The study of lifecycle of three *Pardosa* species *viz.*, *P. birmanica*, *P. leucopalpis* and *P. oakleyi* under laboratory conditions

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Abstract

The lifecycle of three *Pardosa* species (*P. birmanica*, *P. leucopalpis* and *P. oakleyi*) was studied under laboratory conditions. The spiders were reared in clear perplex cages on artificial diet. The cotton swab was soaked in modified diet and kept in the cage. The diet was changed after regular interval (24 hours). After mating ten female spiders were selected for research in separate cages. On hatching newly born spiders ling were climbed on the mother back for 4 days after that they were separated from the mother’s back. A group of seventy spider’s lings was separated randomly from all the cages for research and each spider-ling was introduced into a cage to prevent cannibalism. The diet was given to these spiders lings under same laboratory conditions. Daily observations were noted on each cage to study the developmental stages of *Pardosa* species. The first molting stage was spent on the mother’s back. The selected *Pardosa* species exhibited eight instars to become adult. The total length of male and female was observed at adult stage. The feeding of man-made diet by the ground spiders is an indication that the mass rearing of these spiders could be utilized against to control the various insects’ pest species.

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Introduction
A large part of predatory fauna of agro-ecosystem is organized by Araneaein Punjab. They are known as generalist predators but a few species are known as specialist predators. The spiders are used as biological control with concomitant administration of non-persistent and selective insecticides which limits the application to only specific times to protect spiders to provide favorable results (Mushtaq et al. 2000).

The agriculture is a jugular vein of economy of Pakistan. Various crops like vegetables, crops, wild orchard and ornamental plants of Pakistan are damaged by many phytophagous insects. The insect pests badly affected the all agricultural crops (Siddiqui, 2005).

Pesticides are frequently used for the inhabiting of pest population. The pesticides have been frequently used in Pakistan. The indiscriminate use of pesticides has been increased many folds in recent decades. In Pakistan these pesticides not only affected the human health but also affected the non-targeted species. So these factors are liable for weakening of natural forces which play an important role to control the pest problems (Rana et al., 2010). The aim of the study was to rear the Pardosa species in laboratory conditions and these could be exploited against the pest.

Above mentioned Pardosa species are dominant ones in agro ecosystems of Punjab and play a major role in prey predator relationship. Spider is being the natural enemy of many insect pests in Pakistan. Baseline information on life history and biology is fundamental for ecological work and is of great importance to investigate the predatory potential of a species.

Materials and Methods
Research work was conducted in laboratory experiments. Collection was taken from the fields of Ayub Agricultural Research Institute (AARI) Faisalabad, Postgraduate Agriculture Research Station (PARS), and surrounding of Faisalabad, Pakistan. The laboratory study was done at Arachnology Laboratory, Department of Zoology, Wildlife & Fisheries, GC University, Faisalabad.

Collection of the spiders
The experiment was conducted in the Arachnology laboratory, Department of Zoology, Wildlife and Fisheries GC University Faisalabad. The wondering spiders were collected by simple hand picking method for both identification and further research. Spiders after collection was brought to the laboratory, washed with xylene, preserved in vials (containing 70% alcohol and few drops of glycerin) and used for identification.

Identification
All the spiders were recognized to species level using the keys by Tikader and Biswas (1981), Tikadar and Malhotra (1980), and Barrion and Litsinger (1995).

Measurements
The measurement of the body parts, eggs and cocoon were measured with the help of micrometer. The measurements of total length were done with the help of micrometer for both sexes at adult stage.

