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Morphometric analysis and Reproductive system studies of *Trogoderma granarium* Everts (Coleoptera: Dermestidae)

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Abstract- *Trogoderma granarium* Everts (Khapra beetle) a serious pest of stored grains and stored products with quarantine status is one of invasive species feared around the world. Detection of Khapra beetle live or dead attracts serious trade restrictions and economic fallout, especially in developing countries like India. It attracts strict phytosanitary regulation by many countries in order to restrict the pest at the boundaries. The pest is difficult to control owing to its diapause. It is, therefore, necessary to design a system's approach to tackle this pest. However, to design such a system, precision knowledge regarding identification, biology and reproductive system is essential. Determination of larval instars according to Dyar's law helps to identify this pest in infected samples. Identification of adult khapra beetle on the basis of morphology is difficult due to its similarity with other dermestids, thus study of genitalia will specify the species and also aim in understanding its reproduction and fecundity.

Index Terms- Khapra beetle, Phytosanitary regulation, Quarantine , Biology, Reproductive system

I. INTRODUCTION

The Khapra beetle, *Trogoderma granarium* Everts is one of the most notorious primary insect pests of stored grains and causes direct and various indirect losses (Banks, 1977; Hill, 1983; El Nadi *et al.*, 2001). It is a very serious pest under hot dry conditions, from quarantine point of view, especially in western countries that are of strategic importance to India for exports of cereals, pulses, oilseeds, etc (EPPO,1990 .Data sheets on Quarantine pests-*Trogoderma granarium* Everts). If infestation is severe, the devastation is complete, reducing the grain to mere frass (EPPO, 1990). Its exuviae, shed skin and other body parts are hazardous to human health causing respiratory and skin diseases. The US Government spent about $15 million for its eradication programme, when it was accidentally introduced into USA (Kerr, 1981). In India, many export shipments have suffered heavy losses due to detection of this pest in one or other form. Russia banned imports of plant products from India owing to detection of this pest in a consignment of sesame (Reuters, 2006, HT Media, 2007). Administration of Quality Supervision, Inspection and Quarantine (AQSIQ) Authority of China returned 460 tons of soybeans imported from the Scoular Company, USA due to detection of Khapra beetles, despite the consignment being certified by the Quarantine Authority of US.

For introducing an effective control measure it is important to know the pest by studying its taxonomical characters especially male and female genitalia. Further, the detailed study of its biology helps for implementation of appropriate control measures. Though various workers have extensively studied the Khapra beetle, considerable variations are seen in their reporting of the number of larval instars *viz.* from 4 to 15. Thus, despite detailed studies, an ambiguity still exists, on the number of larval instars that this pest may undergo.

II. MATERIALS AND METHODS

Present study of *Trogoderma granarium* Evert was done at National Institute of Plant Health Mangement, Hyderabad, India (17.3125°N, 78.4000°E) with temperature and humidity range from 23.5°C to 46°C , 37% to 62 %. The culture was maintained for the period of twelve months in large plastic containers with different grain types such as whole wheat, broken wheat (rawa) and rice in the laboratory conditions of 32^0C temperature and 60 % R.H and 12 h photoperiod.The containers were sealed with muslin cloth and was placed in a separately lab as to avoid contamination of culture ,dispersal of larvae or adults due to its quarantine status.

Study of morphology and development:

Eggs of *Trogoderma granarium* Everts were obtained by placing ten pairs of adults in a sterile plastic container on sterilized rice grains. The eggs thus collected daily were segregated and maintained in small petri dishes and used for detailed studies .Newly emerged larvae were placed in a sterile vail with some measured quantity of grains (ten grams).Such twenty four larvae were used as replicates to understand the pattern of growth and development . Monitoring was done at the interval of three days to observe activities like feeding, molting and mortality .Larval exuvia were separated and kept in labeled vials ,each time post molt exuvia were added and maintained .Weakly 5 grams of rice grains were added to vials with larvae .After each consecutive molt the length and head capsule width of the respective larvae were measured with the help of Dinolight camera (40x to 220x) and micrometric scale (1/0.2mm). Each probable combination of instar ranges was compared to expected values obtained from a ratio using Dyar's Law .The pupated larvae were measured for its length ,width and was observed for developmental period and emergence. The frequencies of the measurements based on larval head were plotted to ascertain the number of instars. Standard deviation between the observed head

width and length of the larvae was calculated by SPSS software and variance and difference were calculated by one way ANOVA by using Star software.

