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Larval Development of the Bamboo Borer (*Dinoderus minutus* Fabricius) Using Individual Rearing Method

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ABSTRACT

Detailed information on larval development of the powder-post beetle, *Dinoderus minutus,* was investigated using individual rearing method under laboratory conditions at 27±2°C and 75±5% relative humidity. Five classes of head capsules were recorded, indicating five moults from first instar to prepupa. The mean larval duration recorded was 52.8±0.31 days with 8.04±0.12 days for the first instar larva, followed by 9.74±0.20 days, 13.10±0.17 days, 16.20±0.15 days and 5.72±0.13 days, for the second to fifth instar larvae, respectively. The longest instar stage was the fourth instar, with a development time ranging from 14 to 18 days and the shortest was the fifth instar ranging from 4 to 6 days. The highest growth ratio of larva by body weight was 2.70, which was observed between the first and second instars. This indicated that the maximum feeding rate of the larva occurred between these instar stages. The highest growth ratio, with respect to head capsule and larval length, was 1.35 and 1.34 between the fourth and fifth instar.

Keywords: Dinoderus minutus, bamboo borer, powderpost beetle, Bostrychidae, larval development

INTRODUCTION

Bamboo borers of the family Bostrychidae, particularly *Dinoderus minutus,* cause serious damages to felled bamboo culms as well as finished products (Abood, 2008; Garcia & Morrel, 2009; Abood *et al.,* 2010). The beetles bore their way into seasoned bamboo and attack insidiously. If left unchecked, the infested bamboo would be reduced to a relatively intact outer shell with a fully degraded powdery mass within. The adults and larvae cause direct damages by boring and continuously feeding inside the culm. The presence of beetle holes and powdery material coming out from the entrance and exit holes is a manifestation

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of active *D. minutus* infestations (Liese & Kumar, 2003). The presence of entrance and emergent holes and fine powdery frass is typical of powderpost beetle damage characteristics (Abood & Murphy, 2006) and a manifestation of active *D. minutus* infestations (Liese & Kumar, 2003).

Stebbing (1914) describe the larva of *D. minutus* as pale canary-yellow in colour, curved with swollen thoracic segments and prothorax tapering sharply towards the head with body length ranging from 3.00 to 3.75 mm. Ahmed and Zulfiqr (2006) describe mature *D. minutus* larva as grub-like, curved, and range between 2.50±0.21 mm long. Meanwhile, Garcia and Morrel (2009) reported the duration of larval development to range from 40 to 58 days at 25°C on *Bambusa vulgaris.* Plank (1948), however, reported a wider range of larval development times from 21 to 76 days at the same temperature. Ho (1994) reported that *D. minutus* larva takes approximately four weeks to develop into pupa on infested bamboo although the bamboo species used in the observation was not mentioned. Iwata (1984), who investigated on *Lyctusbrunneus,* reported that grouping head capsules or exuvial mandibles of powderpost beetle larvae provide a reliable and useful method for the determination of larval moults for coleoptarous species. This method was also adopted by Kojima *et al.* (1968) and Suzuki (1983) for Japanese Cerambycid and *Lyctusbrunneus*. Nonetheless, there is no available documented information on detailed larval development of *D. minutus*. The studies by Garcia and Morrell (2009) and Ho (1994) provided a brief description on some aspects of the larval development observed in bamboo.

In this study, three different media were tested as rearing material to observe larval development. The media selected were bamboo powder, cassava powder and frass produced from infested bamboo. The latter was selected based on the preliminary observations which indicated that *D. minutus* preferred bamboo frass to freshly ground bamboo powder. Rajor *et al.* (1995), who worked on the chemical characterization of borer dust reported that frass from the bamboo borer on *Dendrocala musstrictus* is a rich source of glucose 1-phosphate.

This particular study was conducted to meet the following objectives: (i) to determine the suitability of individual rearing method for studies on larval development using three different media, and (ii) to obtain detailed information on weight, length, head capsule width and duration of each larval stage using the most suitable medium.

MATERIALS AND METHODS

Insect Supply

Eggs were collected from paired adult beetles for the first instar larvae collection. The collected eggs were placed in Petri dishes lined with black filter paper. Hatchability for the first instar larvae was observed daily. The first instar larvae, weighing less than 0.10 mg, were carefully removed with a fine brush and a single larva was confined into each capsule stuffed with 0.1 g of the medium.

