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Comparative Study on the Immature Stages of Three *Hieroglyphus* Species (Acrididae: Orthoperta) from Pakistan

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Abstract.- *Hieroglyphus* Krauss is among the most economically important pest of rice, sugarcane, wheat, maize and minor pest of millets and fodder crops in Pakistan. Presently immature stages of *Hieroglyphus perpolita* (Uvarov), *H.oryzivorus* Carl and *H. nigrorepletus* I. (Bolivar) were studied under the laboratory conditions during the years 2005-2007 from Pakistan. The first instar hatched in the form of vermiform larva. In both sexes, normally there are five moults and six nymphal stages and the adult being the seventh stage. Hoppers usually emerge during June-July as soon as monsoon begins. Sex ratio was extremely in the favour of females in all the species were studied. Inner side of hind femur with tinge of red or orange red color was reported in *H.perpolita* on contrary to this; it was light green in *H. nigrorepletus*. Posterior margin of pronotum was recorded almost straight in *H.oryzivorus* while it was obtuse angular in *H.perpolita*. Morphometric studies on the immature stages of these three species showed that there was significant differences in the measurement of different body parts as well as all instars are also slightly differ with each other. The total average developmental period from first instar up to the last instar was recorded 4.21±0.36 days for *H. perpolita,* 5.18±0.43days for *H.oryzivorus* and 5.07±0.47 days for *H. nigrorepletus*. Study of life history, including life span, development time, and sex ratio contributes importantly to understand of insect species.

Key words: Comparative study, immature stages, *Hieroglyphus,* morphometric, moult.

INTRODUCTION

Hieroglyphus Krauss (1877) is among the most economically important pest of rice, sugarcane, wheat, maize and minor pest of millets and fodder crops in Pakistan. Certain species of *Hieroglyphus* present interesting complex, as they appear in swarm (Ghouri and Ahmed, 1960), while some are dimorphic, occurring in both brachypterous as well as in macropterous forms. Beside, its economic importance, it is an insect of considerable biological interest. It has a long egg-diapause of two types ("one-year" and "two-year" diapause) (Roonwal, 1976a) an annual hatching rhythm, a long 5-year egg viability and wing dimorphism.

Numerous papers have been published on the taxonomy and biology of a number of different Othopteran insects including *H. nigrorepletus* and *H. banian*. A few important ones are those of Bolivar (1912), Kirby (1914), Carl (1916), Uvarov (1922), Roonwal (1945, 1976a,b, 1978), Bei-Bienko and Mishchenko (1952), Katiyar (1953), Srivastava (1956), Pradhan and Peshwani, (1961), Jago (1963), Eades (1964), Mason (1973), Siddiqui (1986, 1989) and Moizuddin (2001) without describing the morphological characteristics and identification keys of instars. Janjua (1957) has given an inadequate clarification on breeding habits of *H. oryzivorus,* while Pruthi (1949) has given an account of the number of moults in *H. nigrorepletus* regardless of other description of instars. Although, present authors have already given a series of papers on the various aspects of *Hieroglyphus* spp. (Riffat and Wagan, 2007a, b, c, d, 2008a, b, c) but there is no account available particularly concerning the nymphal stages of *H. perpolita* and *H. oryzivorus.* The present study is, therefore, confined to establish the number of nymphal instars in each species, to describe and illustrate the morphological differences between these instars and to report information on life history, including life span, development time, and sex ratio.

MATERIALS AND METHODS

Collection of grasshoppers

The stock of *Hieroglyphus* spp. adults were collected from the agriculture fields of rice, maize,

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sugarcane, millets, fodder crops and their surrounding vegetation of grasses with the help of traditional insect hand-net (8.89 cms in diameter and 50.8 cms in length) as well as by hand picking. The collection was made during the year 2005-2007 in the months of June to November from various provinces of Pakistan.