Study of Adults Reproductive system :

The newly emerged adults from the observation set were dissected with an insulin syringe (8mm) needle size ,on a glass slide in saline solution (0.9% NaCl).Abdomen of the adult insect were exposed so as to observe the reproductive system which was then separated to observe the reproductive system ,which was further dehydrated with ascending grades of alcohol, clearing the tissue by using xylene and finally fixing in DPX . Micrometry of external and internal genitalia was done by using ocular micrometer and photographed by Optica vision light 2.12 (microscopy digital USB camera) under 40 X through a compound microscope.

III. RESULTS

The development of *Trogoderma granarium* Everts along with adult genitalia is studied under the laboratory conditions. The larvae were active and no larvae diapuase observed during the period of the study .Further, it was noticed that initial instars of the grub were secondary feeders and the primary nature of feeding was developed somewhere near the beginning of an end of the third instar. The adult khapra beetle have wings, but apparently do not fly and feed very little.

Life cycle of *Trogoderma granarium* Everts:

Fecundity:

Copulation took place in 2 or 3 days after adult emergence .Pre-oviposition period was 4 days and average 58 number of eggs was laid by a mated female .The eggs were laid loosely and singly in the frass of wheat grains for a period of 3-5 days. Female *Trogoderma granarium* Everts mated for 4-5 times and the number of eggs laid decreases with the consecutive number of mating.

Longevity:

The adult longevity was 7 to 10 days on an average respectively in case of males and females. The entire life cycle was completed within 45 to 50 days. There is an average of 7 to 8 generations in a year**.**

1. Egg:

The eggs are milky white, turning pale yellowish with age (Fig.a). They are cylindrical in shape, one end is rounded and the other is pointed. On an average, they measured about 0.68 mm (0.633 mm to 0.733 mm) in length and 0.25 mm (in the range of 0.233 mm to 0.267 mm) in width. In the controlled conditions, of the laboratory, the incubation period was of 5 days. The eggs developed yellowish markings towards the end of incubation period. All Eggs hatched from fifth day to seventh day of laying. The larvae showed 5- 7 moultings in development process from first to fifth instar.

2. Larvae :

The length ,head capsule width and duration of each instar ,after every successive molt were measured and recorded (Table 1 and represented in Graph 1 and 2).The principles of Dyar's Law were applied to the recorded values of head capsule to calculate and assure the larval instar.

2.a . First instar larvae (fig b):

The first instar grubs are approximately 1.099 mm (in the range of 0.8 to 1.233 mm) long and 0.33 mm (in the range of 0.233 to 0.633 mm) wide. Hairs originating from the last abdominal segment covered most of the body length. Grubs are uniformly yellowish white, except head. The head capsule measures 0.186 mm wide (in the range of 0.1 mm to 0.267 mm) and 0.111 mm long (in the range of 0.067 mm to 0.167 mm). The duration of first instar grub was 1 to 2 days with an average of 1.6 days.

2.b. Second instar larvae (fig c) :

The larvae are yellowish in colour and measures 1.749 mm long in the range of 1.233 mm to 2.110 mm while the width was 0.485 mm in the range of 0.33 mm to 0 .933 mm. The head capsule is 0.343 mm wide (in the range of 0.267 mm to 0.467 mm) and 0.173 mm long (in the range of 0.1 mm to 0.267 mm) . The duration of this instar is 2 to 3 days with an average duration of 2.7 days.

2.c. Third instar larvae (fig d):

Third instar grubs yellowish golden coloured. They measures on an average 2.453 mm in length (in the range of 2.110 mm to 3.111 mm) and 0.669 mm in width (in the range of 0.5 mm to 0.967 mm). The head capsule is 0.550 mm wide (in the range of 0.433 mm to 0.733 mm) and 0.297 mm long (in the range of 0.267 to 0.467mm). The grubs appear golden brown or red brown and their bodies are distinctively bearing profuse and characteristic hair. The total duration of this stage is 7 to 8 days, with average of 7.5 days.

