdery mildew, *Erysiphe cichoracearum* DC enclosed in plastic petriplates and glass test tubes under laboratory conditions. The observations made in respect of different life stages of the beetle are as presented and discussed with suitable illustrations (Table 1). During the study period, temperatures ranged from 25.7 to 29.4 °C and RH ranged between 39 to 72 per cent.

### Egg

Female of *I. cincta* laid opaque white eggs in clusters on both dorsal and ventral surfaces of the leaves. The number of eggs present in each cluster ranged from 12-41. Eggs were elliptical in shape, measuring 0.62±0.15 mm in length and 0.29± 0.018 mm in width. Incubation period was 3.59±0.55 days. The chorion turned light yellow on the previous day of hatching. In *Thea bisoconotata* Muls., the eggs were laid usually in batches of 6-10, with the incubation period being 6.20 days (Kapur, 1943). However, Chakraborty *et al.*, (1994) reported that in *I. indica* Timb., the native natural predator of white powdery mildew, *Phyllactinia corylea* (Pers.), the incubation period of egg was 4.55 days and eggs measured 0.50x0.37 mm. Satti (2015) reported that in *Psyllobora bisoconotata*, a mean of 6.00 ± 2.86 eggs/batch was observed with incubation period ranging between 1–3 days.

### Grub

During the entire larval period, the grubs moulted thrice and thus completed four larval instars. A brief description of each larval instar and their durations are as detailed below.

### First instar

The grubs underwent four instars. The newly hatched grub measured 1.17±0.09 mm in length and 0.33±0.017 mm in width. Newly hatched grubs crawled slowly all over the sunflower leaf surface in search of food (powdery mildew). They were separated and kept in individual petridishes with mildew infected sunflower leaves. The dorsal surface of cephalic region of the first instar grub was having three dark brown bands. Each thoracic segment contained a

pair of dorsal dark brown markings with tuft of bristles on each marking. Each of the first 9 abdominal segments had 2 pairs of dorsal dark brown spots with minute bristles on each spot. The last 2 segments were fused together and lacked the band but were covered with fine bristles (Plate 1). The duration of first instar was  $2.25 \pm 0.32$  days. The present findings are almost in conformity with the reports of Chakraborty *et al.*, (1994) on the biology of *Illeis indica* on mulberry, wherein it was reported that the duration of first instar grub was  $2.20 \pm 0.536$ , which measured  $1.13 \pm 0.088$  mm in length and  $0.36 \pm 0.009$  mm in width.

### Second Instar

The brown bands on each thoracic segment were transversely elongated. The number of bands on the thoracic as well as abdominal segments were same as that found on the first instar, but they were more prominent and deeper in colour, without bristles. The duration of second instar was significantly longer *i.e.*, ranging between 2.12 to 4.75 days, with a mean of  $3.85 \pm 0.55$  days. The second instar grub measured  $3.06 \pm 0.11$  mm in length and  $0.53 \pm 0.013$  mm in width.

### Third Instar

The third instar grub was almost similar to the second instar in shape and markings on its body, but it was bigger in size measuring  $4.16 \pm 0.03$  mm in length and  $0.76 \pm 0.035$  mm in width. Its developmental period occupied  $4.04 \pm 0.55$  days.

### Fourth Instar

The dimensions of the fourth instar was observed to be  $5.92 \pm 0.09$  mm in length and  $0.98 \pm 0.026$  mm width and the duration of this final larval instar lasted for  $5.23 \pm 0.60$  days. The total developmental period of *I.*

*cincta* during grub stage was completed in 15.37 days, which was ranging between 11.44 to 18.12 days.

Kapur (1943) also recorded four larval instars in the mycophagous coccinellid, *Thea bisoetonotata* Muls., wherein the consecutive instars each occupied on an average of 3.7, 2.8, 3.2 and 3.8 days, respectively. Total larval period was about 13.5 days. The results of the present investigations are almost in conformity with the reports of Chakraborty *et al.*, (1994) on the biology of *Illeis indica* on mulberry, wherein it was reported that the duration of four consecutive larval instars was 2.2, 3.2, 4.3 and 5.2 days, respectively and the total larval period was 14.95 days. The four instars of *Illeis indica* grub measured  $1.13 \times 0.36$  mm,  $1.69 \times 0.45$  mm,  $2.15 \times 0.60$  mm and  $4.03 \times 1.02$  mm, respectively. In case of *Psyllobora bisoetonotata*, Satti (2015) found a gradual increase in the instar durations from the 1<sup>st</sup> larval instar ( $2.80 \pm 0.27$ ) upto the 4<sup>th</sup> instar ( $4.71 \pm 0.51$ ). Slight variations in the above biological parameters as compared to the present findings may be due to variations in geographic location and climatic conditions prevalent during the period of these two different investigations, besides predator-prey species differences.

