Study on the life cycle of *Tribolium castaneum* (Coleoptera: Tenebroinidae) on different cereals.

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Research Article

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Abstract

Background

Tribolium castaneum is one of the most destructive pest of stored grains products around the world. The present study was conducted to observe the life cycle of *Tribolium castaneum* on different cereal flour varieties i.e. *Triticum aestivum* (wheat flour), *Oryzae sativa* (Rice flour), *Zea mays* (Corn flour), *Vigna unguiculata* (Cow peas Flour), *Sorghum bicolor* (Sorghum flour) and *Hordeum vulgar* (Barley flour). From two sources the samples were being collected, i.e. grocery Stores/super mart (Source-I) and flour mills/warehouses (Source-II) at the ideal temperature and relative humidity under laboratorial conditions at the Zoology department, Shah Abdul Latif University Khairpur.

Results

During the present study it was recorded that *Tribolium castaneum* consistently undergoes six larval stages on each cereal flour from source-I and source-II. Among the cereal flours It was noticed that total duration from eggs to adult remains fastest on wheat flour i.e. 41.8 ± 0.80 followed by sorghum flour 52.9 ± 0.90 and lowest on Cow-peas flour 65.5 ± 1.00 followed by corn flour 61.8 ± 0.96 and average total duration from eggs to adult on rice flour 54.9 ± 0.83 followed by Barley flour 53.9 ± 0.82. The length of eggs was 2.9mm and width was 0.7mm were recorded and the length of larva from 1st instar to 6th instar was 5mm to 12mm and width was 2.1mm to 3.7 were recorded and length of pupa was 15.2mm and width was 3.8mm and length of adult was 17.2 mm and width was 4mm were recorded respectively.

Conclusions

The results of this study indicated that cow-peas flour was the least suitable for development of *Tribolium castaneum* as compared to wheat flour. Additionally, postharvest products from source-II (flour mills) were found to be extremely susceptible to *Tribolium castaneum*. Study of life cycle of *Tribolium castaneum* in different cereals flours would be helpful in post-harvesting of the insect pest in various stored grain food materials.

Background

The *Tribolium castaneum* is a notorious group of insect, it includes Beetles and weevils. These belongs to the order Coleoptera. It is one of the largest insect order, recognized and identified about 40% of all known insect species around the world. There are 360000 different species of beetles. Throughout the world, including Sindh, it is the most destructive pest of stored grain products that causes serious damage. About 5–10% of the world's destruction caused by that pest, and about 50% in tropical and sub-tropical regions. According to (Maqsood, 1988; Adams, 1998; Ahmad and Ahmad, 2002) that estimated the damage caused by beetles during the summer months in tropical areas can reach up to 50%. Beetles

infest a wide variety of foods and seriously harm grocery stores, flour mills, and warehouses. These are the most noticeable insects; some of them also have most attracted colors and remarkable forms of beetles. One of the most well-known major pests and notorious pest in the Coleoptera order and family Tenebroinidae is the *Tribolium castaneum*, also known as the red flour beetle. The Latin word tenebrio, which means "who loves to live in darkness". *Tribolium castaneum*, a pest with a globally distributed and present in a variety of foods like wheat, rice, oatmeal, peanuts, chocolate, spices, flours, cereals, oatmeal, beans, and pasta, among others. (Lepseme, 1944) noted that these species can be found in arid and semi-arid regions throughout the world and can survive in temperatures as high as 50^{O}C. One of the most destructive pests and species of the Tenebroinidae family, the red flour beetle is the darkling beetle that lives on stored grain products, according to (Thiaw *et al.*, 2012; Weston and Rattingourd, 2000; Neenah, 2014; Devi and Devi, 2015) observed that can harm a variety of long-lasting stored goods, including barley, corn, flour, millet, wheat, rice, and sorghum (Bennet, 2003). According to (Fedina and Lewis, 2007), this species is a significant and important insect pest in grain processing and storage. According to (Campbell and Hagstrum, 2002), *Tribolium castaneum* can be a significant pest in warehouses, Supermarts, and flour mills. Similarly, (Arthur and Dowdy, 2003) the high temperature increases the numbers of *Tribolium castaneum* on stored product. For the purpose of preventing insect and pest damage to stored goods, airtight storage or sealed storage can be used (Naravvo, 2012). According to (Villers *et al.*, 2008) observation it is one of the safest alternative technological method for protecting stored products. (Anankware *et al.*, 2012).