2.d. Fourth instar larvae (fig e):

Grubs of fourth instar are 4.111 mm long and 0.858 mm wide in the ranges of 3.111 – 4.333 mm and 0.667 – 1.2 mm respectively. The head capsule is 0.855 mm wide and 0.584 mm long in the range of 0.733– 0.933 mm and 0.4 – 0.667 mm respectively. The grubs became darker in their colour as they grow . Fourth instar larval duration is on an average 9.6 days, the minimum being 9 and the maximum being 10 days.

2.e. Fifth instar larvae (fig f):

The grubs are 5.733 mm long in the range of 4.333 mm to 6.2 mm. The body width is 1.413 mm in the range of 1.2 mm to 1.733 mm. The head capsule is 0.978 mm wide (in the range of 0.933 mm to 1.333 mm) and its length on an average is 0.615 mm in the range of 0.533 mm to 0.8 mm. The tail made up of hair was drastically shortened upto this period. The duration is on average 10.5 days with minimum of 10 days and maximum being 11 days.

The total larval period on an average from first to fifth instar is 31.7 days in the range of 29 to 34 days. The duration of later instars progressively increased in comparison with earlier instars (graph 2).

3. Pupa (fig g and h):

Exarate type of pupae is observed with distinctly segmented body. The female pupae is comparatively larger than the males and easily distinguished .On an average the female and male pupa measured 5.3 mm ,3.9 mm in lenght and 0.233 mm, 0.267 mm in width respectively. The Pupal duration is approximately 5 days.

4. Adults (fig i and j):

Adults are approximately 1.6 to 3.0 mm in length and 0.7 to 1.7 mm in width with a retractile head and 11 segments clubbed antennae which fits into the groove present near pronotum. Males are dark brown to black with indistinct reddish brown markings on elytra and are easily distinguished from the females by being darker and smaller in size.

Reprodcutive system of male and female *Trogoderma granarium* Everts :

a. Male reproducutive system:

The male genitalia of *Trogoderma granarium* Everts studied under compound microscope at 40X ,the internal organs (figure 2.a.) comprises a pair of testes ,a pair of vesicular seminalis, two accessory gland namely accessory gland I and II, ejaculatory duct and the external genitalia is made up of, aedeagus, paramere and bridge. Testes are paired, bean shaped (0.13mm x 0.15mm) and attached by accessory gland I to the posterior side, both testes are connected to common ejaculatory duct. Vesicular seminalis (0.27mm) are paired and short joining testes and ejaculatory duct. There are two accessory gland namely accessory gland I (0.13mm x 0.11mm) in a pair placed near the posterior end of testes and accessory gland II is cylindrical, long in 4-5 numbers (0.46mm) attached posteriorly to the ejaculatory duct. The external genitalia (fig.2.b) of *Trogoderma granarium* Everts shows an aedagus attached to tegmen and a bridge which is transversely straight and of equal width along its entire length further concluded with paramere .

a. Female reproducutive system:

The female genitalia of *Trogoderma granarium* Evert (fig.3.a) lies from fifth to ninth abdominal segment and found entangled with digestive system studied under 40X compound microscope .It represents a system composed of telotrophic meroistic ovary (0.16-0.18 mm long) made up of 5-11 numbers of ovarioles, depending on the developmental stage of the ovary (fig.3.b). An immature female shows an undeveloped ovary with 5-7 ovarioles with non-distinguishable ovarioles in it (fig 3.c). Fully developed mature ovary bears 9-11 ovarioles among which numerous developed eggs can be observed found decending towards the ovipositor. Ovary is further connected to common short oviduct which leads into posterior region of curved bursa copulatrix (0.15 mm). Bursa copulatrix is large, sac like structure bearing a corrugated spermtheca (0.06 mm) attached to laterally and a serrated sclerite .Finally busa copulatrix opens into external genitalia or ovipositor (0.11 mm in length). Ovipositor is long extending from 8th abdominal segment and its posterior region can be observed externally.