Preparation of the Rearing Medium

Cassava flour, ground bamboo and bamboo frass were used as the rearing media for the study (Fig.1). Freshly dried cassava and bamboo of the species *Gigantochloascortechinii* were ground to powder while bamboo frass was obtained from the infested bamboo culture of *D. minutus*. All the diet used in the study passed through mesh no. 100 (passing particles of less than 150µ diameter). The newly emerged larvae introduced to cassava flour were taken from the eggs produced by the parent beetles cultured in cassava blocks, while the newly emerged larvae introduced to bamboo powder and frass were taken from the eggs produced by the parent beetle cultured on bamboo; 0.10 g of each diet was weighed and placed into gelatine capsules.

Rearing and Inspection of Larva

Each capsule containing rearing medium and a single larva was mounted horizontally on a plastic container (3.5cm in height and 5.0cm in diameter) stuffed with plasticine of 2.0 cm thickness at the bottom. Ten gelatine capsules were placed in each plastic container that was placed in an incubator at 27±2°C and 75±5% relative humidity. 50 larvae were reared for each diet and their development was observed. Larvae were taken out from the capsules on alternate days to inspect for larval exuviae. Each rearing medium was sieved through mesh no. 100 for the third instar onwards. For the early instar larvae, inspection was carefully done under a stereomicroscope. The duration of the larval instars, body lengths, weight and head capsule widths were recorded as soon as exuvia was recorded using a stereomicroscope equipped with NIS-Elements® image analysis software. The growth ratio for *D. minutus* larvae was determined by means of grouping head capsule widths according to Dyar's rule (Dyar, 1890). Different stages of the larvae were also examined under JEOL JSM-6400 scanning electron microscope for further observation.

Statistical Analysis

Larval mortality data were analyzed using a non-linear regression to observe the trend in mortality for different media used in individual rearing. Data obtained from the most successful rearing medium was used

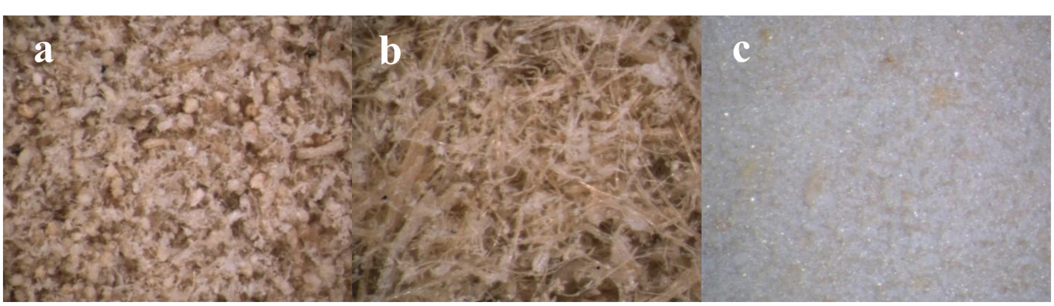


Fig.1: The particles of rearing media (x50); a. Bamboo frass, b. Bamboo powder, and c. Cassava flour

in the analysis on body length and weight, head capsule width and instar duration. One-Way Analysis of Variance (ANOVA) and Duncan Multiple Range Test were used in the analyses.

RESULTS

Mortality of Larva on Rearing Medium

The findings indicated that rearing of larva on bamboo powder resulted with the first mortality on day 2, while complete mortality was observed on day 40. The highest mortality was recorded in the second week of rearing with 30% mortality. Rearing of larva on bamboo frass showed a similar trend in mortality. The highest mortality was also recorded in the second week, with 28% of larva mortality. However, 20% of the larva pupated. Although there was a 10% mortality of larvae on the cassava flour in the first week of rearing, this medium supported the highest survival rate, with 60% successful pupation. Fig.2 shows the trend in the mortality of larva reared on different media.

Table 1 shows the non-linear regression equation of *D. minutus* larval mortality in different rearing media. The results showed curvilinear effect between powder form diet

TABLE 1
The non-linear regression of the larval mortality in different rearing media

Powder state diet	Regression equation	R^2	F value
Bamboo powder	$y=55.53-2.04x+0.01x^2$	0.99	1764.72
Bamboo frass	$y=52.93-1.36x+0.003x^2$	0.98	968.13
Cassava powder	$y=50.91-0.43x-0.002x^2$	0.97	573.98