The main purpose of research plan is to determine how these stored grain beetles are related to each other and how they effect on various types of cereal flour consequently, in growth and development. Additionally, the study will help to initiate pest control measures for stored grain in a proper time. During the present Study on the life cycle of *Tribolium castaneum* (Coleoptera: Tenebroinidae) on different cereals would be useful for post-harvest insect pest control in a variety of grain-based food products that are being stored.

Methods

Flours test

During the present study I have selected six different cereals flours such as *Triticum aestivum* (wheat flour), *Oryzae sativa* (rice flour), *Zea mays* (Corn flour), *Vigna unguiculata* (Cow-peas flour) *Sorghum bicolor* (Sorghum flour) and *Hordeum vulgar* (Barley flour).

These flours were collected from two different sources these flours are required for the Biology of *Tribolium castaneum.* Visited different flour mills, grocery stores and ware-houses and collected 6 months/1 year old cereal flours, from all these infested flours after that found that the stored grain pest. The flour was sieved through 80-mesh sieve to count the eggs and observed other developmental stages. Some flours were available in thinnest powder form and sticky in nature so there did not need to sieve them (Gerken and Campbell, 2020).

Culture of Insects

After collection of live stored grain pests the selected samples of ware-houses/super-mart and flour mills were brought into the laboratory of the Department of Zoology at Shah Abdul Latif University, Khairpur Mir's and these selected flours were sieved with 80-mesh and 60-mesh sieved one by one and take a six 500 gram jars and put the sieved 250 grams of flour in each jar, and the population of *Tribolium castaneum* at least 100 adult insect of *Tribolium castaneum* were placed in each jar of the provided cereals. For the maintenance of culture gave the 5% of the Brewer's yeast and jars were covered with muslin cloth banded with rubber band. The Brewer's yeast used to increase their population or the rate of growth and development and also maintained the standardized temperature 37^0C and RH 65–80% on 16-hours in a day time and 8-hours in a night time cycle.

Insect collection and Identification

After the collection of live stored grain pests took the samples into the laboratory and observed under the binocular microscope model No: BS-3020B and by the help of magnifying glass and take 4-vials for each flour and put 10g of each flour supplied as food in each vial after that the first instar grubs were carefully collected by the help of camel hair brush then separately shifted in glass vials. After some time keep other stages of *Tribolium castaneum* in each vial, such as other instars, Pupa and Adult. After maintenance of the culture in each vials and these vials were continuously monitored and checked humidity and temperature and measure the length and diameter by the using ImageJ (1.53K) Software then after identified the morphological and taxonomical characteristics provided in the literature as well as concerned of supervisor and identified insect pest *Tribolium castaneum*.

Results

Tribolium castaneum commonly called as Red flour beetle have complete life cycle involved egg, larva, pupa and adult stages. The larval stage passed through six instars. Observations on incubation period, larval duration; pupal duration, adult duration; length and breadth of different immature stages and adult were recorded. Present study is aimed to examine the life cycle of *Tribolium castaneum* on six different cereal flour varieties i.e. Triticum aestivum (wheat flour), Oryzae sativa (rice flour), Zea mays(Corn flour), Vigna unguiculata (Cow-peas flour) Sorghum bicolor **(**Sorghum flour) **and** Hordeum vulgar **(**Barley flour) these samples were collected from two different sources such that grocery store and super mart (Source-I) and flour-mills and ware-houses (Source-II) to assess the infestation rate and effects on growth and development of the pest. However, Gerken and Campbell, (2020) worked on the oviposition and development of *Tribolium castaneum*, a major pest of wheat and rice. They used 18 different commercially available flours (almond, amaranth, barley, buckwheat, cassava, coconut, corn, garbanzo, millet, oat, potato, quinoa, rice, rye, sorghum, spelt, teff, and wheat for experiment purposes and to know about the level of infestation and effect on growth and development of the pest.

Taxonomical characteristics of different life stages of Tribolium castaneum

Tribolium castaneum has complete metamorphosis which passes through four stages of life cycle: egg, larva, pupa, and the adult (Fig. 1a-i)

Eggs

Eggs are grayish-yellowish, cylindrical in shape and tiny. The egg's surface is sticky; and food particles can stick to its surface. The tiny eggs hatch in approximately four days under the laboratory conditions. The egg was averagely 2.9mm in length and 0.7mm in width.

