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SEM Description and Life History Traits of Indian Biotype of *Acanthoscelides macrophthalmus* (Schaeffer) (Coleoptera: Bruchidae)

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ABSTRACT

Many insect pests have crossed over the geographical boundaries and became cosmopolitan in distribution through anthropogenic migration and import and export of the comestible consignments. *Acanthoscelides macrophthalmus* (Schaeffer) (Coleoptera: Chrysomelidae: Bruchidae) a recently reported well known pest of *Leucaena leucocephala* (Lamark) de Wit (Fabaceae) from Indian subcontinent has been studied for life history traits under laboratory conditions. *Acanthoscelides macrophthalmus* utilizes aphagously developing and developed seeds for oviposition and larval development. Eggs were laid either on the pod surface, over a seed, or directly on exposed seeds. Female laid an average of 38.3 ± 8.01 eggs and produced 27.4 ± 6.77 offsprings. Larvae grew and molted inside the host seed and adult bruchid emerged out after cutting a circular window in testa of the seed and pod as the case may be. Total development was completed in 41.6 ± 7.18 days and newly emerged insects became sexually mature after one hour of emergence. All the four larval instars were studied Scanning Electron Microscopically (SEM) and head, antenna, clypeolabial complex, leg etc., were described separately and taxonomically important structure like setae, sensillae trichodea, microtrichia etc., beared by integumentary system were projected accordingly.

Key words: *Acanthoscelides macrophthalmus*, pest, *Leucaena leucocephala*, life history, larval morphology

INTRODUCTION

Pulse beetle are small sized (1.0-6.0 mm) insects belonging to family Bruchidae. More than 1700 species under 62 genera are known worldwide (Romero and Johnson, 2004). Bruchids are well known pests of stored as well as field legumes all over the world. Globally about 30 species of bruchids are serious pests and 9 of them are cosmopolitan in distribution (Kingsolver, 2004). Bruchids feed on the seeds of 34 families of kingdom Plantae and about 85% of them feed on the seeds belonging to family Fabaceae, 4% to Arecaceae and Convolvaceae each, 2% to Malvaceae and remaining 5% to other plant families (Johnson, 1985).

Adults lay eggs on the seed of a host plant suitable for development of the larvae and immature stages are spent inside. Larvae are voracious feeders and feed entirely within seed, making their detection and control difficult, while adults live free in nature and feed on pollen and nectar (Gupta *et al.*, 2009). The internal mode of life protects them from variations of temperature and humidity and enables them to be carried unnoticed during trade across the international boundaries (Thakur, 2012b).

Acanthoscelides Schilsky is one of the largest genera of the tribe Acanthoscelidini containing more than 340 species globally (Johnson, 1990). However, only two species, *Acanthoscelides obtectus* (Say) and *A. macrophthalmus* (Schaeffer) (Coleoptera: Bruchidae) have been reported so far from Indian subcontinent. The present species, *A. macrophthalmus* was described by Schaeffer (1907) and placed under genus *Bruchus* but Johnson (1968) rightly put this species under genus *Acanthoscelides*.

In India, no study has been conducted so far on the biology, life cycle and other aspects of *A. macrophthalmus* except taxonomy, distribution and pest status (Thakur, 2012a). The aim of present investigation is to study the life history and larval morphology of *A. macrophthalmus* on its natural host plant, *L. leucocephala* to develop the database for the control of *A. macrophthalmus* where it presented a potential threat to propagation for plant in many countries (India, Burma, Cambodia, Thailand etc.) and also to control the plant production in other countries (Taiwan, Australia, South Africa, Northern America etc.) where it posed a weed problem. The present study may provide useful information for propagation or bio-control of plant by utilization of this bruchid species.

MATERIALS AND METHODS

Sample collection: The infested and uninfested pods of *L. leucocephala* were collected from different localities of Himachal Pradesh viz., Hamirpur, Bilaspur, Solan and Una to establish laboratory cultures. Cultures were maintained at controlled conditions of temperature ($27.6 \pm 2.5^{\circ}\text{C}$) and relative humidity ($70.2 \pm 1.03\%$) in biological oxygen demand incubator (DB-2025).