### Pre-pupa

This was non-feeding stage, slow moving, whitish with yellow tinges, clump shaped and measuring  $4.81 \pm 0.12$  mm in length and  $1.77 \pm 0.018$  mm in width. Pre-pupal period lasted for  $1.83 \pm 0.57$  days. Earlier, Jagadish and Jayaramaiah (2004) reported that the prepupal period was 0.30 days in case of *Coccinella septumpunctata* Fab. on *Myzus nicotianae* Blackman. Whereas, Patro and Sontakke (1994) recorded that the prepupal period of *Coccinella transversalis* Fab. lasted for 0.61 days. Chakraborty *et al.*,

(1994) reported that pre-pupa measured 3.65x1.10mm, with a duration of 1.35 days in case of *I. indica* on *Phyllactinia corylea* (Pers.).

### **Pupa**

Pupa of *I. cincta* was elliptical in shape, yellowish or white coloured and measured 4.05±0.04 mm in length and 2.04±0.022 in width, its pupal period occupied 5.02±0.59 days. Kumar *et al.*, 2000 recorded that the pupal period of *Illeis indica* on *Phyllactinia corylea* was 6-7 days, whereas Chakraborty *et al.*, (1994) reported it to be 6.8 days, with 0.75x1.59 mm dimension. Kapur (1943) reported the pupal period of *Thea bisoctonotata* Muls. to be 4 to 7 days, with an average of 5.20 days.

### **Adult**

Adults were light yellow coloured measuring 5.07±0.04mm in length and 3.52±0.033mm in width for males and 7.01±0.07mm in length and 4.01±0.029 mm in width for females. During adult emergence, it lifted the pupal case vertically and came out by bursting the pupal case. However, in *Cryptolaemus montrouzieri*, the pupal case remained in horizontal position even after beetle emergence (Mani and Thontadarya, 1987a). Adult body was less convex and narrowed towards the apex. Pronotum contained black spots. These features are in close agreement with the observations of Chakraborty *et al.*, (1994). The mean adult longevity of an adult of *I. cincta* was 9.09±0.85 days for male and 13.66±1.30 days for female. Similar findings were also reported by Chakraborty *et al.*, (1994) in case of *I. indica* Timb, wherein females were having higher longevity (31.1 days) than males (7.45 days). Younis *et al.*, 2003 reported that longevity of *Vibidia duodecimguttata* Poda was 98.8±46.86 days

for females and 79.9±39.69 days for males. Ahmad *et al.*, (2003) found that longevity of *Psyllobora bisoctonotata* was 47.25±19.69 days for males and 54.25±14.26 days for females.

### **Reproduction**

The average pre-mating and mating (copulation) periods were 2.96±0.46 days and 14.36±0.49 minutes, respectively (Table 2). The pre-mating period recorded in the present study closely agrees with that of *Illeis indica* as reported by Chakraborty *et al.*, (1994) wherein the pre-mating period was 3 days. Khinchi *et al.*, (2013) reported that the pre-mating period and mating period of *Coccinella septempunctata* was 6.55 days and 51.85 minutes, respectively.

### **Oviposition**

Pre-ovipositional, ovipositional and post-ovipositional periods for *I. cincta* averaged 6.08±0.33, 2.88±0.23 and 4.03±0.42 days, respectively. Kumar *et al.*, (2000) reported that fertilized females of *I. indica* lay eggs after about 7.03 ±0.144 days, whereas, *Psyllobora confluens* (Fab.) females showed an ovipositional period of 32.70 days (Cividanes *et al.*, 2007).