Laboratory culture of adults and nymphs

For the study of life cycle of immature stages of insect adults specimens collected from various fields were reared in crowded conditions in cages at room temperature and under normal lighting conditions. Each cage was provided with cup containing sieved garden sand for oviposition. Fresh drops of water were added daily to keep the sand moist. Green shoots of fresh maize leaves were clipped and placed into 50ml conical flask filled with water. Egg-pods were collected and then buried in the soil by following the method of Pradhan and Peshwani (1961). The hoppers hatched out from these egg's pod were used for the subsequently results.

The insect were raised up to the adulthood in cage (Length 16.5 Width 13.5 cms) as well as in separate glass jars then these medium were placed under laboratory (25°-23/N 68°-24/ E) condition where the temperature fluctuated between 28±2°C to 39±2°C with relative humidity of 26% to 61%. These temperature and relative humidity regimes are similar to field conditions. However, for determining the numbers of moult and duration of nymphal instars up to adult the first instar hoppers were reared singly in ordinary jam bottles. All the jars were marked separately and a *Zea may* was provided as host plant. Same method was adopted for all the *Hieroglyphus* species *viz., H. perpolita, H. oryzivorus* and *H. nigrorepletus.*

Statistical analysis

Data obtained from experimental groups were subjected to one-way analysis of variance (ANOVA) (SPSS 10.0 Soft-Ware) with repeated measures and significant means were determined using Duncan's New Multiple Range Test (DNMRT) and treatment means were compared using the Least Significant Difference Test (LSD).

RESULTS

Key for separation of hopper stages of *Hieroglyphus* spp.

1. Elytron and wing rudiments, when present directed downward (Fig. 1A-D)..... 2
- Elytron and wing-rudiments turned upward (Fig.1E and F) 5
2. Elytron and wing rudiments not developed, (Fig. 4A) antennae with 10-13 segments ..... *First instar*
- Elytron and wing rudiments evident, antennae with usually 13 or more than 13 segments 3
3. Antennae usually with 13-22 segments, elytron and wing rudiments faintly marked directed downwards (Fig.1A) External genitalia feebly marked..... *Second instar*
- External genitalia well marked. Antennae 13- 25 segments 4
4. Antennae with 13-25 segments, elytron-and-wing-rudiments directed downward, slightly back, smooth to weak rugose (Fig. 1C) ..... *Third instar*
- Antennae with 19-27 segments, elytron-and-wing-rudiments directed downward and noticeably back, conspicuously rugose (Fig. 1D) ..... *Fourth instar*
5. Elytron and wing-rudiments not extending beyond first abdominal segment (Fig.1E) .....*Fifth instar*
6. Elytron and wing-rudiments extending well beyond first abdominal segment (Fig.1F) ..... *Six instar*

Key to species of immature *Hieroglyphus*

1. Inner side of hind femur with tinge of red or orange red color 2
- Inner side of hind femur with light green in color ....*nigrorepletus*
2. Posterior margin of pronotum almost straight (Fig.3)..... .....*oryzivorus*
- Posterior margin of pronotum obtuse angular (Fig.4) ....*perpolita*

Morphological description of hopper stages

The vermiform larva

The freshly hatched larva (vermiform larva) is covered all over with a thin, colorless and semitransparent cuticular membrane which is quickly cast-off (the intermediate moult) with this intermediate moult the larva enters the first intar. In both sexes, normally there are six moults and six nymphal stages (excluding the vermiform larva) and the adult being the seventh stage.

Hieroglyphus oryzivorus

First instar (Fig. 3. A)

Newly hatched nymph yellowish in color having reddish- brown spots and patches along the middle of the back. Pronotum straight. Head and

pronotum extend similar in size; some yellowish pitches on dorsum of pronotum and abdomen. Femur pale green with brownish distal end. Tarsi bluish grey with minute spines and hairs. Cerci poorly developed sex can be differentiated easily.