Maintenance of culture: Along with 50 seeds of *L. leucocephala* one pair of newly emerged male and female of *A. macrophthalmus* were conducted in 10 replications in different petridishes. The seeds of petridish from each batch were replaced with new seeds daily until the death of the female. The seeds from each batch were then observed daily until the next generation of adults. Total number of eggs laid by one female, eggs laid in one day, oviposition period, longevity, duration of larval and pupal period and number of adults emerged were observed. In a separate experiment eggs laid on each day were kept separately in petridishes and mean development period was calculated.

Scanning electron micrographs: Larval specimens were preserved in ethanol and glycerol (9:1). Scanning electron micrographs of some specific regions were taken with SEM QUANTA-250, model No. D9393 of FEI make in ESEM mode.

Slide preparation: Temporary mount of prothoracic plate of first instar larvae was prepared in 10% potassium hydroxide (KOH). First instar larva was killed with ethyl acetate vapours and then placed on clear glass slide along with a few drops of 10% KOH. Slide was covered with cover slip after 10-15 min in order to spread larval body contents evenly. The prothoracic plate thus exposed was separated from other body parts and washed 2-3 times with distilled water followed by dehydration. Slide was then observed carefully under microscope for its species specific structure.

Measurements and illustration: Measurements were taken by standardizing eyepiece micrometer with stage micrometer fitted in microscope. The 0.1 mm scale line and illustration of prothoracic plate was drawn by using graph eyepiece fitted in stereoscopic binocular microscope.

RESULTS

Adults of *A. macrophthalmus* were found almost throughout the year with decreased population during January and February. Adult bruchid measures 3.51 ± 0.46 mm in length and 1.95 ± 0.30 mm in width and rust red in colour. Adults are sexually dimorphic. Body size, elytral pattern and shape of pygidium of males were different from females. Larger and more serrated antennae were possessed by males than their female counterparts. Adults were strong flier and remained active most of the time but frequently seen feign to death when disturbed. Females reproduced aphanogously.

Freshly emerged adults copulate at any time, about one hour after their emergence. During copulation, male normally raises fore and mid legs to hold the female. Copulation lasted for 8-12 min. Gravid female lays eggs on pods and directly on seeds of host plant if exposed. Normally eggs were laid singly on seeds but multiple ovipositions occurred when number of seeds was limited. Pods contain many eggs generally laid in the vicinity of developing seeds. Oviposition lasted for 5-10 days and rates of egg laying was highest on 2nd and 3rd day of oviposition. Eggs were oval in shape and shining white, but become yellowish white after hatching. Scanning electron micrograph of egg shows the presence of hexagonal plates on the egg membrane (Fig. 1). Incubation period was about 5-8 days. Female live longer than male and laid an average of 38.3 ± 8.01 eggs (Table 1).

Larva undergoes four moults before pupation. First, second, third, fourth larval instars and pupal developmental duration was completed in an average of 6.6 ± 2.07 , 7.4 ± 1.84 , 7.9 ± 1.79 , 8.3 ± 1.16 and 10.2 ± 2.29 days, respectively. Total development duration was 41.6 ± 7.18 days (Fig. 2).

Table 1: Life history traits of *Acanthoscelides macrophthalmus* on *Leucaena leucocephala* (values as Mean \pm SD)

Mating duration (min)	8-12
Female longevity (days)	12.2 \pm 1.75
Male longevity (days)	10.2 \pm 1.28
Oviposition period (days)	6.8 \pm 1.75
No. of eggs laid/female	38.3 \pm 8.01
Incubation period (days)	6.0 \pm 1.05

