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# Morphology of Immature Stages of *Schistocerca gregaria* with Special References to its Size Variations

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**ABSTRACT**

Adults as well as immature of *Schistocerca gregaria* are voracious vegetation consumer and destructive pest to the useful plants. Morphology and morphometry of immatures of desert locust in relation to gaining of body length and weight at each consecutive stage was carried out for the purpose of investigating the role of immatures in destruction of plants in Thar Desert, Sindh. Hatchlings of solitarious *S.gregaria* were light green at the time of emergence, but turned over to black with yellow strips after two hours of hatching, when they were placed in crowded condition. However, they remained green, when they were in solitary condition. Solitarious hoppers were passed through five nymphal stages during development into adult in field as well as in laboratory condition. Comparative morphometry revealed that in relation to previous stages 2nd and 3rd nymphal stages gained much more length and weight as compared to 4th and 5th nymphal stages. Correlations between length and weight of hoppers of each nymphal stage were positive. It could be concluded that 4th and 5thinstars were voracious in consuming vegetation and having more chances of survival than 1st, 2nd and 3rd instars. Finally this study suggests that 4th and 5th instars, which were neglected previously, are the potential enemies of agro economy in Thar Desert.

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**Authors' Contribution**
**AAS performed the experiments and collected the samples. RS designed the study, analysed the data and complied the results.**

**Key words**
**Desert locust, Nymphal instars, Destructive pest, Comparative morphometry, Correlation analysis, Variance, Agro economy and outbreak.**

## INTRODUCTION

*S. gregaria* is a destructive pest of vegetation stretching from Mauritania to the Thar Desert of Pakistan and India. Solitarious phase remain calm but gregarious phase may produce swarms which consume all the vegetation where ever they land down and cause loss in agro-economy of the world. About eight major plagues which have destroyed crops adversely, had been reported for last two centuries from Africa, the Middle East and Southwest Asia (Meinzingen, 1993; El Hassan, 2000).

Desert locust exhibits incomplete metamorphosis with three stages: egg, nymph and adult. Nymph passes through different developmental stages which vary from each other depending on ecological conditions (Steedman, 1990). Desert locust may turn out to be sexually mature in a few weeks or a few months, according to environmental conditions. Notable scholars Husain and Ahmad (1936), Steedman (1990), Chapman (1998), Maeno and Tanaka (2008), Riffat and Wagan (2010, 2011, 2015) and Samejo and Riffat (2016) unearthed so many reproductive and other aspects of desert locust but, detail morphology and statistical analysis of body size of immatures need some attention yet. So, this study is carried to carve out a portrait of how body size of immatures is concerned with agro economy.

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## MATERIALS AND METHODS

*Sampling*

Adults of desert locusts were collected from pearl millet and cluster bean crops in fields of Thar Desert during July 2016 and fetched to laboratory for rearing.

*Rearing of desert locust*

Adult male and female of desert locust were reared in wooden rearing cage measuring up to 2ft x 2.5ft x 3ft height, width and length, respectively. The cage was woven with wire mesh on all sides except the bottom which was not wired but fixed on soil. On top a cloth in the form of tube was fitted for providing food and cleaning. The cages were checked daily for cleaning and changing the food. As nymph emerged in cage they transferred into another rearing cage for further development.

*Rearing cages for nymphal development*

Cages for nymphal development was made of plastic jars which were perforated with small pores on all sides for maintaining fresh air. Tube-like clothes were fitted on mouths of jars and bottom of the jars was filled with clean soil. Only one nymph was kept in each jar and soft leaves and seedlings were provided for feeding. Jars were checked daily for observing molts and whenever, molts were found in the jars they were collected and date and time was noted.

*Measurement of body weight and length*

Immatures of desert locust were taken from rearing cages, weighed and measured after each molt. Immatures were also collected from field and sorted out into various nymphal stages then weighed and measured. Record of weight and length was maintained properly for statistical analysis.