IV. DISCUSSION

Among enlisted invasive species, *Trogoderma granarium* Everts (Khapra beetle) has a quarantine status for many countries around the world. Presence of live or dead specimen in any consignments of trade immediately triggers alarm leading to rejection and huge economic losses. Many countries including India has to undergo export restrictions due to presence of this notorious pest. Larvae being the damaging stage cause direct and indirect effect on environment .In year 2006 ,Mohammad Anwar and his co-researchers observed the larval stages of *Trogoderma granarium* Everts to cause seviour damage, adding impurities and other inclusion resulting in deteriorating quality of the stored products .Their nature of going into diapause in unfavorable condition for months to year and further reinfestation, stands as a major barrier for selection and application of appropriate control measures . In such a scenario ,it is of prime importance to know and understand the biology of pest which will help in proper detection, identification and will lead to better implementation so as to restrict and kill distructive quarantine pest khapra beetle.

In present work an effort is made to elucidate the biology, determination of larval instars and study of genitalia of *Trogoderma granarium* Everts. Life cycle study in controlled laboratory condition leads to normal development of insects and entire development is completed in 45-50 days. This observation was almost similar with that of Karnavar, G.K.1972 while studying the mating behavior and fecundity in *Trogoderma granarium* Everts (Coleoptera: Dermestidae). The khapra beetle adults have wings, but apparently do not fly and feed very little, if at all and newly emerged are active. The initial larval instars acted only as secondary feeders and the primary nature of feeding was developed somewhere near the beginning and end of the third instar. No larval diapause is observed and reasons contributing to this may be due to frequently division of the culture and avoiding the overcrowding. Some researchers such as H.D.Burges,1963 observed that in normal climatic condition of store house the larvae of trogoderma goes into diapause for several days to months and even a year. Total number of larval instars is considered by morphometry of head width which revealed five instars in male and six instars in some female insects. Readings were analyzed and conformed by using Dyar's law as applied by Paul .Dallara,et.al.,2012 to the walnut twig beetle and also in determination of larval instars of the rice weevil by A.E.O'Donnell ,1967. The duration of larval instars is observed which is directly related to the growth of larvae and number of instar thus, important to consider the amount and level of infestation caused by them. As higher larval instars took more days to develop and simultaneously feeds more causing severe damage to the grains.

Adults are considerably less destructive but eggs laid by them add in the level of infestation and pest population. Presence of adult insects of khapra beetle in the infested grains can be mistaken with other dermisted species due to resemblance in morphological characters. Thus, study of reproductive system will specify the species as well add knowledge about its reproduction and fecundity. Ewa Mroz, 2012 specified species of hemipteran fly *Psallus* Fieber on the basis of male genitalia.

Similarly, Mahmut Erbey et al.,2010 described *Lixus cardui* Oliver according to morphology of male and female genitalia. In 2005, Vaclar stejskal along with his co workers have identified and discribed the *Trogoderma* species (*T.longistosum and T.variable*) on the basis of morphological character an genitalia. Similarly in the identification guide provided by USDA-APHIS-PPQ identifiers and in ISPM 27 different species of *Trogoderma* are distinguished based on morphological charactera and details of genitalia .In most of the records, female external and internal genitalia are discribed but details regarding the ovary have to be include. With respect to male khapra beetle only external genitalia is detailed and needs more specifications regarding the internal genitalia. For the same in the present study we have tried to detail the internal as well as external genitalia of *Trogoderma granarium* Everts, which would further help to understand the reproduction of the species leading to better control measure than present today.

V. CONCLUSION

Biology and reproductive system of *Trogoderma granarium* Everts (Khapra beetle) including external genitalia of adults is described in the present paper .Khapra beetle is rated as quarantine pest and is one of most 100 invasive pest species .Being a threat of introduction into many non khapra beetle contries, phytosanitatry measures are followed which restricted this pest on boundries. In countries where khapra beetle has already invaded and established itself, have trade restrictions reflecting in economy of that country. For this it is important to implant an effective control measure against this pest, which is possible only if one have proper knowledge of it. As external morphological characters can be confused with other similar species, reproductive system with external genitalia specifies the species additionally detailing information about its internal organs of reproduction.