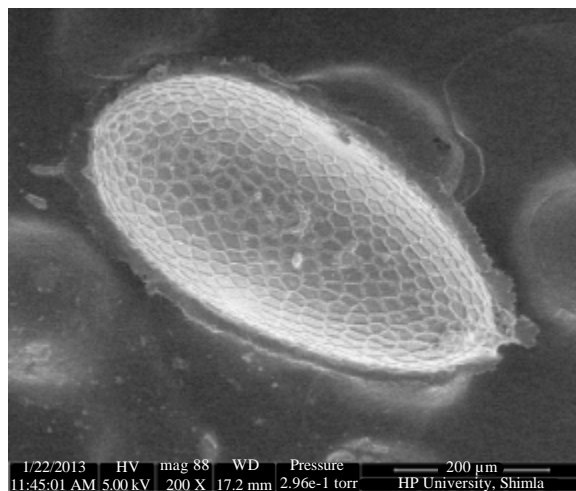


Fig. 1: Scanning electron micrograph of egg of *Acanthoscelides macrophthalmus*

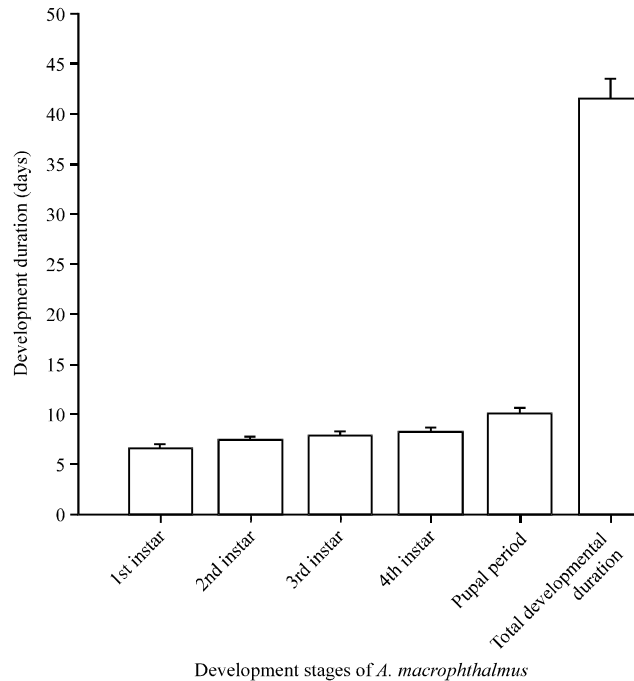


Fig. 2: Developmental duration of different stages of *Acanthoscelides macrophthalmus*

A single female produced an average of 27.4 ± 6.77 offsprings, consisting of 14.7 ± 4.19 females and 12.7 ± 3.02 males out of the total 38.3 ± 8.01 eggs laid. Since the first instar larvae bore into the seed feed, grow and moult into successive instars entirely within seed, no evidence of their presence appears except a minute penetration hole which also plugged by faecal matter of larval instar.

Prothoracic plate: In first instar larva of *A. macrophthalmus*, a brown coloured chitinized X-shaped prothoracic plate was observed. This plate had anterior, median and posterior arms and five pairs of sensilla trichodea. Prothoracic plate also had two pairs of teeth on median arm and five teeth on each posterior arm (Fig. 3). First instar larva penetrated into seed with the help of this prothoracic plate.

Larval description: Four larval instars of *A. macrophthalmus* were identified by head capsule width and moult. It is a frequently used method to identify larval stages in terms of their instars. However, data overlapping between adjacent instars usually leads to inaccurate estimation. Therefore, scanning electron micrographs of four larval instars were taken to describe their morphological characteristics. The main observed differences were in the body size, setae, number of segments of legs and amount of sensilla trichodea on body and antenna.