*Correlation coefficient of body weight and length*

Formula for correlation coefficient:

$$r = \frac{\sum_{i=1}^{n}(xi-\bar{x})(yi-\bar{y})}{(n-1)s_x s_y} = \frac{\sum_{i=1}^{n}(xi-\bar{x})(yi-\bar{y})}{\sqrt{\sum_{i=1}^{n}(xi-\bar{x})^2 \sum_{i=1}^{n}(yi-\bar{y})^2}}$$

Note: when r >0 we say that the sample data pairs are positively correlated, and when r <0 we say that they are negatively correlated.

*Statistical analysis*

A relationship between body weight and length was analyzed by correlation scatter chart and correlation coefficient to determine that how much these instars gained body weight and length at each nymphal stage. Variance analysis of each nymphal stage was carried out to demonstrate variations of body weight and length among individual immatures of a single nymphal stage.

## RESULTS

This study confirmed that hatchling of solitarious desert locust passed through the five nymphal stages in laboratory as well as field conditions. Morphometric comparisons were calculated and correlations between length and weight of each nymphal stage were made. Hatchlings of different stages were spotted in the various fields of Thar Desert from August to October mostly. Mean and standard deviation of body length and weight of 1st to 5thinstars of *S. gregaria* were calculated (Table I). 2nd instar (14.36±1.26) assumed more than twice the length of the 1st instar (6.93±0.58) whereas in 3rd, 4th and 5th instars each stage gained more than half of their previous stage. Besides, 2nd instar acquired three times more weight than the 1st instar while, 3rd instar four times more than the 2nd instars. However, 4th and 5th instars gained two and quarter times more than their previous stages. From this result it is believed that 2nd and 3rd instars gained much more length and weight as compared to 4th and 5th instars.

Moreover the correlation between the length and weight of the hatchlings was significantly positive (Fig. 1). In the 4th and 5th instars body length was strongly correlated to body weight (r=0.931972; n=20; P<0.001 in 4th instar; r=0.970063; n=20; P<0.001 in 5th instar), in 1st and 3rd moderately correlated (r= 0.609737; n=20; P<0.01 in 1st instar; r=0.619175; n=20; P<0.01 in 3rd instar) whereas in 2nd instar correlation was weak (r=0.179913; n=20; P>0.05).

*Morphology of nymphal instars*

*S. gregaria* had incomplete metamorphosis with three main developmental stages: egg, nymph and adult. Nymphal stage passed through five moults before emerging as adult. All nyphmal instars varied morphologically with each other.

*1st instar*

The first instar was whitish green in color and dull when newly hatched but, after 2 h turned into black with yellow spots and stripes and became highly active. Head seemed larger and wider than thorax and abdomen. Eyes were oval with one eye strip and reddish in color in crowd-reared, whereas green in isolated-reared. Antennae was segmented filiform with rounded and thick pedicle and was black in gregarious phase while, green in solitarious. Pronotum was not so distinct but had three transverse sulci

**Table I.- Length and weight (Mean±SD) and coefficients of correlation between length and weight of 1st to 5thinstars of *S. gregaria*.**

| | Nymphal instars | | | | |
|---|---|---|---|---|---|
| | First | Second | Third | Fourth | Fifth |
| Body length(mm) | 6.93±0.58 | 14.36±1.26 | 20.07±0.80 | 31.31±2.43 | 41.78±2.06 |
| Body weight (mg) | 12.93±2.035 | 38.92±3.52 | 155.52±22.06 | 373.61±30.03 | 843.57±101.58 |
| Correlation coefficients of length and weight (r) | 0.609737** | 0.179913*** | 0.619175** | 0.931972* | 0.970063* |

*Correlation is significant at P<0.001; ** Correlation is significant at P<0.01; *** Correlation is significant at P>0.05.

which were bent forwarded laterally on both sides and posterior end of the pronotum was compressed not raised medially. Mesothorax and metathorax were indistinct without elytron and wing rudiments. Hind femurs were larger having light black spots with green background color in isolated, but in crowd-reared dark black bands with yellow background color were observed. Hind tibia was green with smaller and thinner spines. This stage was smaller in length with light weight so one could not find them easily in the field (Fig. 2Aa, Ab).