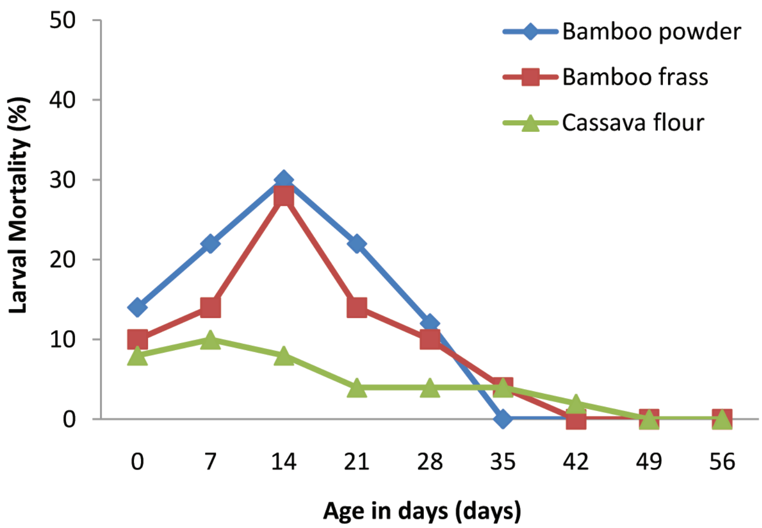


Fig.2: The percentage of larval mortality reared on different media

and the mortality rate of larva throughout the rearing period.

Larval Development

At hatching, young larva inside the chorion bore its way through by a fairly active movement of the terminal abdominal segment. Once the chorion is broken, the larva emerges, posterior end first, and wriggles its way out by contraction of body muscles (Fig.3). The newly hatched larva of *D. minutus* appears creamy white in colour while the head is reddish brown. An observation on the larval development shows morphological changes with succeeding instars (Fig.4 to Fig.6). The maxillary and labial palpi showed prominent changes in length and intensified in colour due to sclerotization. In addition, setae were observed at the posterior abdominal tip and head.

Results from larval development on the most successful rearing medium (cassava flour) showed five classes of head capsule widths representing five moults (Table 2). Table 2 shows the dimension and weight from larval to pupa and the growth ratio obtained using Dyar's Rule. The grouping of the head capsule widths was tested for conformity to Dyar's Rule with the *t*-test method used by Oke and Odebiyi (2008).

The results showed highly significant differences ($p<0.0001$) between succeeding instar stages on larval body length, body weight, head capsule width and duration. The mean head capsule width, body length and body weight for the first instar were 81.45±0.63 µm, 739.26±4.46 µm and 0.06±0.001 mg, respectively (Table 2). The calculated head capsule width for the first instar was 80.74 µm, with 0.87% difference between the observed and the calculated head capsule widths. The ratio increment of the head capsule from the first to the second instar was 1.13. Meanwhile, the duration ranged between 7 to 9 days, with a mean of 8.04 ±0.12 days (Table 3). The ratio of the increment of body weight was the highest from the first to the second instars (2.70).

Fig.3: Eclosion into the first instar larva (X80)