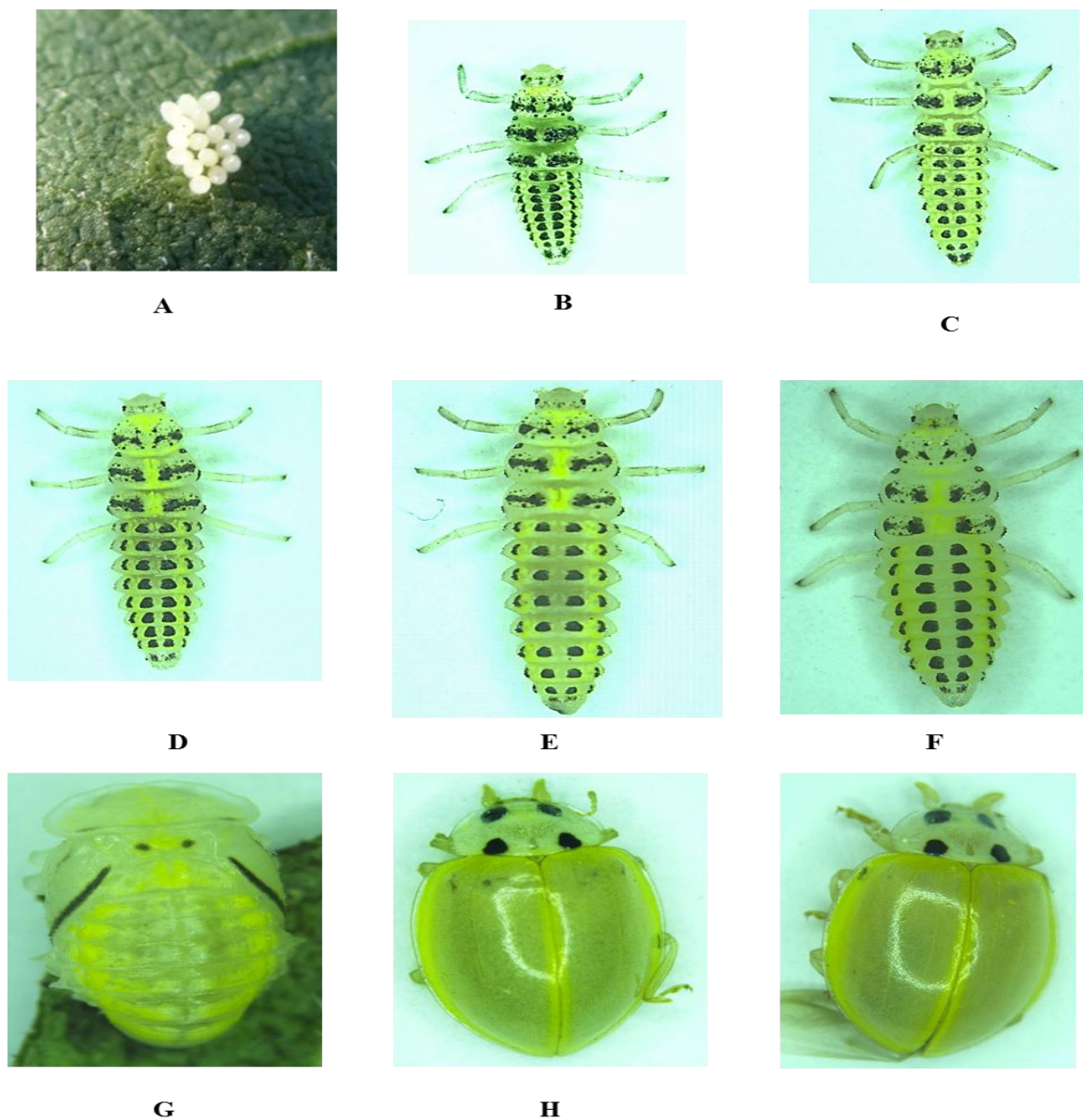
Tank and Korat (2007a) recorded that mean pre-ovipositional, ovipositional and post-ovipositional periods were 2.61 ± 0.76, 14.38 ± 2.36 and 3.23 ± 0.72 days, respectively in case of *Cheilomenes sexmaculata* (Fab.).

### **Fecundity**

Fecundity of female *I. cincta* varied between 12-41, with an average of 25.10±9.80 eggs. The fecundity of a gravid female of *Illeis indica* was reported to be 18.40 by Chakraborty *et al.*, (1994).