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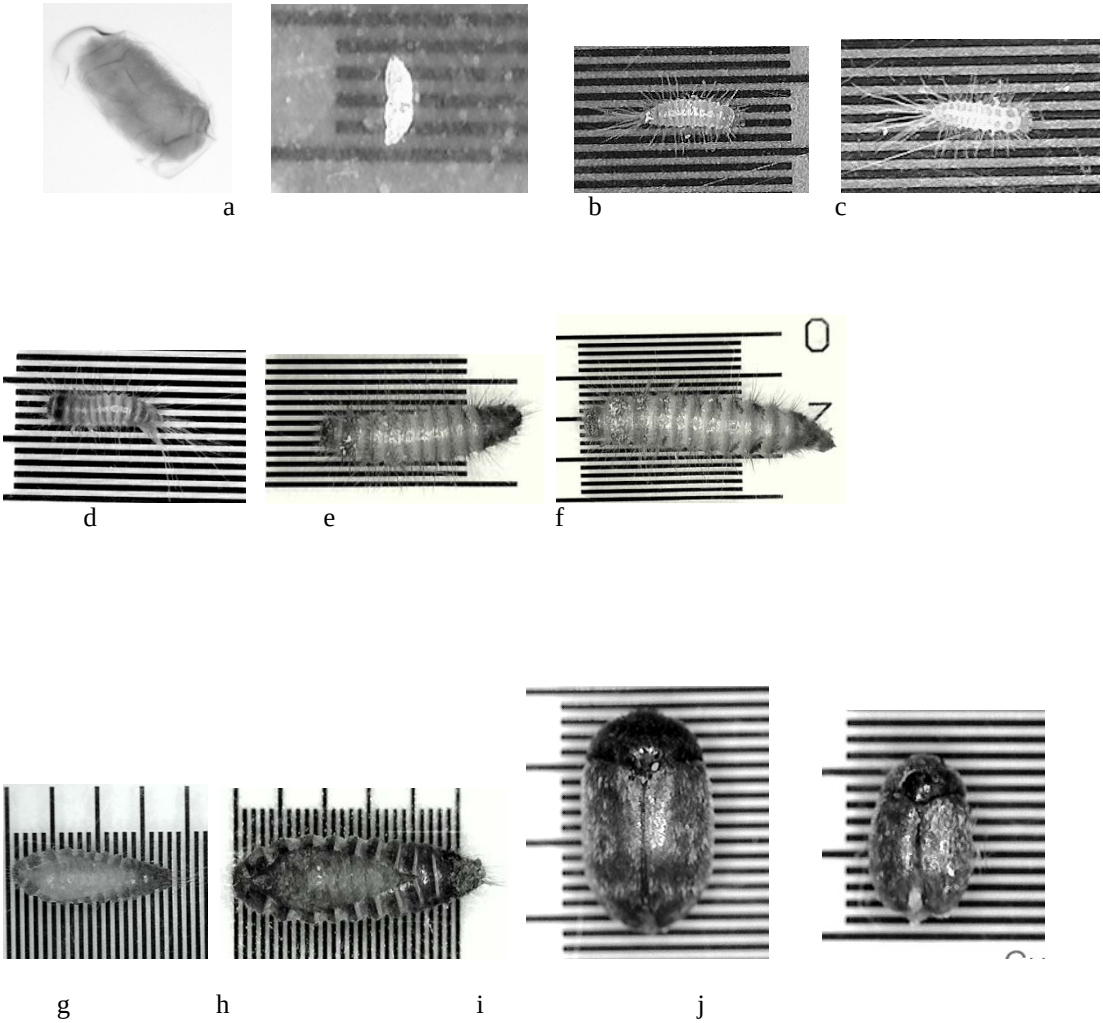
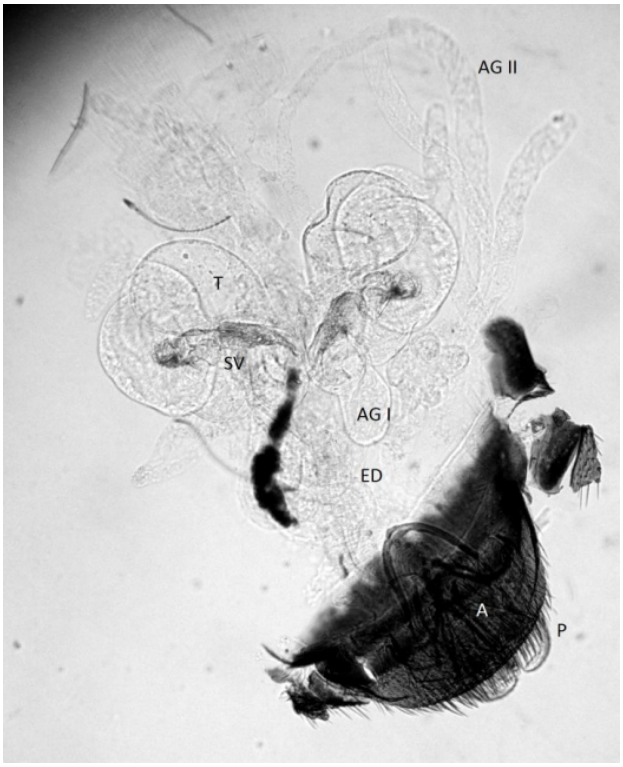