First instar: Body of first instar was 0.48 mm long and 0.20 mm wide. Mouth parts and surrounding areas were heavily sclerotized and pigmented (Fig. 4a). General colour of the larva was white but prothoracic plate was brown. Clypeolabrum had six blunt-tipped sensilla (S) on labral portion and two sensilla on clypeal portion (Fig. 4b). Antenna had one segment composed of

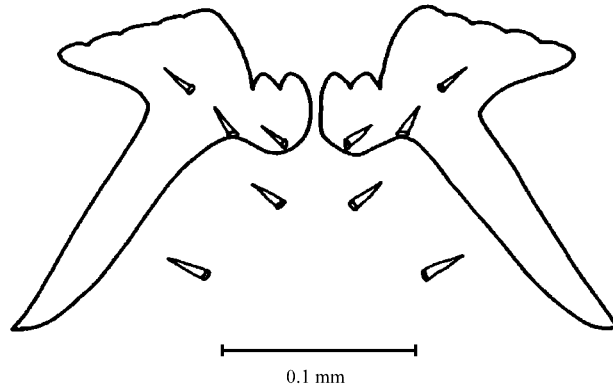


Fig. 3: Prothoracic plate possessed by first instar larvae of *Acanthoscelides macrophthalmus*

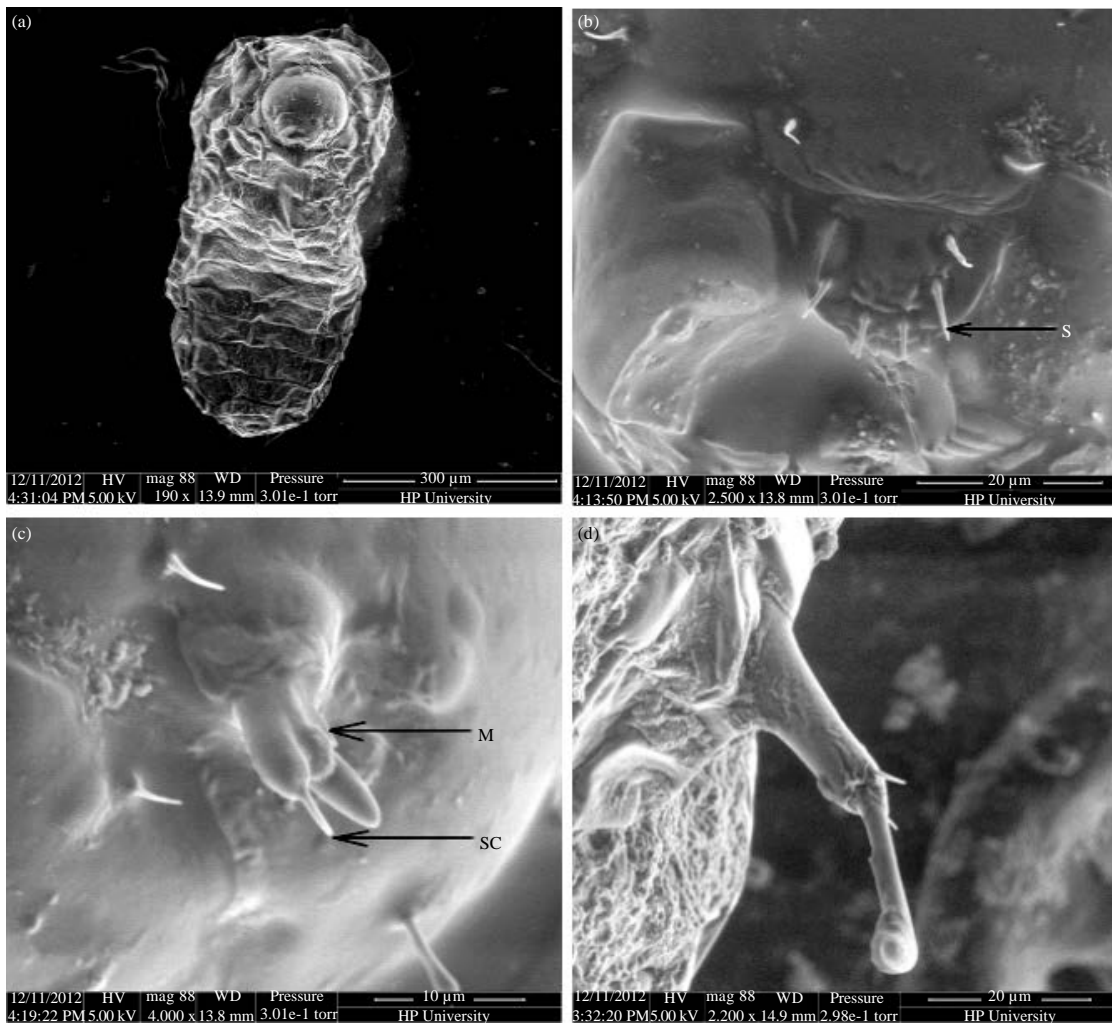


Fig. 4(a-d): Scanning electron micrographs of first instar larva of *Acanthoscelides macrophthalmus*, (a) Ventral view, (b) Clypeolabrum showing blunt-tipped Sensilla (S) on clypeus and labrum, (c) Antenna showing Microtrichia (M) and Sensillum Chaeticum (SC) and (d) Leg