**Fig.1** Different life stages of *Illeis cincta*



A-Egg mass, B-1<sup>st</sup> instar, C-2<sup>nd</sup> instar, D- 3<sup>rd</sup> instar, E-4<sup>th</sup> instar, F-Pre-pupa, G-Pupa, H- Adult female and I-Adult male

**Powdery mildew area cleared by larval and adult stages of *I. cincta***

Scores	Extent of leaf area covered with powdery growth
0	No powdery mildew on leaves
1	Powdery mildew specks covering 1% or less leaf area
3	Powdery lesions covering 1-10 % of leaf area
5	Enlarged powdery lesions covering 11-25% of leaf area
7	Powdery lesions coalesce to form big patches covering 26-50 % of leaf area
9	Powdery patches covering 51 % or more of the leaf area

**Table.1** Biology of *Illeis cincta* on sunflower under laboratory conditions

Stage of life cycle	Length (mm)	Breadth (mm)	Duration (days)
<b>Egg</b>	0.62±0.15 (0.45-1.01)	0.29±0.018 (0.29-0.34)	3.59±0.55 (3-4.55)
<b>Grub</b>			
<b>Ist instar</b>	1.17±0.09 (1.00-1.30)	0.33±0.017 (0.31-0.36)	2.25±0.32 (2-2.75)
<b>2<sup>nd</sup> instar</b>	3.06±0.11 (2.80-3.19)	0.53±0.013 (0.52-0.56)	3.85±0.55 (2.12-4.75)
<b>3<sup>rd</sup> instar</b>	4.16±0.03 (4.10-4.21)	0.76±0.035 (0.68-0.81)	4.04±0.55 (3.12-4.75)
<b>4<sup>th</sup> instar</b>	5.92±0.09 (5.77-6.05)	0.98±0.026 (0.96-1.05)	5.23±0.60 (4.00-5.87)
<b>Pre-pupa</b>	4.81±0.12 (4.59-4.98)	1.77±0.018 (1.73-1.79)	1.83±0.57 (1-2.75)
<b>Pupa</b>	4.05±0.04 (3.98-4.10)	2.04±0.022 (2.01-2.08)	5.02±0.59 (4.25-5.87)
<b>Adult longevity</b>			
<b>Male</b>	5.07±0.04 (5.00-5.13)	3.52±0.033 (3.46-3.57)	9.09±0.85 (8.25-10.37)
<b>Female</b>	7.01±0.07 (6.88-7.12)	4.01±0.029 (3.99-4.09)	13.66±1.30 (11.25-15.62)

NB: Range is in parenthesis

**Table.2** Reproductive parameters of *I. cincta* on sunflower under laboratory conditions

Parameters	Duration (in days)
<b>Pre-mating period</b>	2.96±0.46 (2.00-3.62)
<b>Copulation period</b>	14.36±0.49 (min) (14-15)
<b>Pre-ovipositional period</b>	6.08±0.33 (5.50-6.62)
<b>Ovipositional period</b>	2.88±0.23 (2.50-3.25)
<b>Fecundity</b>	25.10±9.80 (12-41)
<b>Post oviposition period</b>	4.03±0.42 (3.25-4.62)
<b>Total developmental period</b>	Female:39.47; Male:34.90

NB: Range is in parenthesis

**Table.3** Number of spores consumed and the mycelial area cleared by the different instar grubs and adults of *I. cincta*

Stage of <i>I. cincta</i>	Number of spores consumed ( $\times 10^3$ )	Mycelial area cleared (cm <sup>2</sup> )
<b>I instar grub</b>	<b>24.10a</b>	<b>0.42a</b>
<b>II instar grub</b>	<b>43.30b</b>	<b>0.62b</b>
<b>III instar grub</b>	<b>63.60d</b>	<b>1.17d</b>
<b>IV instar grub</b>	<b>56.50c</b>	<b>0.91c</b>
<b>Adults</b>	<b>64.30d</b>	<b>1.22d</b>
<b>F-test</b>	<b>*</b>	<b>*</b>
<b>SEm</b>	<b>0.99</b>	<b>0.05</b>
<b>CD</b>	<b>2.83</b>	<b>0.15</b>

NB: n=10, \*-Significant at p=0.05

**Table.4** Consumption of powdery mildew by, *Illeis cincta* adults on 4cm<sup>2</sup> powdery mildew infected leaf area when released at different numbers