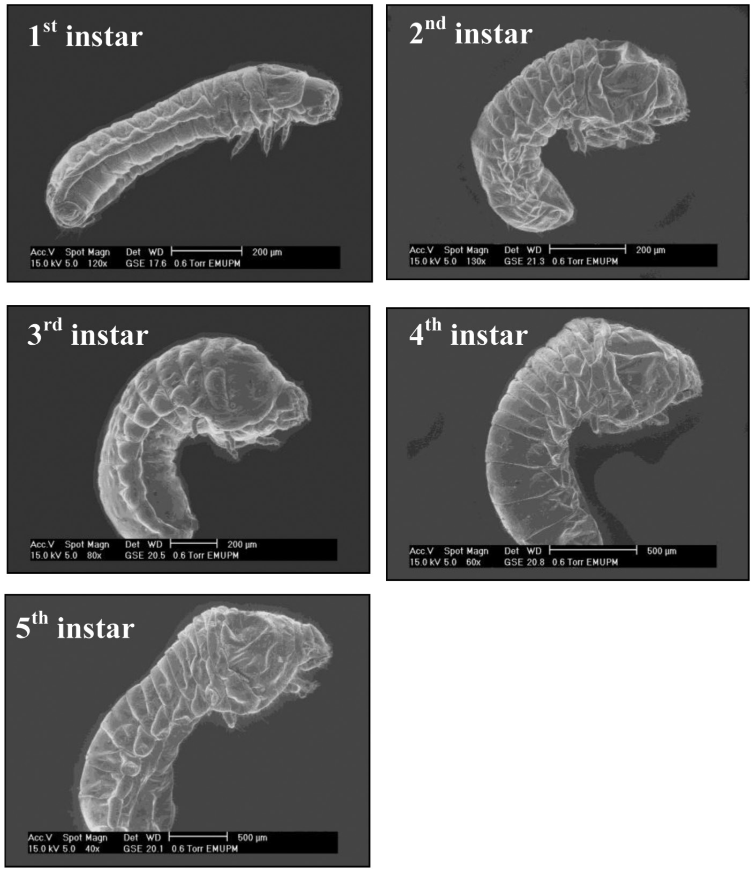
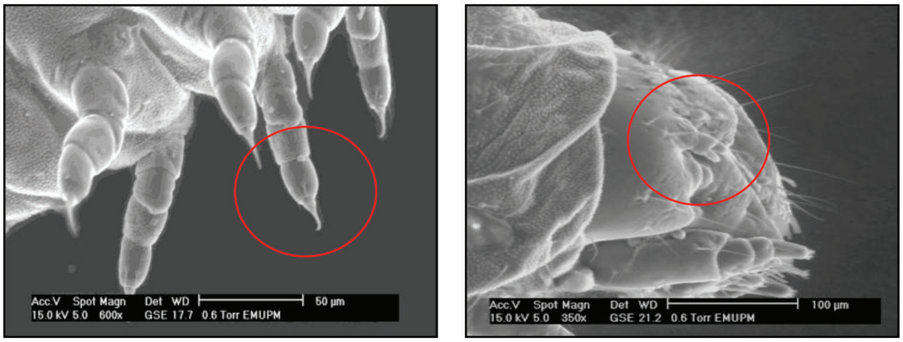


Fig.4: Scanning electron micrograph of the first to fifth instar larva

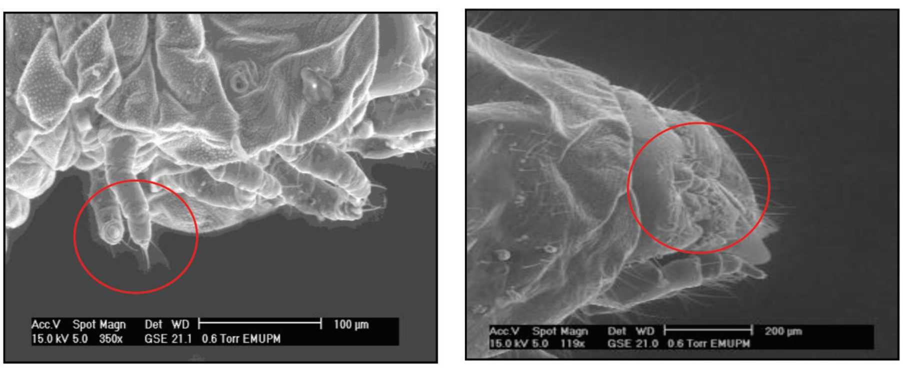
The mean head capsule width, body length and body weight of the second instar were 91.85±0.81, 893.97±5.07 µm and 0.17±0.002 mg, respectively, while the calculated head capsule width was 91.04 µm (Table 2). The difference between the observed and the calculated head capsule widths was 0.88% and the ratio of increment was 1.25. Duration of the second instar ranged between 8 to 12 days, with a mean of 9.74 ± 0.20 days (Table 3). The ratio of the increment of larval body length from the second to third instar was 1.06, which was the lowest ratio compared to the other stages.

The mean head capsule width, body length and body weight of the third instar were 114.74±0.71, 937.50±7.72 µm and 0.27±0.002 mg, respectively, while the calculated head capsule width was 114.14 (Table 2). There was a 0.52% difference between the observed and the calculated head capsule widths. The ratio of increment of the head capsule was 0.77. The duration of the third instar ranged between 12 to 15 days, with a mean of 13.1 ± 0.15 days (Table 3).