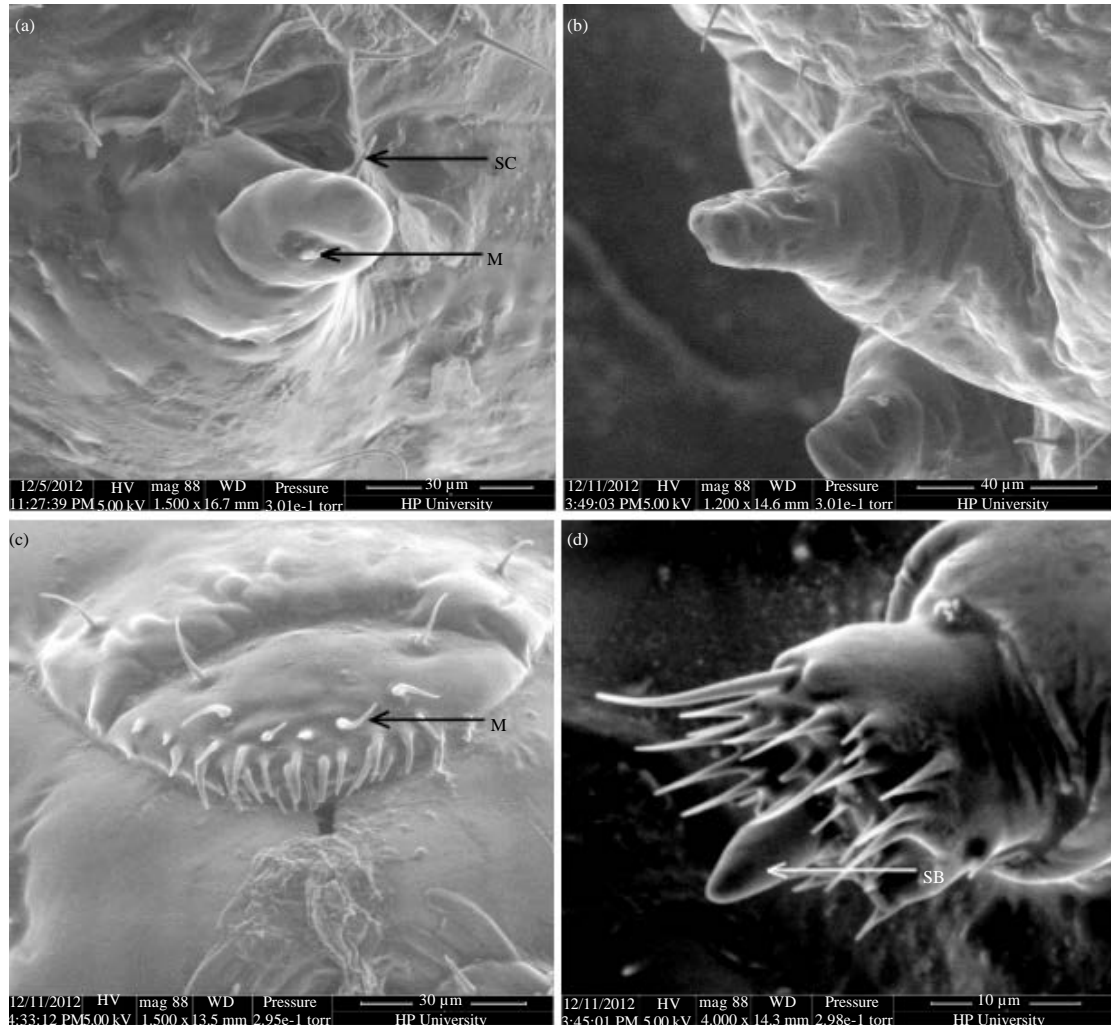


Fig. 5(a-d): Scanning electron micrographs of (a) Antenna, (b) Leg of 2nd instar larva, (c) Clypeolabrum showing Microtrichia (M) on Labrum and (d) Antenna showing elongate Sensillum Basiconicum (SB) and multiple rows of microtrichia of 3rd instar larva of *Acanthoscelides macrophthalmus*

single elongated medial and smaller lateral microtrichia (M) and Sensillum Chaeticum (SC) on an elongated base (Fig. 4c). Leg had two segments with an enlarged base, a pair of elongate decurved sensilla trichodea located on distolateral margins (Fig. 4d).

Second instar: Antenna had one segment composed of two medial and one elongated lateral Microtrichia (M). One Sensillum Chaeticum (SC) emerged from a short, stalk-like base arising from antennal surface (Fig. 5a). Clypeolabrum had five blunt tipped sensilla on labrum and two sensilla on clypeal portion. Leg was fleshy with four vague segments and single sensillum trichodeum at the end (Fig. 5b).

Third instar: The head capsule was heavily pigmented near mouth parts. Clypeolabrum had approximately thirty transversely blunt-tipped sensilla on labrum and two sensilla on clypeal region. Several microtrichia (M) were also distributed near the peripheral C-shaped arc (Fig. 5c). Antenna had one segment composed of one elongate medial and two smaller lateral microtrichia on opposite sides. Antenna also had one elongate Sensillum Basiconicum (SB) on a stalk-like base. Multiple rows of Microtrichia (M) were prominent on the distal dorsal surface of antenna (Fig. 5d).

Fourth instar: Body of fourth instar was 2.71-3.03 mm long and 1.41-1.67 mm wide, fleshy, C-shaped, widest at metathoracic and abdominal segments and tapering posterior (Fig. 6a). Facial view had clypeolabrum which shows mouth parts (Fig. 6b). Clypeolabrum had ten transversely