Treatment	Mycelial area at 0 hrs		Mycelial area left unconsumed after											
			12 hrs		24 hrs		36 hrs		48 hrs		60 hrs		72 hrs	
	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%
<b>T1</b>	4	100	1.94	97.50	1.31	65.50	0.42	21.00	0.23	11.50	0.12	6.00	0.00	0.00
<b>T2</b>	4	100	1.82	90.50	1.28	64.00	0.39	19.50	0.18	9.00	0.04	2.00	0.00	0.00
<b>T3</b>	4	100	1.62	81.00	1.19	59.50	0.26	13.00	0.12	6.00	0.00	0.00	0.00	0.00
<b>T4</b>	4	100	1.14	57.00	0.97	48.50	0.12	6.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>T5</b>	4	100	2.07	103.50	2.26	112.50	2.36	118.00	2.40	120.00	2.47	123.56	2.50	124.56
<b>F-test</b>	-	-	*	*	*	*	*	*	*	*	*	*	*	*
<b>SEm±</b>	-	-	<b>0.05</b>	<b>0.80</b>	<b>0.04</b>	<b>0.73</b>	<b>0.04</b>	<b>0.83</b>	<b>0.05</b>	<b>0.59</b>	<b>0.05</b>	<b>0.43</b>	<b>0.03</b>	<b>0.51</b>
<b>CD</b>	-	-	<b>0.15</b>	<b>2.31</b>	<b>0.13</b>	<b>2.11</b>	<b>0.16</b>	<b>2.40</b>	<b>0.14</b>	<b>1.71</b>	<b>0.15</b>	<b>1.25</b>	<b>0.10</b>	<b>1.47</b>

\*-Significant at p= 0.05

- T1: Leaf lamina having 4 cm<sup>2</sup> of powdery mildew patch enclosed with 2 beetles
- T2: Leaf lamina having 4 cm<sup>2</sup> of powdery mildew patch enclosed with 3 beetles
- T3: Leaf lamina having 4 cm<sup>2</sup> of powdery mildew patch enclosed with 4 beetles
- T4: Leaf lamina having 4 cm<sup>2</sup> of powdery mildew patch enclosed with 5 beetles
- T5: Leaf lamina having 4 cm<sup>2</sup> of powdery mildew patch enclosed with no beetles

**Table.5** Consumption of powdery mildew by, *Illeis cincta* third instar grubs on 4cm<sup>2</sup> leaf area when released at different numbers

Treatment	Mycelial area at 0 hrs		Mycelial area left unconsumed after											
			12 hrs		24 hrs		36 hrs		48 hrs		60 hrs		72 hrs	
	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%
<b>T1</b>	4	100	1.91	95.50	1.19	59.56	0.31	15.50	0.12	6.00	0.00	0.00	0.00	0.00
<b>T2</b>	4	100	1.79	89.50	0.95	55.56	0.14	7.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>T3</b>	4	100	1.73	86.56	1.06	53.13	0.09	4.50	0.00	0.00	0.00	0.00	0.00	0.00
<b>T4</b>	4	100	1.12	56.00	0.79	39.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>T5</b>	4	100	2.07	103.50	2.27	112.56	2.36	118.00	2.42	120.00	2.48	123.56	2.50	124.56
<b>F-test</b>	-	-	*	*	*	*	*	*	*	*	*	*	*	*
<b>SEm±</b>	-	-	<b>0.05</b>	<b>1.13</b>	<b>0.06</b>	<b>1.47</b>	<b>0.03</b>	<b>0.72</b>	<b>0.08</b>	<b>0.32</b>	<b>0.03</b>	<b>0.25</b>	<b>0.04</b>	<b>0.32</b>
<b>CD</b>	-	-	<b>0.16</b>	<b>3.25</b>	<b>0.18</b>	<b>4.23</b>	<b>0.10</b>	<b>2.07</b>	<b>0.25</b>	<b>0.92</b>	<b>0.11</b>	<b>0.72</b>	<b>0.12</b>	<b>0.93</b>

\*-Significant at p= 0.05

- T1: Leaf lamina having 4 cm<sup>2</sup> of powdery mildew patch enclosed with 2 grubs
- T2: Leaf lamina having 4 cm<sup>2</sup> of powdery mildew patch enclosed with 3 grubs
- T3: Leaf lamina having 4 cm<sup>2</sup> of powdery mildew patch enclosed with 4 grubs
- T4: Leaf lamina having 4 cm<sup>2</sup> of powdery mildew patch enclosed with 5 grubs
- T5: Leaf lamina having 4 cm<sup>2</sup> of powdery mildew patch enclosed with no grubs

**Table.6** Consumption of powdery mildew by five adult beetles of *Illeis cincta* on different mycelia area of Powdery mildew infected leaves