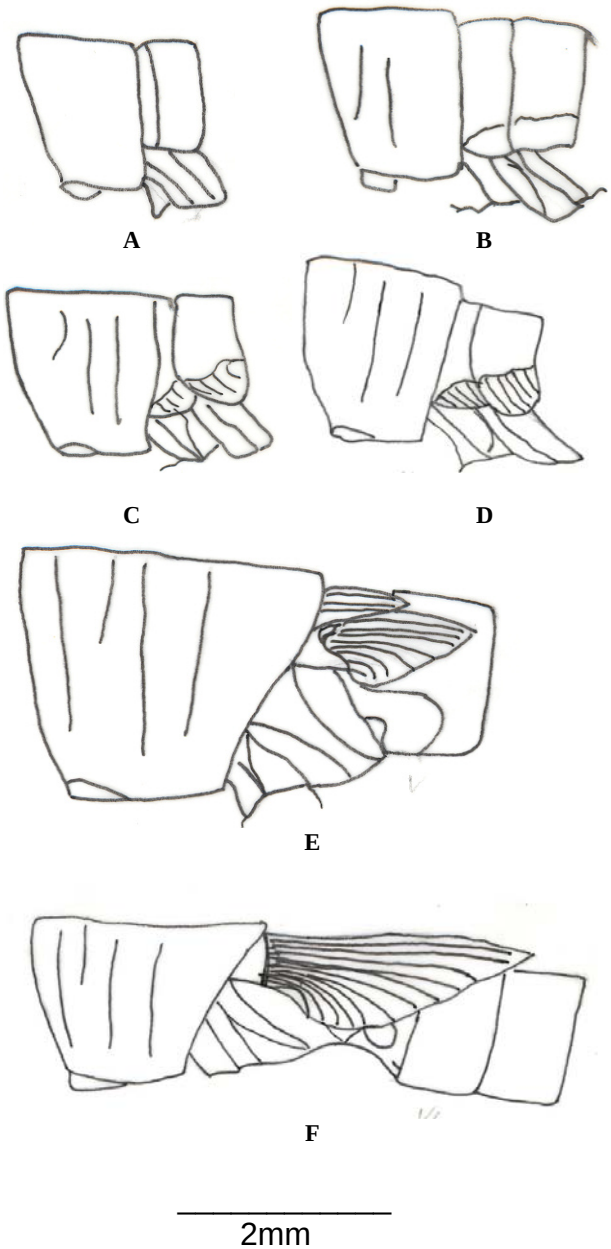


Fig. 1. Wing pads of *H. perpolita* (Uvarov, 1932); A, first instar; B, second instar, C, third instar; D, Fourth instar; E, fifth instar; F, sixth instar (Scale line=2mm)

Second instar (Fig. 3B)

Elytron-and wing-rudiments developed little more; but still pointing downward laterally. Femur with reddish brown marking; abdomen yellowish-green with no marking on the dorsal as well as on the ventral surface; Cerci developed and it is very unique character of *H.oryzivorus* to differentiate the sexes.

Third instar (Fig. 3C)

Eyes dark black from the half while rest position is reddish. A yellowish line middorully all along the pronotum. Black marking on the femur may be more or less obliterated. Cerci well developed; anterior section elongated and wider whiles the apically becoming narrow inflated lateral in the middle. Elytron-and-wing-rudiments little more developed and still pointing downward. Phallic complex slightly begin to appear at this stage.

Fourth instar (Fig. 3D)