Figure 1 . Developmental stages of *Trogoderma granarium*
a. Egg b.First instar larvae c. Second instar larvae. d.Third instar larvae. e. Fourth instar larvae.
f. Fifth instar larvae. g.Pupae of male. h. Pupae of female. i.Adult male. j.Adult female .



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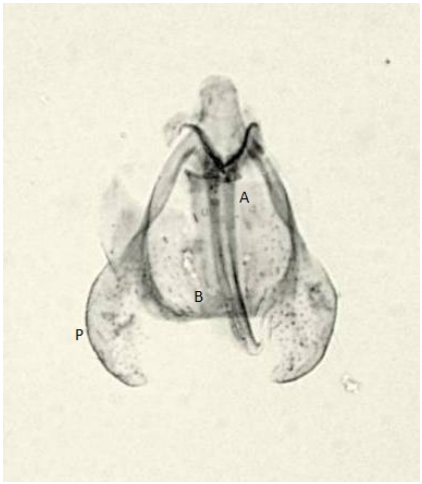


b

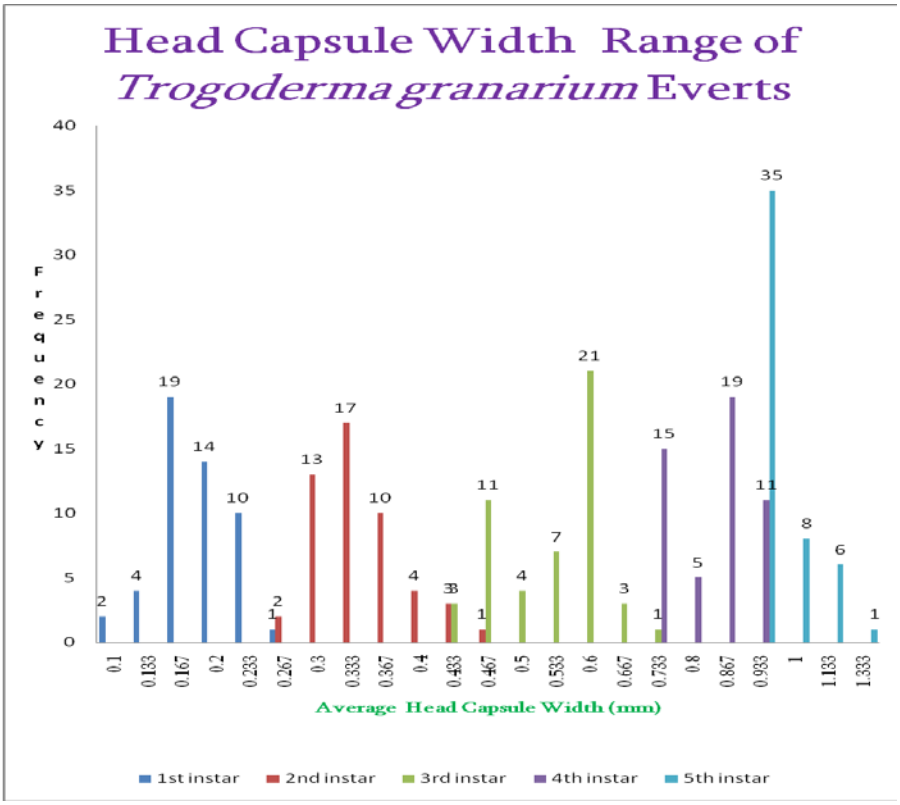
Figure 2 : a. Male Reproductive system of adult *Trogoderma granarium* Everts
b. External genitalia of *Trogoderma granarium* Everts
T: testes , SV: seminal vesicle , AD I :accessory gland 1 ,AD II: accessory gland 2
ED :ejaculatory duct , A: aedeagus , P:paramere ,B: bridge