Treatment	Mycelial area at 0 hrs		Mycelial area left unconsumed after											
			12 hrs		24 hrs		36 hrs		48 hrs		60 hrs		72 hrs	
	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%
TR1	2	100	1.25	62.50	1.97	98.50	0.00	0.00	0.00	0.00	-	-	-	-
TR2	4	100	2.05	51.25	2.93	73.28	3.90	97.50	0.00	0.00	-	-	-	-
TR3	6	100	2.68	44.66	3.49	58.15	4.57	76.15	5.67	94.56	-	-	-	-
TR4	8	100	2.97	37.12	4.61	57.62	5.26	66.76	7.81	97.70	-	-	-	-
F-test	-	-	*	*	*	*	*	*	*	*	-	-	-	-
SEm±	-	-	0.08	1.16	0.08	0.97	0.07	0.74	0.09	0.45	-	-	-	-
CD	-	-	0.28	3.37	0.25	2.85	0.22	2.16	0.27	1.32	NS	-	NS	-

\*-Significant at p= 0.05

TR1: Leaf lamina having 2 cm<sup>2</sup> area infected with powdery mildew enclosed with 5 beetles  
 TR2: Leaf lamina having 4 cm<sup>2</sup> area infected with powdery mildew enclosed with 5 beetles  
 TR3: Leaf lamina having 6 cm<sup>2</sup> area infected with powdery mildew enclosed with 5 beetles  
 TR4: Leaf lamina having 8 cm<sup>2</sup> area infected with powdery mildew enclosed with 5 beetles

**Table.7** Consumption of powdery mildew by five third instar grubs of *Illeis cincta* on different mycelia area of Powdery mildew infected leaves

Treatment	Mycelial area at 0 hrs		Mycelial area left unconsumed after											
			12 hrs		24 hrs		36 hrs		48 hrs		60 hrs		72 hrs	
	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%	cm <sup>2</sup>	%
TR1	2	100	1.42	71.00	1.95	97.44	0.00	0.00	-	-	-	-	-	-
TR2	4	100	2.55	64.28	3.45	86.57	3.95	98.28	-	-	-	-	-	-
TR3	6	100	4.21	70.15	5.06	84.42	5.89	98.16	-	-	-	-	-	-
TR4	8	100	4.98	62.22	7.13	89.14	7.79	97.30	-	-	-	-	-	-
SEm±	-	-	0.08	0.93	0.09	0.99	0.07	0.50	-	-	-	-	-	-
CD	-	-	0.24	2.69	0.28	2.88	0.21	1.46	-	-	-	-	-	-

\*-Significant at p= 0.05

TR1: Leaf lamina having 2 cm<sup>2</sup> area infected with powdery mildew enclosed with 5 beetles  
 TR2: Leaf lamina having 4cm<sup>2</sup> area infected with powdery mildew enclosed with 5 beetles  
 TR3: Leaf lamina having 6cm<sup>2</sup> area infected with powdery mildew enclosed with 5 beetles  
 TR4: Leaf lamina having 8cm<sup>2</sup> area infected with powdery mildew enclosed with 5 beetles

However, the mean female fecundity of *Vibidia duodecimguttata* (Poda) female beetle was  $74.93 \pm 48.61$  eggs (Younis *et al.*, 2003).

Ahmad *et al.*, (2003) reported the mean fecundity of *Psyllobora bisoetonotata* (Muls.) female was  $102 \pm 88.50$  eggs. The total capacity of oviposition of *P. confluens* (Fab.) was in the range of 16.8 to 439.9 eggs (Cividanes *et al.*, 2007).

### **Total developmental period**

Under laboratory conditions, in case of females of *I. cincta*, the total developmental period was 39.47 days and for male it was 34.90 days. Kumar *et al.*, (2000) reported that the life cycle of *Illeis indica* was completed in 38.50 days. In *Psyllobora bisoetonotata*, the mean total lifecycle from egg to adult stage was  $25.28 \pm 1.57$  days (Satti, 2015). However, in *Cryptolaemus montrouzieri* the total life cycle occupied on an average of  $68.92 \pm 2.37$  days for males and  $78.58 \pm 2.98$  days for females (Dumaniya *et al.*, 2013).

### **The feeding potentiality of *I. cincta* on powdery mildew fungus under laboratory conditions**

Results with regard to the consumption of powdery mildew spore by individual larval instars and adult beetle were recorded and found that maximum number of spores were consumed by adults (64.30/spore individual/12hrs) followed by third instar grub (63.60 spore/individual/12hrs), fourth instar grub (56.5/spore individual/12hrs), second instar grub (43.30/spore individual/12hrs) and first instar grub (24.10/spore individual/12hrs), in that decreasing order (Fig. 1). Similarly, the mycelial area cleared by adult beetle was maximum ( $1.22 \text{ cm}^2$  within 24 hours)

followed by third instar grub ( $1.17 \text{ cm}^2$ ), fourth instar grub ( $0.91 \text{ cm}^2$ ), second instar grub ( $0.62 \text{ cm}^2$ ) and first instar grub ( $0.42 \text{ cm}^2$ ) (Table 3). There was a significant difference in the number of spores consumed and mycelial area cleared by each life stage of *I. cincta*. Adult beetles and third instar grubs were statistically on par with respect to number of spores consumed and mycelia area consumed.