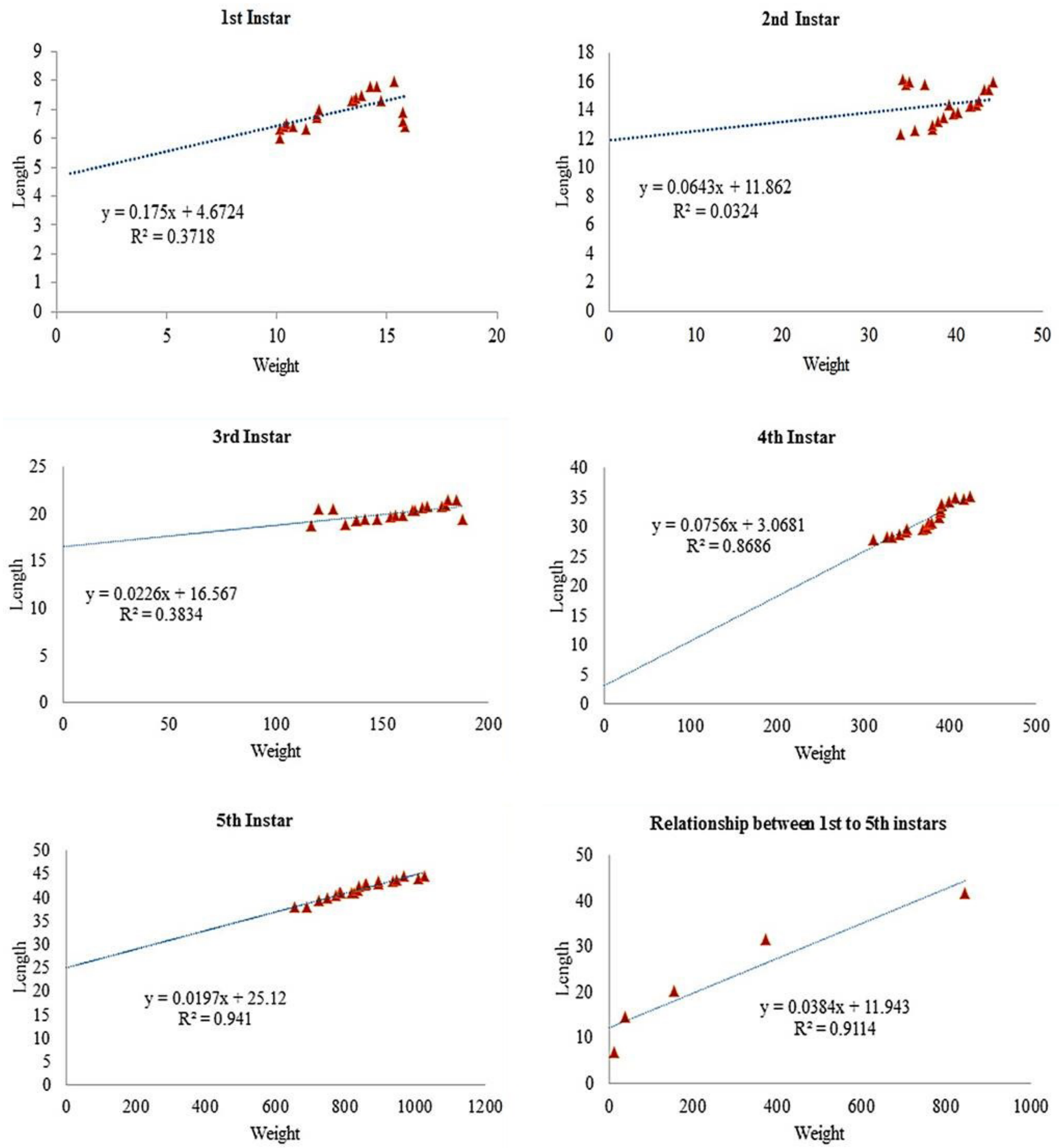


Fig. 1. Correlation relationship between body weight and body length from 1st instar to 5th instar.