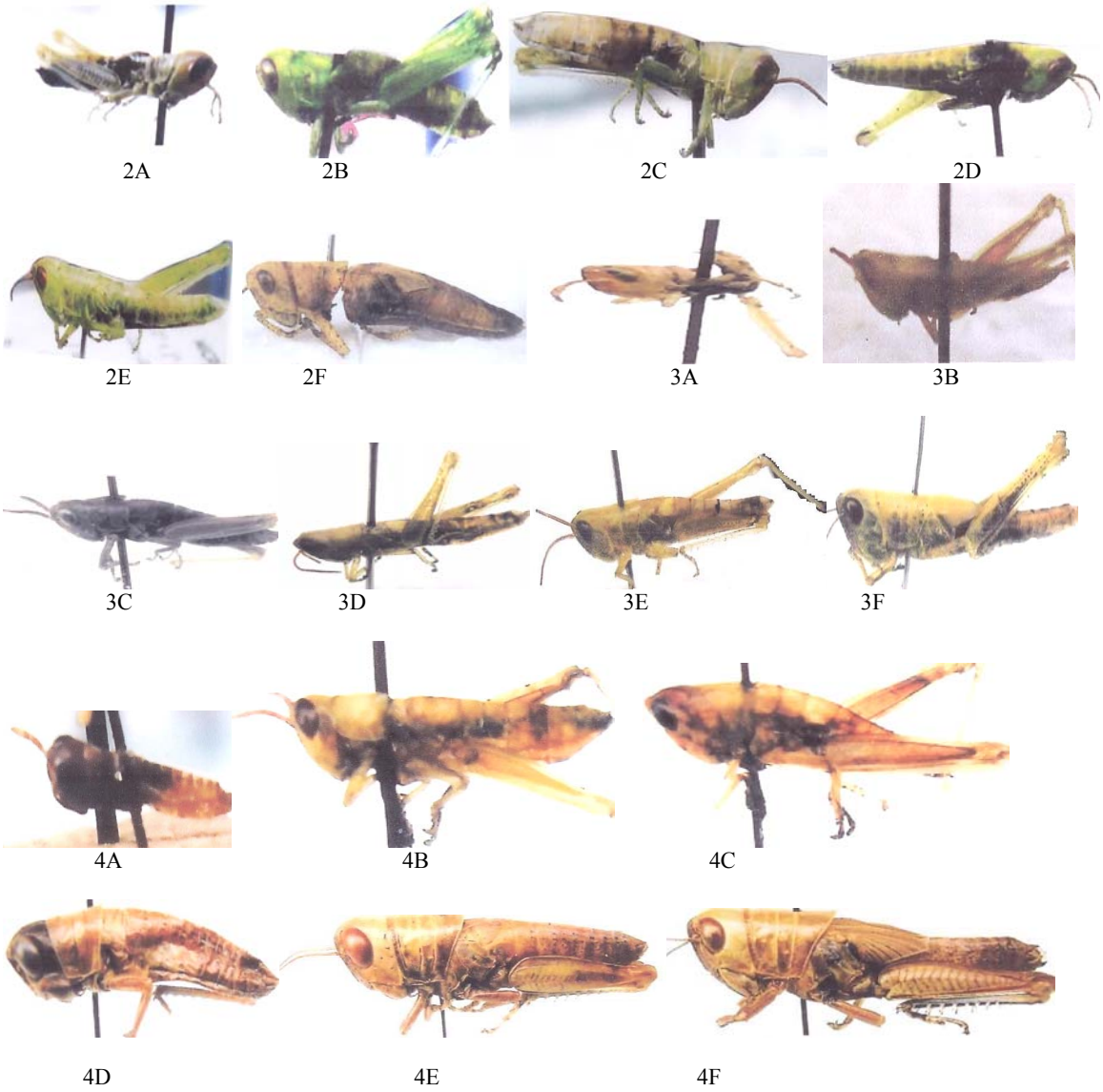
Fastigium of vertex with strong depression. Wing rudiments become slightly bigger; brownish dots on the femur, distal end of femur black; tarsi greenish with spines hairs. Male genitalia extending a little backward. In female the anterior and posterior ovipositor valves a little more developed but do not yet overlap the posterior valves; middle valves slightly developed.

Fifth instar (Fig. 3E)

Reddish dots on the fastigium of vertex. Pronotum slightly saddle-shaped. Side of abdomen dark brown, ventral side yellowish green in color and covered with hair. ♂ genitalia extending well beyond the hind end of the following sternum. In ♀ the ovipositor valves extending backward further more.

Six instar (Fig. 3F)

Eye color mostly similar to fifth instar. Small brown dots also on the ocelli; abdomen yellowish green in color. Femur slender reddish from inner side. Apices of folded tegmina and wings approximately level with first abdominal segment. Male genitalia extending further more while in female anterior and posterior ovipositor valves developed little more while middle valve cannot complete yet.