### **Consumption of powdery mildew by adults of *I. cincta* on $4 \text{ cm}^2$ powdery mildew mycelial area when released at different numbers**

The mycelial area cleared by both the third instar grubs and adults were measured. Results with regard to the consumption of powdery mildew mycelia by *Illeis cincta* adults after every twelve hours, upto 72 hrs are depicted in Table 4. Mycelial area at zero hour *i. e.* at the initiation of experiment, when number of beetles were varied between 2 to 5 and kept enclosed with infected leaves of  $4 \text{ cm}^2$  area in the respective treatments are furnished in Table 4. The data revealed that after 12 hours in T<sub>4</sub>-treatment a significant reduction was recorded ( $1.14 \text{ cm}^2$ ) in mycelial area, when five beetles were kept enclosed with  $4 \text{ cm}^2$  of infected leaf area, followed by T<sub>3</sub>, T<sub>2</sub> and T<sub>1</sub>, in which mycelial area was reduced to 1.62, 1.82 and  $1.94 \text{ cm}^2$  by four, three and two beetles enclosed, respectively. After 24 hours, the maximum mycelial area was reduced in the treatment-T<sub>4</sub> ( $0.97 \text{ cm}^2$ ) whereas the least reduction in the area was obtained in treatment-T<sub>1</sub> ( $1.31 \text{ cm}^2$ ). After 36 hours, the maximum mycelial area was reduced in the treatment-T<sub>4</sub> ( $0.12 \text{ cm}^2$ ), whereas the least reduction in the area was obtained in treatment-T<sub>1</sub> ( $0.42 \text{ cm}^2$ ). Similarly, after 48 hours, the mycelial areas were in the range of  $0.12 - 0.23 \text{ cm}^2$  in the treatments-T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> except in the

treatment-T<sub>4</sub> where the mycelial area was completely consumed by the beetles. The mycelial areas were recorded in the range of 0.04 - 0.12 cm<sup>2</sup> in the treatments-T<sub>1</sub> and T<sub>2</sub> after 48 hours and in T<sub>3</sub>, mycelial area was completely consumed. Total consumption of mycelial area was observed after 60 hours in the treatments T<sub>1</sub> and T<sub>2</sub> with 2 and 3 beetles, respectively (Table 4).

However, in the untreated control the powdery mildew patch having 4 cm<sup>2</sup> at initial stage (0 hours) was increased with the passage of time *i.e.* 3.50 per cent after 12 hours, 12.50 per cent after 24 hours, 18.00 per cent after 36 hours, 20.00 per cent after 48 hours, 23.56 per cent after 60 hours and 24.56 per cent after 72 hours.

#### **Consumption of powdery mildew by third instar grubs of *Illeis cincta* on 4cm<sup>2</sup> powdery mildew mycelial area when released at different numbers**

In another set of experiments to determine the mycelial area cleared by third instar grubs, revealed that the mycelial area at zero hour *i.e.* at the initiation of the experiment when two to five grubs were kept enclosed with infected leaves (of 4 cm<sup>2</sup> area) in the respective treatments are presented in Table 5. The data revealed that after 12 hours, significant reduction in mycelial area was recorded in T<sub>4</sub> (1.12 cm<sup>2</sup>), wherein five beetles were kept enclosed, followed by T<sub>3</sub>, T<sub>2</sub> and T<sub>1</sub> in which mycelial area was reduced to 1.73, 1.79 and 1.91 cm<sup>2</sup> when four, three and two beetles were enclosed, respectively. After 24 hours, the maximum mycelial area was reduced in the treatment-T<sub>4</sub> (0.79 cm<sup>2</sup>) whereas the least reduction in the area was obtained in treatment-T<sub>1</sub> (1.19 cm<sup>2</sup>). After 36 hours, complete mycelial area was cleared in the treatment-T<sub>4</sub>, whereas reduction in the area was obtained in treatment-T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> to the extent of 0.31,

0.14, 0.09 cm<sup>2</sup>, respectively. Similarly, after 48 hours the mycelial area was completely cleared in T<sub>2</sub> and T<sub>3</sub>, However in T<sub>1</sub>, 0.12 cm<sup>2</sup> of mycelial area was present. Total consumption of mycelial area was observed after 60 hours in the treatment T<sub>1</sub> (Table 5).