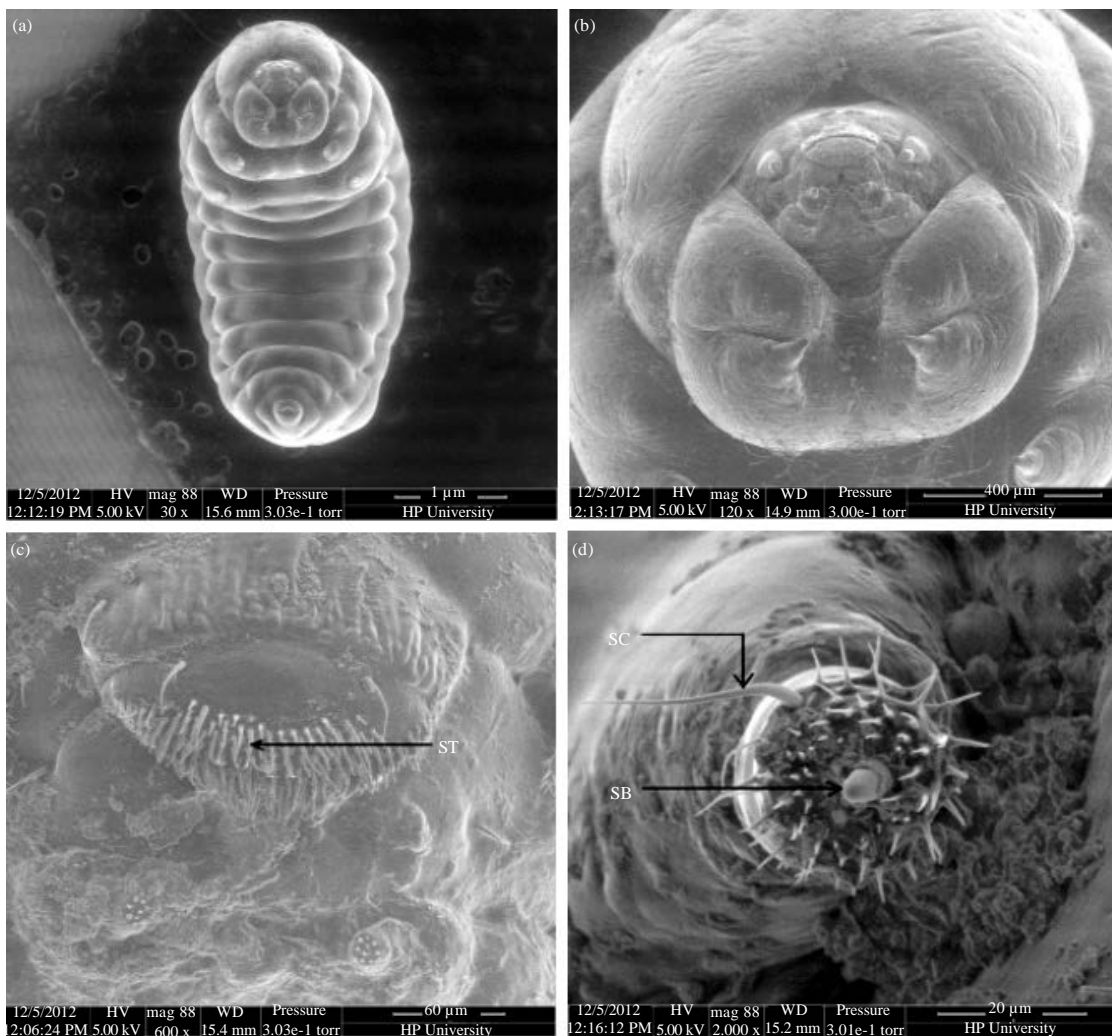


Fig. 6(a-d): Scanning electron micrographs of fourth instar larva of *Acanthoscelides macrophthalmus*, (a) Ventral view, (b) Facial view showing mouth parts, (c) Clypeolabrum showing Sensilla Trichodea (ST) and (d) Antenna showing Sensillum Basiconicum (SB) and Sensillum Chaeticum (SC)

blunt-tipped Sensilla Trichodea (ST) and six sensilla on labrum (Fig. 6c). Antenna had one segment with an enlarged medial and smaller lateral Sensilla Basiconica (SB) and also had one elongate Sensillum Chaeticum (SC) (Fig. 6d).

The present study revealed that *A. macrophthalmus* is serious pest of *L. leucocephala*. The data represented in this study provide some baseline information regarding the life cycle and larval morphology. In many regions where *L. leucocephala* is considered as weed, *A. macrophthalmus* may be used as a biocontrolling agent to suppress the widespread growth of plant. While, in other regions where this plant is utilized for commercial purposes this beetle is required for conservation of this plant. Scanning electron micrographs of four larval instars was taken to describe their morphological characteristics which help in accurate identification of adjacent instars.

DISCUSSION

Acanthoscelides macrophthalmus has been found to be the only bruchid species of the Indian subcontinent that developed inside the seeds of *L. leucocephala*. This insect is multivoltine and complete many generations in a year. Sexual dimorphism was observed in adults. Male beetles were smaller in size with vertical pygidium whereas, female possessed sub vertical pygidium and larger in size. Similar observations were recorded in *A. macrophthalmus* (Thakur, 2012a). A close observation revealed that freshly emerged adults copulate any time after emergence. Copulation lasted for 8-12 min. But in *A. obtectus* copulation lasted for 4-5 min (Thakur, 2012b) and in *Callosobruchus maculatus* and *C. analis* copulation period of 7-10 min has been recorded (Arora, 1977).