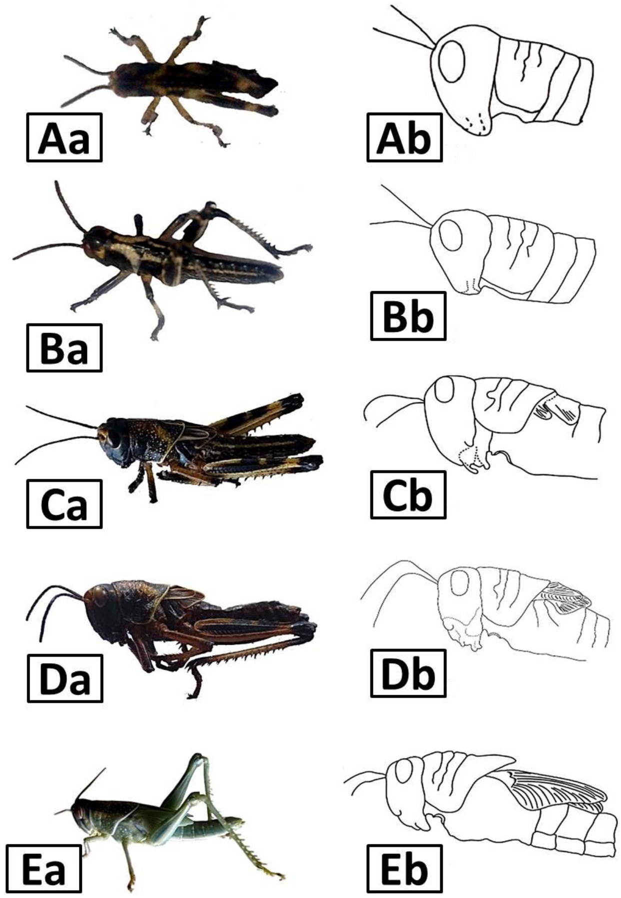


Fig. 2. Morphology of 1st to 5th immature instars (Aa, Ab, Ba, Bb, Ca, Cb, Da, Db, Ea, Eb) of *S. gregaria.*

*2nd instar*

Body coloration was similar to 1st instar but black with yellow spots and stripe on body surface, head seemed large and clear. Eyes possessed two eye-stripes and were dark in gregarious. Pronotum was also distinct and raised medially having three longer transverse sulci. Median carina on pronotum was distinct. Meso and meta-thorax were distinct but wing buds were still absent. Hind femurs were well developed at this stage and seemed larger, thicker and steeper. Tibia was also looked stronger than those of 1st instars with larger and clearer spines. Nevertheless, it was hard to differentiate the second instar from the first, but authors experienced that the size of head, the length and the weight of body were much larger and greater in second instar than first instar (Fig. 2Ba, Bb).

*3rd instar*

Body coloration become darker with yellow spots and stripes in crowded-reared, whereas green with yellow spots in isolated. Head developed prominently larger and wider with larger and robust antennae. Fastigium of vertex looked like trapezoidal with shallower depression. Eyes were extracted out concavely and become more oval with three eye stripes. Pronotum grew larger and seemed robust with three sulci which were deeper, larger and clearer. Median carina raised so high that steeped up the median and posterior end of the pronotum became rounded. This instar was easy to recognize by two pairs of wing buds which could be seen protrusive from underneath, the pronotum on both lateral sides of the thorax obliquely. Elytron slender shaped rounded obliquely posteriorly about at the angle of 45 degree, while wings were triangular lower margin was downward. Veins were still not developed on both the elytron as well as the wings. Wing pads were extended up to 1st abdominal segment. Genital plates were distinct in each sex at this stage. Femur and tibia with spines grew larger and stronger and were able to create mightier pull for jumping and popping. This instar attained much more length and weight compare to earlier stages (Fig. 2Ca, Cb).