Figure 3 : a. Female genitalia of adult *Trogoderma granarium* Everts
b. Telotrophic meroistic ovary with ovarioles
c. Single Ovariole
OV : ovary , OVL : ovariole , S :spermathica , SL:sclerite,
BC : bursa copulatrix, OVP : ovipositor

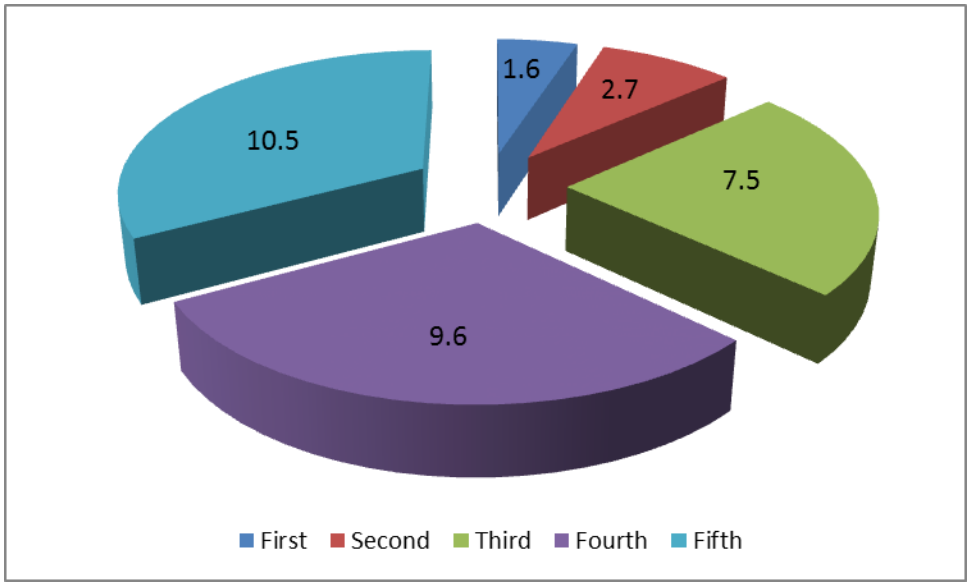
Tables: **Table 1. : Different developmental parameters of larval stage (*Trogoderma granarium* Everts) .**

Instar	Average Duration of Larval growth (days)	Observed Length of larvae (mm)	Observed head capsule width (mm)	Calculated head capsule width (observed width x Average ratio of increase in mm)	Average Rate of growth of head capsule (mm)
First	1.6	1.7±0.258	0.186 ± 0.036	nil	nil
Second	2.7	2.0±0.729	0.343 ± 0.044	0.186 x 1.6115=0.300	1.844
Third	7.5	3.52±0.414	0.550 ± 0.072	0.300 x 1.6115=0.483	1.603
Fourth	9.6	4.65±0.914	0.855 ± 0.076	0.483 x 1.6115=0.778	1.555
Fifth	10.5	5.22±0.741	0.978 ± 0.333	0.778 x 1.6115=1.254	1.444

Head capsule width of larvae/instar		Length of larvae/instar	
CV	11.03%	CV	2.89%
SED	0.038	SED	0.063
F	5.35	F	1.56



Graph 1.: Determination of larval instars in *Trogoderma granarium* Everts



Graph 2.Average Duration of growth between each Larval instars of *Trogoderma granarium* Everts.