Larval instars

It was observed that as beetles passes from instars to instars, its width of head increases and movement of instars become slow when it’s going to moult. The larvae are slender, cylindrical and are a cream or yellow color with brown heads. Each larva has six legs with two pointed structure at the back. The larvae feed hungrily and eventually grow to the size of rice grains. The larval period lasts from 15 to more days.

1st instar

It was observed that as beetles passes from instars to instars, its width of head increases and movement of instars become slow when it’s going to moult The color of body is cream white translucent with light brown head, dark brown eye and six leg and hair like appendages were appeared. The Size was approximately 5mm in length and 2.1mm in width.

2nd instar

The body colour is yellowish white, cylindrical coated with hairs, hair like appendages, the colour of head is light brown, and has six legs and the last three abdominal segments have two dark pointed structures. This instar approximately 6.3 mm in length and 2.3 mm in width.

3rd instar

The body colour is yellowish Its size is larger than second instar, approximately 8.2mm in length and 2.6mm in width movement is fast but slower than second instar the characteristics are similar to the second instar and the last three abdominal segments have dark spots.

4th instar

It is also similar to the third instar but increases in size and weight (thick) the head is enlarged dark brown in colour, last three segments are also dark in colour and at the end of the segments pointed structure is present that is known as cerci, the main function of cerci are sensation. On the abdomen of many species of insect Paired like appendages are present and the last three abdominal segments are darker in colour. Its size was approximately 9.5mm in length and 2.7mm in width.

5th instar

It is also similar but different in size approximately 10mm in length and 3.2mm in width and colors, has dark pointed 3-ends segments of the abdomen, it has a pair of cerci at the end. The body is pointed and head is enlarged and curved. Each Instar has 12 segments and 2 antennae, segmented hair like body.

6th instar

The duration is ranged from 4 to12 the size of sixth instar is increased and thick in structure and head is enlarged and have wings, immobile and crescent shaped. Before transformed into pupal stage, the feeding was being stopped by last larval instar. The average minimum, maximum and humidity were 30^0C-36^0C and 42–67%respectively.

Pupa

The resting and starvation stage is pupal stage. The larval instar covers itself in a pupal case it is a thin cover called the pupal cuticle. Before converting into pupation the pupa have dark wings, legs and well developed eyes and its dorsal side had covered with hair.

Adult

The adult beetles had a reddish-brown color, and their bodies were flat and curved on the sides. The thorax had slightly curved sides, and the head bears antennae, six legs, and biting mouth parts. The head was visible from above and did not a beak. Their antennae are capitate, and the last three segments are wider than the preceding segments. The fore femur's posterior side is covered in a setiferous patch on males, but not on females. Size approximately 17.2 mm in length and 4 mm in width. A female beetle can produce 300 to 400 eggs in her lifetime, and an adult can live up to three years.

Morphometric and metrological record of Tribolium castaneum

The morphometric analysis and meteorological record on different stages of *Tribolium castaneum* on different cereal flours were observed (Table. 1). It is showing the 1st stage egg was averagely 2.9 mm in length and 0.7 mm in diameter. The duration of eggs in different cereal was 1–9 days at center minimum and maximum temperature and humidity 20^0C to 24^0C and 26–49% were recorded respectively. Newly hatched larvae (1st Instar) were approximately 5mm in length and 2.1 mm in diameter duration of 1st instar in different cereal was 2–8 days at center minimum and maximum temperature and humidity 22^0C to 27^0C and 30–51% were recorded respectively. 2nd Instar larvae were approximately 6.3 mm in length and 2.3 mm in diameter duration of 2nd Instar in different cereal was 2–8 days at center minimum and maximum temperature and humidity 24^0C to 29^0C and 33–55% were recorded respectively. 3rd Instar larvae were approximately 8.2mm in length and 2.6mm in diameter duration of 3rd Instar in different cereal was 3–9 days at center minimum and maximum temperature and humidity 26^0C to 30^0C and 35–58% were recorded respectively. 4th Instar larvae were approximately 9.5 mm in length and 2.7 mm in diameter duration of 4th Instar in different cereal was 4–9 days at center minimum and maximum temperature and humidity 27^0C to 32^0C and 39–61% were recorded respectively.