The first instar



The third instar



The fifth instar

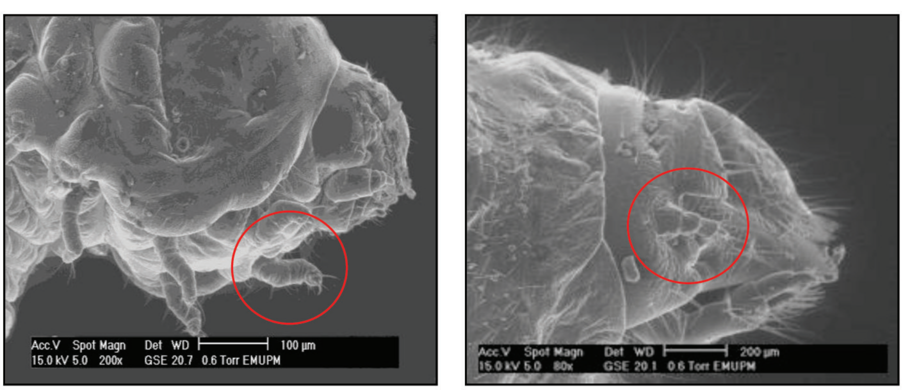
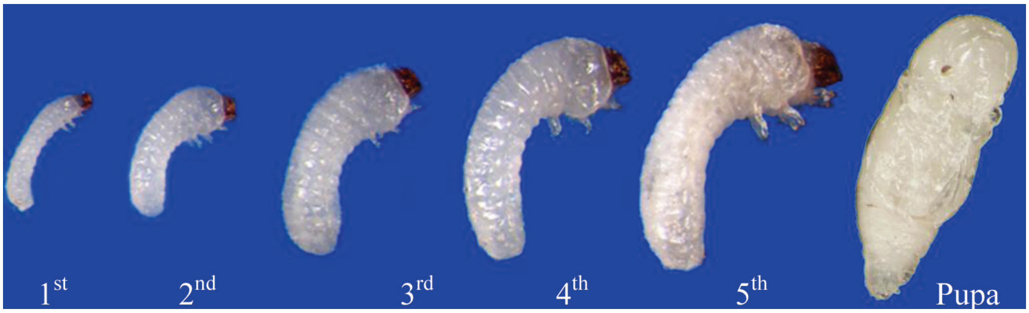


Fig.5: Scanning electron micrograph of changes in leg (left hand side) and antennae (right hand side) in the course of larval development



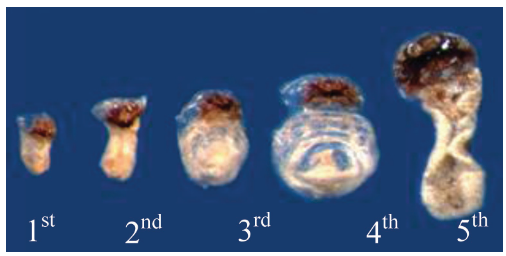


Fig.6: The stereomicrograph of the (a) first instar larva to pupa (X40) and (b) exuviae of larvae (X80)

The mean head capsule width, body length and body weight for the fourth instar were 147.92±1.56, 1224.97±6.96 µm and 0.39±0.002 mg, respectively (Table 2). The difference between the observed and the calculated head capsule widths was 0.3%, and the increment ratio of head capsule was 1.29 (Table 2). The duration of the fourth instar was the longest compared to the other stages, i.e. ranging between 14 to 18 days, with a mean of 16.2 ± 0.15 days (Table 3).

The mean head capsule, body length and body weight of the fifth instar were 199.52±1.45, 1644.53±10.73 µm and 0.61±0.008 mg, respectively, with a head capsule increment ratio of 1.35 (Table 2). There was a 0.08% difference between the observed and calculated head capsule widths. The fifth instar, which was the final instar, took 4 to 7 days with a mean duration of 5.72 days (Table 3) before developing into a pupa. The duration of this instar was the shortest of all the stages.

External Morphology

The head of *D. minutus* larva is the most prominent in the first instar and it gradually recedes beneath the enlarged prothoracic segment. The overall body form was fairly linear in the first instar and attained a more defined curvature in the proceeding instars (Fig.4). The maxillae are located below the mandibles. A distinct claw is present at the terminal leg and is clothed with fine papillae in the later stages. While the antenna in the first instar shows the antacoria and two terminal segments, in the third and fifth instars, four antennal segments are present (Fig.5). The abdomen of *D. minutus* larva consists of ten segments, with spiracles present on the lateral side of urotergites.