However, in the untreated control the powdery mildew patch having 4 cm<sup>2</sup> at initial stage (0 hours) was increased with the passage of time *i.e.* it increased by 3.50 per cent after 12 hours, 12.50 per cent after 24 hours, 18.00 per cent after 36 hours, 20.00 per cent after 48 hours, 23.56 per cent after 60 hours and 24.56 per cent after 72 hours.

#### **Consumption of powdery mildew by five *Illeis cincta* adults released on mycelial area of different dimensions**

In another set of experiment, consumption of powdery mildew mycelial area having different areas at different time intervals. When a constant number of five beetles and five grubs were kept enclosed with the infected leaf area having 2 cm<sup>2</sup>, 4 cm<sup>2</sup>, 6 cm<sup>2</sup> and 8 cm<sup>2</sup> dimensions was studied. The results revealed that the least time duration of 24 hours was required to consume almost 98.50 % of 2 cm<sup>2</sup> of mycelial patch in the treatment-TR<sub>1</sub> in presence of five adult beetles. In treatment-TR<sub>2</sub> it took 36 hrs to clear 97.50 per cent of the mycelial area, whereas the maximum time of 48 hours was needed by the same number of beetles to consume the entire 6 and 8 cm<sup>2</sup> area of laminae in the treatments, TR<sub>3</sub> and TR<sub>4</sub> (Table 6).

#### **Consumption of powdery mildew by five third instar grubs of *Illeis cincta* released on mycelial area of different dimensions**

Similarly, when five third instar grubs were allowed to feed on the mycelia, least time was required to consume 2 cm<sup>2</sup> of mycelial

area within 24 hrs with 97.44 per cent, whereas, the time required was more to consume larger area of the mycelia. In treatment, TR<sub>2</sub>, TR<sub>3</sub> and TR<sub>4</sub> it took 36 hrs to clear 98.28, 98.16 and 97.30 per cent of the mycelia, respectively (Table 7).

Overall the results indicated that, the third instar grubs were superior in consumption of powdery mildew by taking less time to clear more mycelial area, than in case of the adults. Five grubs took 36 hrs to clear a mycelial area of 4 cm<sup>2</sup>, whereas the same mycelial area was cleared in 48 hrs by adult beetles. This clearly showed that the third instar grubs of *I. cincta* had greater feeding potential as they consumed more mycelia in shorter period of time, when compared to the adults.

The results of present investigations are in almost in conformity with the results of Soyly and Yigit (2002) who reported that the grubs of *Psyllobora bisoconotata* were found to reduce 92 per cent of the conidial density in leaf sections fed upon by the beetles. Third and fourth instar grubs were the most efficient consumers in terms of leaf area cleaned per unit time. Thite *et al.*, (2013) also reported that the larval stages of *Illeis cincta* are voracious feeders of anamorphs of powdery mildew disease than the adult beetles. Increase in natural populations of mycophagous insects may help in reduction of anamorphs of powdery mildew. This according to the authors will definitely lower further spread of the disease.

Illahi *et al.*, (2011) reported that five beetles of *Halysia tschitscherini* cleared the growth of mulberry powdery mildew mycelia, of grade II (1-5 % leaf lamina covered with powdery mildew mycelia) within 36 hours. Their observations indicated that the beetles have the potential to consume the highest

rating grade V (*i.e.* 96.40 cm<sup>2</sup>) of powdery mildew infection within 60 hours.

Present findings derives its support from the results of earlier work by Sutherland and Parrella (2006), who reported that neonate larvae of *Psyllobora vigintimaculata*, when exposed for 192 hours to the mycelial area gave a significant reduction in powdery mildew growth, as compared to untreated control, and leaf discs exposed to 3<sup>rd</sup> instar larvae for 96 hours showed a significant decrease in the infected leaf area.

Thus, on the basis of feeding behaviour and potential, it can be concluded that the mycophagous coccinellid beetle, *Illeis cincta* may be utilized as a biocontrol agent as it has the capacity to consume the mycelial patches of powdery mildew on the leaf lamina without disturbing the ecological balance in the sunflower ecosystem.

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