The 5th Instar larvae were approximately 10mm in length and 3.2 mm in diameter duration of 5th Instar in different cereal was 2–11 days at center minimum and maximum temperature and humidity 29^0C to 34^0C and 40–65% were recorded respectively. 6th Instar larvae were approximately 12 mm in length and 3.7 mm in diameter duration of 6th Instar in different cereal was 4–12 days at center minimum and maximum temperature and humidity 30^0C to 36^0C and 42–67% were recorded respectively. Pupa were approximately 15.2 mm in length and 3.8 mm in diameter duration of pupa in different cereal was 3–13 days at center minimum and maximum temperature and humidity 32^0C to 38^0C and 44–70% were recorded respectively. Adult red flour beetles were approximately 17.2 mm in length and 4 mm in diameter duration of 6th Instar in different cereal was 5–14 days at center minimum and maximum temperature and humidity 34^0C to 40^0C and 46–75% were recorded respectively. (Table 1).

Variation in growth and development of Tribolium castaneum

The variation in growth and development of *Tribolium castaneum* on six different cereal flour varieties were noted (Table. 2). The eggs hatching was recorded fastest on wheat flour i.e. 3 ± 0.55 followed by i.e. 4.4 ± 0.81 on the sorghum flour. However, the slowest eggs hatching rate were recorded on cow-peas flour i.e. 5.6 ± 1.17 followed by corn flour and average eggs hatching rate were recorded on rice flour i.e. 5.2 ± 1.16 followed by barley flour i.e. 4.6 ± 0.83 occurs in their all life stages respectively. The 1st instar hatching was recorded fastest on wheat flour i.e. 3.8 ± 0.66 followed by i.e. 3.5 ± 0.81 on the sorghum flour respectively. However, the slowest 1st instar hatching rate were recorded on cow-peas flour i.e. 5.9 ± 0.56 followed by corn flour i.e. 5.2 ± 0.73 and average 1st instar hatching rate were recorded on rice flour i.e. 4.2 ± 0.58 followed by barley flour i.e. 5.1 ± 0.93 occurs in their all life stages respectively. The 2nd instar hatching was recorded fastest on wheat flour i.e. 3.3 ± 0.54 followed by i.e. 4.5 ± 1.07 on the sorghum flour. However, the slowest 2nd instar hatching rate were recorded on cow-peas flour i.e. 5.3 ± 0.62 followed by corn flour i.e. 5.3 ± 0.86 and average 2nd instar hatching rate were recorded on rice flour i.e. 5.6 ± 0.78 followed by barley flour i.e. 4.5 ± 0.59 occurs in their all life stages respectively.

The 3rd instar hatching was recorded fastest on wheat flour i.e. 4.4 ± 0.60 followed by i.e. 4.6 ± 0.97 on the sorghum flour. However, the slowest 3rd instar hatching rate were recorded on cow-peas flour i.e. 7.2 ± 0.86 followed by corn flour i.e. 6.4 ± 0.81 and average 3rd instar hatching rate were recorded on rice flour i.e. 5.8 ± 0.66 followed by barley flour i.e. 4.9 ± 0.51 occurs in their all life stages respectively. The 4th instar hatching was recorded fastest on wheat flour i.e. 3.8 ± 0.80 followed by i.e. 5.6 ± 0.68 on the sorghum flour. However, the slowest 4th instar hatching rate were recorded on cow-peas flour i.e. 5.3 ± 0.94 followed by corn flour i.e. 5.4 ± 1.21 and average 4th instar hatching rate were recorded on rice flour i.e. 5.2 ± 0.63 followed by barley flour i.e. 5.8 ± 1.16 occurs in their all life stages respectivelyThe 5th instar hatching was recorded fastest on wheat flour i.e. 3.4 ± 0.40 followed by i.e. 6.2 ± 0.73 on the sorghum flour. However, the slowest 5th instar hatching rate were recorded on cow-peas flour i.e. 7.6 ± 1.21 followed by corn flour i.e. 7 ± 1.14 and average 5th instar hatching rate were recorded on rice flour i.e. 5.5 ± 0.80 followed by barley flour i.e. 5 ± 0.84 occurs in their all life stages respectively.