Figs. 2-4. Different instar of *H. nigrorepletus*, I. Bolivar (2), *H. oryzivorus*, Carl,1916 (3), *H. oryzivorus*, Carl,1916 (4).
(Enlarged), 2A, First instar, (Female); 2B, Second instar (Female); 2C, .Third instar (Female); 2D, Fourth instar (Female); 2E, .Fifth instar (Female); 2F, Sixth instar (Male) (Enlarged view)
3A, First instar (Male); 3B, Second instar (Male); 3C, Third instar (Female); 3D, Fourth instar (Female); 3E, Fifth instar (Female); 3F, Sixth instar (Male) (Enlarged view)
4A, First instar (Male), 4B, Second instar (Female); 4C, .Third instar (Male); 4D, Fourth instar (Female); 4E, Fifth instar (Female); 4F, Sixth instar (Female) (Enlarged view)

Hieroglyphus perpolita

First instar (Fig. 4A)

Soon after the emergence the color of compound eyes is reddish with light brown eyes stripes. Anterior edge of the pronoun straight while the posterior slightly rounded. Small and inconspicuous wings rudiments scarcely developed on the lateral region. Thin yellowish patches on inner side of the femur. Distal joint of the hind tibia brown, while minute spines begin to appear on both the sides of tibia. Cerci developed along with thin hairs.

Second instar (Fig. 4B)

Eyes reddish brown; fastigium of vertex with irregular brown dots. Wing rudiments slightly bigger but on the lateral side. Yellowish irregular patches on the femur became prominent. Concealed genitalia organs were not differentiated easily in this stage. As the nymph advance in age, the color changes to yellow though some green forms can also be seen.

Third instar (Fig. 4C)

Eyes dark brown. Wing rudiments bigger and downwards. Male genitalia and female anterior and posterior ovipositor valves slightly begin to develop. Brown parallel lines running on the abdomen (with exception of few cases) Yellowish brown stripes on the inner side of femur. Spines with blackish tip become sharp.

Fourth instar (Fig. 4D)

The anterior third of the eye gradually attain blackish color while the rest of the eye has a yellowish brown color. Elytron and wing- rudiments small but distinct, pointing downward, veins on the wings become slightly prominent. Male phallic complex with little more in progress and female anterior and posterior ovipositor valves further developed but not yet complete. Femur from inner side slightly reddish. Tibia yellowish grey with hairs; spines becomes sharp.

Fifth instar (Fig. 4E)

Elytron-and-wing rudiments turned upward, outer pods usually do not extend beyond first abdominal segments. Hind femur gradually becomes reddish which is the indication towards the maturity. Male and female genitalia further developed. Some nymphs become yellowish in color at this stage and subsequently assume a yellowish brown.

Sixth instar (Fig. 4F)

Wings rudiments directed upwards; outer pods reach second abdominal segments. The longitudinal vein of forewing prominent, reach to the second abdominal segment and is covered by the wing rudiment. Tibia yellowish-grey in color with 8-9 sharp spines. Cerci well developed; basically cylindrical; slightly inflated apically. Male genitalia extending further more while in female ovipositor valves little are left towards the completion. Reddish color from the inner side of the femur becomes more prominent.

Comparison of different body parameters in various developmental stages of H. peroplita *and* H. oryzivorus

Morphometric means of the various instars of *H.perpolita* clearly showed that there was significant difference in the measurement of different parameter of male and female (Table Ia). In the first instar of *H. perpolita* there was difference in the length of femur and total body length of female followed by male. In the second instar, antennal segment and length of femur was highest in female compared with male but remain other parameters were equal. As far as third instar is concerned different in the total body length of female compare with male. In case of forth instar body length of female was greater than male. In the fifth instar length of antenna, pronotum and total body length was greater however, other parameters were insignificant. Conversely, in the sixth instar all parameters maximum except no disparity was reported in the length of antenna and pronotum.

There were no statistical difference in the first instar of *H. oryzivorus* excluding the length of femur and antennal segment in female was greater than male (Table Ib)**.** There is significant difference in the length of pronotum and total body length of female compare with male in 2nd instar. In the third instars all the parameters were insignificant except that antennal segments were more in female. As far as forth instar is concerned, antennal length and total body length of female was greater than male. In the fifth instar length of pronotum and body length was greater in female. In case of sixth instar all body parameters were significantly large in female.