TABLE 2
The morphometrics of larva to pupa and growth ratio

Stage		Body length (µm)		Body weight (mg)		Head capsule width (µm)		
		Mean±SE	Ratio	Mean±SE	Ratio	Mean±SE	Ratio	Calculated
Instar	1st	739.26±4.46a (649.32-802.57)	-	0.06±0.001a (0.059-0.071)	-	81.45±0.63a (73.37-89.57)	-	80.74
	2nd	887.55±5.07b (820.78-949.21)	1.20	0.17±0.002b (0.135-0.198)	2.70	91.85±0.81b (80.01-98.77)	1.13	91.04
	3rd	937.49±7.72c (801.74-1067.00)	1.06	0.27±0.002c 0.246-0.305)	1.59	114.74±0.71c (104.55-123.80)	1.25	114.14
	4th	1224.97±6.96d (1104.55-1299.10)	1.31	0.39±0.002d (0.362-0.413)	1.41	147.92±1.56d (130.01-167.59)	1.29	147.48
	5th	1644.53±10.73e (1446.87-1778.46)	1.34	0.61±0.008e (0.504-0.716)	1.58	199.52±1.45e (175.91-221.63)	1.35	199.37

Means followed by the same letter in the same column are not significantly different ($p \geq 0.05$, Duncan Multiple Range Test). N=50

TABLE 3
The developmental periods from the first instar larva to pupa on cassava flour

Stages		Period (days)		
		Mean±SE	Min	Max
Instar	1^{st}	8.04 ± 0.12d	7	9
	2^{nd}	9.74 ± 0.20c	8	12
	3^{rd}	13.10 ±0.15b	12	15
	4^{th}	16.20 ±0.15a	14	18
	5^{th}	5.72 ± 0.13e	4	7
Overall larval duration		52.80±0.31	49	58

Means followed by the same letter in the same column are not significantly different ($p \geq 0.05$, Duncan Multiple Range Test). N=50, ns=non-significant

DISCUSSION

Cassava flour provides a successful medium for individual rearing of *D. minutus* larva and facilitates observations on their development. Higher mortality on bamboo powder and bamboo frass is attributed to significantly lower starch contents compared to cassava flour. Ground bamboo powder has larger more particulate matter (Fig.1) and is believed to adversely affect the delicate larval surface morphology. The results clearly show five classes of head capsules, thus indicating five moults with the total larval development duration of 52.80±0.31 days. There is an accompanying highly significant difference ($p<0.001$) in the larval body length and weight.

This result differs from the findings by Garcia and Morrel (2009), who reported that *D. minutus* larvae underwent four stadia during its development on the bamboo blocks of *Bambusa vulgaris* at 30°C. It should be noted that in this particular study, all the observations were made using individual rearing method using a nutrient rich medium. Thus, the results are based on the head capsule widths, presence of larval exuviae, larval body increments and growth ratio. These parameters, however, were not reported by Garcia and Morrel (2009). It was observed that the fourth instar larva was capable of developing into prepupa when no food was offered. This resulted in smaller sized adult beetles as compared to those that underwent five instar stages. The development of powderpost larva under individual rearing method has been adopted by Iwata and Nishimoto (1984) on *Lyctusbrunneus*. In the study, *L. brunneus* underwent four to six instars in the course of development. Iwata and Nishimoto (1984) reported that the difference in the instar number is caused by hereditary or endocrinological factors. Esperk *et al.* (2007) reported that food quality and quantity are the most common factors affecting the number of instar in various insects.

The observations on the larval morphology described in this study showed similar characteristics to those reported by Stebbing (1914). In this study, the growth ratio for head capsules increased with succeeding instars. The result shows that the first instar larva exhibited high values in the growth ratio of body length and weight. The growth ratio in body length was significantly ($p<0.05$) higher between the first and second instar compared to that between the second and third instar. In term of body weight, the highest growth ratio recorded was between the first and second instar.

Dyar (1890) reported that the width of the head capsule increases in a regular geometrical progression in successive instars by a ratio of 1.4. This development has been termed as the Dyar's law, and the recorded ratio of 1.26 for *D. minutus* showed a considerable variation from this value. A study by Oke and Odebiyi (2008) on the flea beetle, *Podagricasjostedti* (Coleoptera) resulted in three instar stages for larva and recorded a ratio of 1.54 for the width of head capsule.

CONCLUSION

The overall larval development period was 52.8±0.75 days, with the longest instar stage ranging from 14 to 18 days for the fourth instar larvae and the shortest instar stage was the fifth instar, ranging from four to six days. Five classes of head capsules were identified, and these indicated five moults from the first instar to the pupa stage. The highest growth ratio (2.70) in terms of body weight, indicating the highest feeding rate, was between the first and second instar. Meanwhile, the highest growth ratio (1.34) in terms of body length and head capsule width (1.35) was found to occur between the fourth and fifth instars.

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