The 6th instar hatching was recorded fastest on wheat flour i.e. 5.9 ± 0.75 followed by i.e. 7.7 ± 0.54 on the sorghum flour. However, the slowest 6th instar hatching rate were recorded on cow-peas flour i.e. 8.6 ± 0.93 followed by con flour i.e. 8 ± 0.71 and average 6th instar hatching rate were recorded on rice flour i.e. 6.5 ± 0.63 followed by barley flour i.e. 8.2 ± 0.66 occurs in their all life stages respectively. The pupa emergence were recorded fastest on wheat flour i.e. 6.8 ± 1.93 followed by i.e. 7.8 ± 1.39 on the sorghum flour. However, the slowest pupa emergence rate were recorded on cow-peas flour i.e. 9.2 ± 1.43 followed by corn flour i.e. 9.4 ± 1.08 and average pupa emergence rate were recorded on rice flour i.e. 8.2 ± 1.07 followed by barley flour i.e. 7 ± 1.03 occurs in their all life stages respectively. The Adults emergence were recorded fastest on wheat flour i.e. 7.4 ± 1.93 followed by i.e. 8.6 ± 1.12 on the sorghum flour. However, the slowest Adults emergence rate were recorded on cow-peas flour i.e. 10.8 ± 1.32 followed by corn flour i.e. 10 ± 1 and average Adults emergence rate were recorded on rice flour i.e. 9.6 ± 1.03 followed by barley flour i.e. 8.8 ± 0.85 occurs in their all life stages respectively(Table. 2)

Discussion

Present study is aimed to examine the life cycle of *Tribolium castaneum* on six different cereal flour varieties i.e. Triticum aestivum (wheat flour), Oryzae sativa (rice flour), Zea mays(Corn flour), Vigna unguiculata (Cow-peas flour) Sorghum bicolor **(**Sorghum flour) **and** Hordeum vulgar **(**Barley flour) these samples were collected from two different sources such that grocery store and super mart (Source-I) and flour-mills and ware-houses (Source-II) to assess the infestation rate and effects on growth and development of the pest. However, Gerken and Campbell, (2020) worked on the oviposition and development of *Tribolium castaneum*, a major pest of wheat and rice. They used 18 different commercially available flours (almond, amaranth, barley, buckwheat, cassava, coconut, corn, garbanzo, millet, oat, potato, quinoa, rice, rye, sorghum, spelt, teff, and wheat for experiment purposes and to know about the level of infestation and effect on growth and development of the pest. However, It was noticed that total duration from eggs to adult remains fastest on wheat flour i.e. 41.8 ± 0.80 followed by sorghum flour 52.9 ± 0.90 and lowest on Cow-peas flour 65.5 ± 1.00 followed by corn flour 61.8 ± 0.96 and average total duration from eggs to adult on rice flour 54.9 ± 0.83 followed by Barley flour 53.9 ± 0.82 respectively.

Conclusion

It is concluded that the Biology of *Tribolium castaneum* on six various cereals flour obtained from two sources cultured under optimum laboratorial conditions. *Tribolium castaneum* consistently give rise to six larval stages on all cereal flours of sources-I and II. It was noticed that total duration from eggs to adult remains fastest on wheat flour i.e. 41.8 ± 0.80 followed by sorghum flour 52.9 ± 0.90 and lowest on Cow-peas flour 65.5 ± 1.00 followed by corn flour 61.8 ± 0.96 and average total duration from eggs to adult on rice flour 54.9 ± 0.83 followed by Barley flour 53.9 ± 0.82.

Declaration

Competing interests: The authors declare no competing interests.

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Tables

Table. 1 Morphometric analysis and Meteorological record of various developmental stages of *Tribolium castaneum* on different cereals with combined sources

S.No:	Life stages	Duration range	Length (Mean) mm	Diameter (Mean) mm	Average temperature		Average humidity	
					Min	Max	Min	Max
1	Eggs	1-9 days	2.9mm	0.7mm	20°C	24°C	26%	49%
2	1^{st} instar	2-8 days	5mm	2.1mm	22°C	27°C	30%	51%
3	2^{nd} instar	2-8 days	6.3mm	2.3mm	24°C	29°C	33%	55%
4	3^{rd} instar	4-10 days	8.2mm	2.6mm	26°C	30°C	35%	58%
5	4^{th} instar	2-9 days	9.5mm	2.7mm	27°C	32°C	39%	61%
6	5^{th} instar	2-11 days	10mm	3.2mm	29°C	34°C	40%	65%
7	6^{th} instar	4-12 days	12mm	3.7mm	30°C	36°C	42%	67%
8	Pupa	3-13days	15.2mm	3.8mm	32°C	38°C	44%	70%
9	Adult	5-14 days	17.2mm	4mm	34°C	40°C	46%	75%

Table. 2 showing variation in the growth and development of *Tribolium castaneum* on different cereal flour varieties with combined sources