Table II suggested that nymphal stages of *H. perpolita* accomplished their development in significant short period of time as compare to other species were studied however, there was no significant difference between the nymphal duration of *H. oryzivorus* and *H. nigrorepletus*. Sex-determination of various instars showed that sex ratio was extremely in the favor of female in all the species of *Hieroglyphus* were studied. (Table III).

Table I.- Morphometric mean used to distinguish various instars of *Hieroglyphus* spp.

Parameter (mm)	I st Instar (n=30)		2nd Instar (n=30)		3rd Instar (n=30)		4th Instar (n=30)		5th Instar (n=30)		6th Instar (n=30)	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
a) *H. perpolita*												
Antennal segment	12.6±0.52	13.4±0.60	20.8±2.0	22.4±0.4	24±0.36	24.6±0.94	25.4±0.9	26.2±0.9	27.56±0.6	27.92±0.4	27.9±0.8	28.02±0.8
Antennal length	1.43±0.60	1.54±0.14	4.5±0.90	5.1±0.60	6.65±1.2	7.62±0.54	9.8±1.16	10.3±1.3	10.23±1.2	10.9±1.2	11.25±1.5	11.6±1.29
Length of pronotum	1.08±0.13	1.28±0.19	3.81±0.50	4.3±0.17	5.25±0.6	5.96±3.00	6.4±0.44	6.65±0.3	9.3±1.04	9.65±0.39	9.8±0.4	10.19±0.4
Length of femur	2.19±0.13	2.75±0.59	10.94±0.9	12.4±0.43	12.9±1.10	14.7±1.60	15.0±0.8	15.06±0.8	19.68±1.6	19.8±1.5	20.35±0.9	20.76±1.2
Total body length	6.2±0.38	7.19±6.70	21.5±1.70	21.7±1.8	25.53±2.6	29.9±2.0	29.26±2.3	34±0.10	35.6±3.5	39.01±1.1	36.5±2.9	41.8±2.10
b) *H. oryzivorus*												
Antennal segment	11.1±0.50	11.53±0.7	13.66±0.4	13.73±0.4	14.33±0.6	15.6±0.4	22.53±0.4	23.26±0.9	24.9±0.7	25.45±0.4	26.26±0.6	27.4±0.4
Antennal length	1.19±0.14	1.35±0.50	1.26±0.24	1.40±0.3	1.30±0.30	1.54±0.14	6.18±0.14	7.3±0.50	8.65±1.1	9.0±0.81	8.9±0.90	10.39±0.4
Length of pronotum	1.12±0.10	1.30±0.20	1.44±0.03	1.59±0.09	1.65±0.04	1.67±0.04	3.8±0.30	4.0±0.30	4.0±0.2	5.06±0.3	4.94±0.6	6.8±0.50
Length of femur	2.0±0.10	2.66±0.70	3.71±0.20	3.91±0.20	4.0±0.15	4.15±0.2	9.8±0.20	9.91±0.30	14.2±1.2	15.2±0.7	14.8±1.5	18.6±1.10
Total body length	5.89±0.4	6.10±0.56	6.46±0.14	7.6±0.23	8.25±0.6	8.3±0.4	22.3±1.5	22.66±1.3	26.4±4.3	27.4±2.0	30.53±2.2	36.8±2.9

Table: II.- Developmental duration of immature stages of *Hieroglyphus* spp. on *Zea mays*.