Female of *A. macrophthalmus* started laying eggs one day after copulation. Similar observations of egg laying were recorded in same species by Effowe *et al.* (2010). Eggs of *A. macrophthalmus* were oval in shape and white in colour when laid freshly but turn yellowish white after hatching. Similar observations were recorded in *A. macrophthalmus* by Thakur (2012a). In *A. obtectus*, freshly laid eggs were milky white but become transparent before hatching (Thakur, 2010).

Generally, one egg was laid on single seed, but multiple ovipositions occurred when number of seeds was limited. However, one or two adults emerged normally but maximum three adults emerging from one seed have been recorded. Thakur (2010) observed that in *A. obtectus*, eggs were usually deposited singly and does not glued to pods or seeds but scattered irregularly among host seeds.

Oviposition period lasted for an average of 6.8 ± 1.75 days and the average number of eggs laid by single female of *A. macrophthalmus* was 38.3 ± 8.01 . Effowe *et al.* (2010) observed that oviposition period of same species lasted for 6.5 ± 2.2 days and single female laid an average of 43.1 ± 13 eggs. Oviposition period of *A. obtectus* lasted for an average of 7.0 ± 0.77 days and number of eggs laid per female were 53.2 ± 7.91 on the seeds of kidney beans (*Phaseolus vulgaris*) (Thakur, 2010).

The mean longevity of *A. macrophthalmus* male and female was 10.2 ± 1.28 and 12.2 ± 1.75 days, respectively. Similarly, Shoba and Olckers (2010) recorded that longevity of newly emerged adult of *A. macrophthalmus* varied from 2-20 days with an average of 11.9 ± 3.5 days. The average incubation period lasted for 6.0 ± 1.05 days. Effowe *et al.* (2010) have recorded the average incubation period of 4.52 ± 0.84 days in same species.

First, second, third, fourth larval and pupal developmental duration lasted for an average of 6.6 ± 2.07 , 7.4 ± 1.84 , 7.9 ± 1.79 , 8.3 ± 1.16 and 10.2 ± 2.29 days, respectively. However, first, second,

third, fourth larval and pupal developmental duration of the same species was observed 4.2, 4.7, 5.3, 5.2 and 5.7 days, respectively (Effowe *et al.*, 2010). Life cycle was completed in 41.6 ± 7.18 days. Effowe *et al.* (2010) recorded the total developmental period of 33.39 ± 2.87 days. The present study revealed that gravid female of *A. macrophthalmus* produced 27.4 ± 6.77 offsprings, comprising 14.7 ± 4.19 females and 12.7 ± 3.02 males as compared to 36.7 ± 11.3 offsprings observed by Effowe *et al.* (2010).

Scanning electron micrographs of four larval stages of *A. macrophthalmus* were taken and differences among them were observed. Antenna of first instar larva had one segment composed of single elongate lateral microtrichia and one sensillum chaeticum on elongate base. Clypeolabrum had six blunt tipped sensilla on labral and two sensilla on clypeal region. Leg had two segments with elongated base. Similar observations of first instar larva of *A. macrophthalmus* were also recorded by Wu *et al.* (2012). Pfaffenberger (1986) described the first instar larva of *Gibbobruchus mimus* (Say) using SEM and described similar structures.

Antenna had one segment in second and third instars. Labrum had five blunt tipped sensilla in second instar. In antenna one elongate sensillum chaeticum and one elongate sensillum basiconicum was present on stalk like base in second and third instar larvae respectively. Fourth instar larva was larger in size among all the larval instars. Antenna had one segment with an enlarged median and smaller lateral sensilla basiconica and also had one elongate sensillum chaeticum. Clypeolabrum had numerous transversally blunt tipped sensilla trichodea on labral region. Similar observations of *A. macrophthalmus* larva were recorded by Wu *et al.* (2012).

A. macrophthalmus is serious pest of *L. leucocephala*. In many regions where *L. leucocephala* is considered as weed, *A. macrophthalmus* may be used as a biocontrolling agent to suppress the widespread growth of plant. In other regions where this plant is utilized for fodder, food, furniture and other commercial purposes, its conservation is required hence present study will be helpful to plan strategies accordingly.

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