*4th instar*

Body coloration was also black with yellow spots and stripes like previous instars and body size was prominent. Head become more distinct with well-developed mouth parts and granulated integument. Granules on integument were bright yellow with black background in crowded-reared, whereas in isolated dull yellow with green or yellow back ground. Four eye-stripes developed in the eyes that were clearly seen in isolated instars but in crowded instars were not clearer. Pronotum was raised medially, compressed laterally and sulci grew longer on both lateral side. Fine granules were seen on pronotum also and light band with dark ridges appeared on dorsal and laterals sides. Posterior medial tip of pronotum became slightly angular and posterior margin of pronotum looked yellow in crowded instars and light in isolated instars. The wing buds were larger and more lucid but still shorter than the length of the promotum. Wing pads were extended posteriorly up to second abdominal segment and covered the tergite of 1st abdominal segment. Inner margins of the wing buds were lifted uprightly. Veins developed in elytron as well as in wings that provided beauty to the wings. Spines on the tibia grew larger and tips of spines were black in color in both crowded and isolated instars. Long-distanced jumping by this instar was observed in fields due to powerful hind femur and tibia. Sexes could be recognized easily by differentiated genital plates (Fig. 2Da, Db).

*5th instar*

Body coloration was also black with yellow spots and stripes in crowded instars, while isolated instars were greenish yellow with blond body. Integument was granulated like 4th instars, granules were more prominent on the pronotum. Head was oval; more rounded dorsally, with clear demarcation sutures on frontal and lateral sides, having well grown maxilla, mandibles, labium and labrum on ventral surface. Antenna became larger and more tactile with rounded, thick, moveable and ball like pedicle. Interestingly pronotum was more compressed dorsally as well as laterally with raised median carina, transverse sulci were deeper, longer and bended irregularly. Posterior medial tip of the pronotum seemed rounded with slight angular appearance. Pronotum was laterally so compressed that it was looking like collar between head and thorax, because anteriorly head was wider than anterolateral compressed part of pronotum, similarly posterolateral part of pronotum along with the pronotum was also wider. Wing pads were grown longer than the length of pronotum, but still could not be used for flight. Wings and elytron were extended posteriorly up to the 4th abdominal segment, covered tergites of three abdominal segments like leaf and veins were developed very well having dark color which rendered the natural beauty to fascinate the beholder. Hind femur and tibia became powerful enough to jump to long distance or to escape from predators and collector, spines on tibia were able to pierce or stab in skin of hands, if anyone wanted to catch and hold them in hands. This instar was lager in size and heavier in weight (Fig. 2Ea, Eb).

## DISCUSSION

This study expands what is known about morphology and statistical analysis of immature instars of *S. gregaria*. During this study it was analyzed that length and weight of hatchlings in 1st instar fluctuate with variance of 4.144 and 0.341, respectively which coincide with the finding of Husain and Ahmad (1936) and Maeno and Tanaka (2008) who reported earlier that hatchling of locusts vary in size even if they emerge from a single egg pod. Correlation between length and weight of 4th and 5th instars was analyzed as strongly positive which indicate that in these instars increase in length was significantly correlated with gain of weight. However correlation was not strong from 1st to 3rd instars as compare to 4th and 5th (Fig. 1). Moreover it is believed from this analysis that gaining of length and weight was not constant in each consecutive nymphal stage from 1st to 5th (Table I) which negates the findings of Chapman (1998) who reported that growth rate of nymphal stadia remain constant.

Present study confirmed morphological characteristics of nymphal instars which were reported previously by Steedman (1990). Furthermore, hatchlings of solitarious *S. gregaria* were light green and more in numbers at the time of hatching. But most of the hatchlings of same egg pod turned over to black with yellow strips about two hours after hatching from small plastic jar cages. Whereas, hatchling of solitarious *S. gregaria* from large wooden cage were fewer in number and green in color and did not turned black. Hunter-Jones (1958) reported that hatchlings of solitarious *S. gregraia* were green and lighter than those from gregarious which were almost black. Uvarov (1966) believed that nymphal coloration in *S. gregaria* is reversible and may be changed during life of individuals when they are crowded or isolated at early stage.