Life stages	Wheat Flour	Rice Flour	Corn Flour	Cow-peas Flour	Sorghum Flour	Barley Flour
	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E
	Range	Range	Range	Range	Range	Range
Egg	3±0.55	5.2 ± 1.16	5.1± 1.08	5.6 ± 1.17	4.4± 0.81	4.6 ± 0.83
	1-3 days	2-7days	2-8days	3-9 days	2-5 days	2-6days
1st instar	3.8 ± 0.66	4.2 ± 0.58	5.2± 0.73	5.9 ± 0.56	3.5 ± 0.81	5.1± 0.93
	2-3days	2-5days	3-5days	4-6 days	2-4 days	3-4days
2nd instar	3.3 ± 0.54	5.6 ± 0.78	5.3 ± 0.86	5.3 ± 0.62	4.5 ± 1.07	4.5 ± 0.59
	2-4days	3-6days	3-7 days	4-7days	2-6 days	3-6days
3rd instar	4.4 ± 0.60	5.8 ± 0.66	6.4 ± 0.81	7.2 ± 0.86	4.6± 0.97	4.9 ± 0.51
	4-6days	2-8days	4-8 days	5-10days	3-7 days	3-9days
4thinstar	3.8± 0.80	5.2 ± 0.63	5.4 ± 1.21	5.3± 0.94	5.6 ± 0.68	5.8± 1.16
	5-6days	2-7days	2-8 days	3-9days	4-7days	2-7days
5th instar	3.4 ±0.40	5.5 ± 0.80	7 ± 1.14	7.6 ±1.21	6.2 ± 0.73	5 ± 0.84
	3-5days	3-9days	2-9days	4-11 days	5-8days	3-7 days
6th Instar	5.9 ± 0.75	6.5 ± 0.63	8 ± 0.71	8.6 ± 0.93	7.7 ± 0.54	8.2 ± 0.66
	4-8days	5-9days	6-10 days	7-12 days	6-9 days	6-10 days
Pupa	6.8 ± 0.97	8.2 ± 1.07	9.4 ± 1.08	9.2 ± 1.43	7.8± 1.39	7 ± 1.05
	3-9days	5-10days	6-12 days	5-13 days	3-9days	4-10days
Adult	7.4 ± 1.93	9.6± 1.03	10 ± 1	10.8 ± 1.32	8.6± 1.12	8.8 ± 0.85
	5-10days	6-12days	7-13 days	6-14 days	4-11days	5-12 days
Total duration	41.8± 0.80	54.9± 0.83	61.8± 0.96	65.5 ± 1.00	52.9 ± 0.90	53.9± 0.82

Figures

Figure. 1 Post-Embryonic developmental stages of *Tribolium castaneum* on different cereals from (a to i)

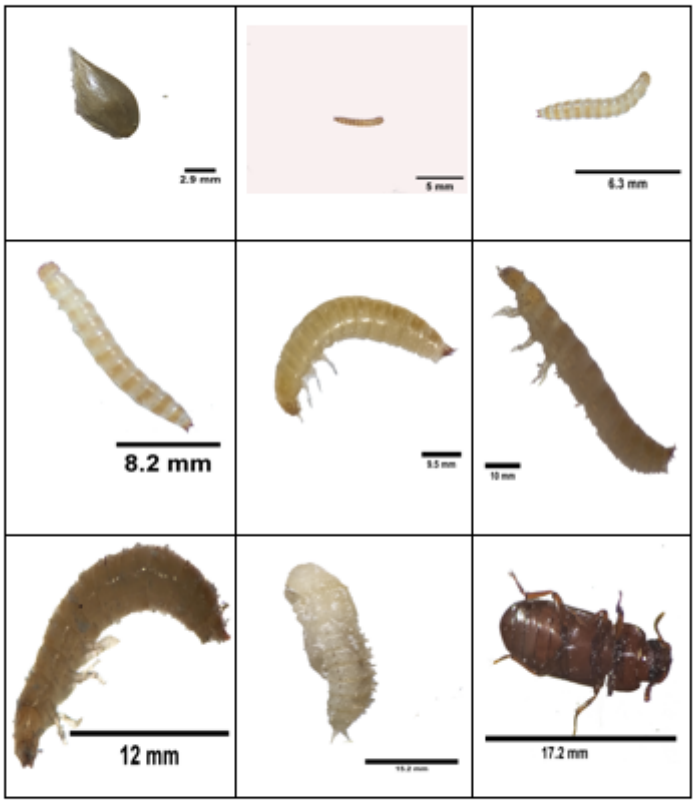

Figure 1. a) Egg b) 1st instar c) 2nd Instar d) 3rd instar e) 4th instar f) 5th instar g) 6th instar h) Pupa i) Adult

Figure 1

See image above for figure legend.