	Developmental period of instar in days (Mean ± SD)							
Species	**I**	**II**	**III**	**IV**	**V**	**VI**	**Average duration**	**Total period**
H.perpolita	4.02±0.22 [b]	4.06±0.21 [b]	4.51±0.02 [b]	4.27±0.14 [a]	3.70±0.21 [a]	4.72±0.17 [d]	4.21±0.36 [a]	25.38[a]
H. oryzivorus	4.7±0.36 [a]	5.67±0.18 [a]	5.45±1.08 [a]	4.76±0.50 [a]	5.6±0.79 [a]	4.92±0.02 [a]	5.18±0.43 [a]	31.10 [a]
H.nigrorepletus	4.37±0.0 [b]	5.02±0.14 [a]	5.73±0.05 [b]	4.79±1.20 [b]	5.64±0.38 [c]	4.92±0.82 [a]	5.07±0.47 [b]	30.48[b]

Table: III.- Sex-determination of various instars in *Hieroglyphus* spp.

	Developmental Stages											
Species	**I st instar**		**2nd instar**		**3rd instar**		**4th instar**		**5th instar**		**6th instar**	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
H.perpolita	6.32%	6.00%	5.11%	5.50%	6.69%	6.90%	9.27%	11.22%	11.16%	13.96%	9.22%	8.58%
H. oryzivorus	7.69%	9.0%	7.23%	6.02%	8.34%	9.46%	6.49%	7.42%	7.60%	7.23%	7.05%	16.30%
H.nigrorepletus	4.27%	7.37%	7.40%	10.77%	10.61%	13.25%	9.96%	11.06%	6.83%	6.67%	4.92%	6.83%

DISCUSSION

Usually, the different structures taken into consideration for determining the nymphal instars among the Orthoptera are, the length of body, the antennae, the legs, the genitalia, the pronotum, the tegmina and wings, the number of eye stripes, and a few less important morphological features such as coloration, etc. However, Roonwal (1952) gives importance to variation in the number of the antennal segments during the postembryonic development of *H. nigrorepletus* and some other Acridids. At the present all the parameters were studied except the numbers of eye stripes. While, Chesler (1938), gave systematic description of the identification of the various nymphs of Acrididae on the basis of the length of body, hind femora, antennae and wing rudiments. Similarly, Richards (1961) reported that length of various parts of legs, the body and cerci, as determining factors. Coleman and Kunhi Kannan (1911), Karandikar (1945) and Albrecht (1955), have shown that the development of tegmina and wings is of great important in determining the hopper instars. Present study is agreed on this account.

At the present significant differences was observed in measurements on instars one of the reasons, for this irregularity of growth rate throughout the instars might be due to the different rates of growth between the various body parts and between instars in the same part. Nymphal stages of *Hieroglyphus* species revealed that earlier stages are more epidemic to crops then that of adults possibly the reason for this lack of high flying ability. All the nymphs are slightly differ in form and coloration to each other but there is no significant difference in morphological features except that posterior margin of pronotum was recorded almost straight in *H. oryzivorus* while it was obtuse angular in *H. perpolita*. Furthermore, inner side of hind femur with tinge of red or orange red color in *H. perpolita* opposing this, it was light green in *H. nigrorepletus*. The life-cycle of *Hieroglyphus* spp. takes more than (4.82±0.531 days) for completion and pass through six nymphal instars.

However, significant variation in the numbers of moults reported by previous authors. As Pruthi (1949) stated that *H. nigrorepletus* cross through fifth nymphal instar to turn out to be adult whereas Srivastava (1956) reported that *H. nigrorepletus* moult just seventh time. Pradhan and Peswani (1961), Roonwal (1976b) and Riffat and Wagan (2007) observed it go by six nymphal instars. Presently it was reported that *H. perpolita* and *H. oryzivorus* also pass through six nymphal instars like that of *H. nigrorepletus*. However, the above difference in *H. nigrorepletus* might be due to effect of different climatic conditions of the region.

Study on the life-cycle of *H. perpolita* and *H. oryzivorus* reported for the first time. However, for the detailed description of nymphal instar morphometeric body parts comparison of *H. nigrorepletus* please see our previous paper (Riffat and Wagan, 2007a) nevertheless, its illustration, (Fig. 2) separation and identification keys are being presented here for the first time.

The results obtained are valuable in acquiring a better understanding of the economic importance of this genus and knowledge attained on the life-cycle of these insects in comparative manner will be valuable in planning more appropriate and effective control measure in near future.

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