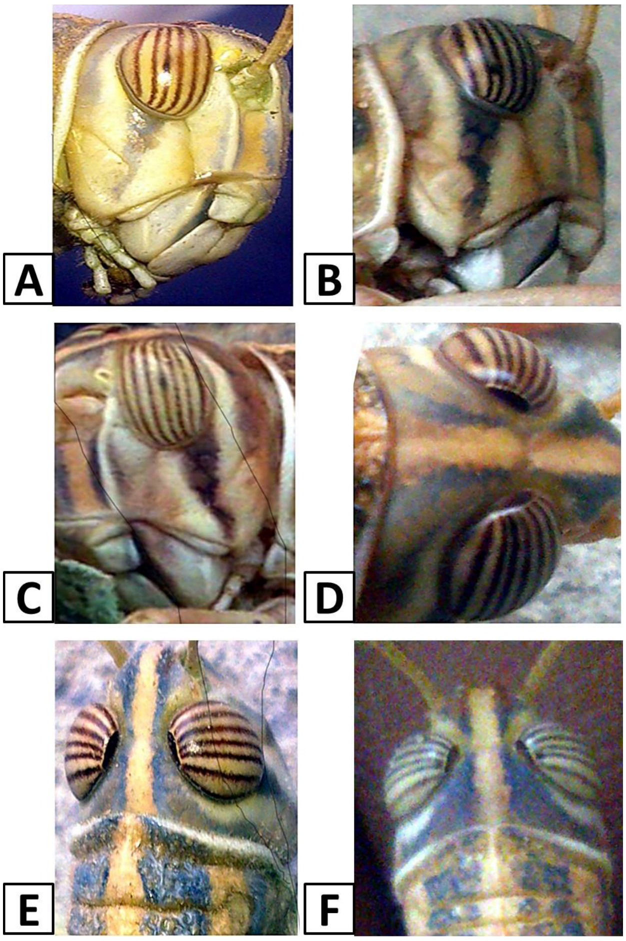


Fig. 3. Number of eye strips in the eyes of various adults of *S. gregaria* collected from fields as well as laboratory. A, B and C are lateral views of eye of three different specimen of *S. gregaria* and D, E and F are the dorsal views of eye of the same.

In present study five nymphal stages of *S. gregaria* were confirmed by counting number of eye-strips in the eyes of adults (Fig. 3). In insects eyes-strips are strongly correlated with number of moults through which adult has gone and first instar possess one eye strip which is then further added during each moult reported by Volkonsky (1937). Solitarious *S. gregaria* may have an extra 6th moult while, gregarious phase have five moults and do not undergo the extra 6thmoult as believed Maeno and Tanaka (2004) who confirmed this by eye strip formula. Number of instars may be counted by number of eye strips in the eyes of adult by formula N+1, where N stands for number of instars and 1 is for adult. Subfamily Cyrtacanthacridinae including Schistocerca, Nomadacris, Anacridium and other genera possess the key characteristic which is strong correlation between number of nymphal instars and number of eye-strips for recognizing how many instars species have (Albrecht, 1955, 1967; Pener, 1991; Uvarov, 1966). But in this study it is observed that adults of solitarious *S. gregaria* possess six eye-stripes. According to formula N+1, here 5+1 indicated that they had undergone through five instar and do not had extra 6th moult (Fig. 3).

## CONCLUSION

It could be concluded that in solitarious desert locust developmental stages were five and these stages were confirmed by counting eye-strips in the eyes of adults. It was difficult to distinguish between 1st and 2nd instars. Gaining of body weight was fast in early developmental stages. Correlation between assuming body weight and length was strongly positive in 4th and 5th instars. It is presumed that 4th and 5thinstars were very aggressive in consuming vegetation and having more chances of being survival of fittest. 1st, 2nd and 3rd were prone to high mortality. Finally this study suggests that 4th and 5th instars are the potential enemy of agro economy and further study is required for monitoring activities of these instars in the field.

*Statement of conflict of interest*

The authors declare no conflict of interest.

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