Rearing of Pardosa species
The identified male and female Pardosa spider was kept in separate mating chamber (Fig. 1) pair-wise and after mating the male was removed. The collection of the spider were taken from fields of Ayub Agriculture Research Institute (AARI) Faisalabad and surrounding of Faisalabad District by hand picking method in plastic vials and taken to Arachnology Laboratory, Department of Zoology, Wildlife and Fisheries, GC University Faisalabad. After incubation, these spider-lings were replaced to the rearing cages (10 x 7 x 5 cm) (Fig. 2). There were cages of clear perplex freezer dishes (sandwich boxes), 2 cm diameter ventilation hole was covered by mesh No. 4.0 milk strainer cloth (brass screen). Moisture was constantly given to the inner side of the cage by cotton swab.

To avoid from cannibalistic behavior, spiders were placed in separate cages and reared on man-made
diet with some modification conditions optimization (Amalin et al.1999; Amalin and Pena 2003). The diet was a combination of 100 ml homogenized milk, 100ml soya-bean oil, one fresh chicken egg yolk and 5ml honey. The cotton swab was soaked with the artificial diet and place in the cage. Diet was replaced after regular period (48 hours). Laboratory conditions were adjusted at 27±2 °C, 70±5 RH and 12:12 L: D photoperiod.

Ten sexually matured female spiders were chosen for investigation. On hatching, newly borne spiderlings were attached on the back of the female spider for 4 days. After detachment of spiderlings from the back of the mother, a group of 70 spiderlings were randomly separated from all cages for examination and each spiderling of the group was shifted to a separate cage to prevent cannibalism. The spiderlings were nourished on man-made diet under same laboratory conditions. Daily observations were noted on each cage to know the complete picture of development from selected Pardosa species. The number of days spent before each molt was noted.

Results
ANOVA for the number of days at each in star level
The interaction between three species had significant difference for the number of days spent at each molting stage. The interaction between spider species and their instars were significant for the number of days at each instars level 1st instars to adult stage of these Pardosa species were also significant for the number of days spent at each molting stage (Table.1). There was a great variation among the duration of the each instar in Pardosa birmanica. 1st instar required (4.6 ± 0.559) days to change into second instar, 2nd instar spent (5.6± 0.509) days in the next molting stage, 3rd instar required another (7.4± 0.244) to change into 4th instar. Spider stayed for (17± 1.37) days in 4th instar stage. The 5th instar change into 6th instar in (12± 0.707) days, The 6th instar change into 7th instar in (21.6± 1.4) P. birmanica spent (21 ± 0.547) days in the 7th instar and finally 8th instar changed into adult in (18± 1.7029). P. leucopalpis had shown higher variation among the duration of the each instar. The 1st instar required (4.4 ± 0.509) days to change into 2nd instar. The 2nd instar spent (7± 0.447) days in the next molting stage. 3rd instar required (9.4 ± 0.509) days to change into 4th instar. The 4th instar took (19.2 ± 0.663) days to change into 5th instar also spent (13± 0.547) days to change into next molting stage. The 6th instar spent (26.2± 1.88) days to change into 7th instar level. P.leucopalpis took (20± 0.707) days in the 7th instar and lastly 8th instar changed into adult (15±0.707) days into adult. P.oakleyi had shown higher variation among duration of various instars. The 1st instar required (4 ± 0.374) days changed into 2nd instar. The 2nd instar took (3.8±0.2) days to change into 3rd instar. 3rd instar spent (8 ± 0.316) days to go into 4th molting stage. The 4th molting stage required (16± 0.717) days spent before 5th molting stage. The 5th instar spent (14.8± 0.734) to change into 6th molting stage. The 6th instar spent (20.4± 1.56) days to change into 7th instar level. P. oakleyi took (22.2± 1.82) days in the 8th instar and lastly 8th instar changed into adult (17.2 ± 1.65) days into adult. In case of P. birmanica there was a non-significant difference for the numbers of days spent of at 1st and 2nd instars while the number of days spent at the 3rd, 4th and 5th instars were statistically non significant but significant difference was observed between 7th and 8th instars. The overall comparison among the different instars of P. birmanica indicated that the 5th instars were significantly differing from the rest of the instars.

| Table 1. ANOVA for the number of days spent by each stages (1st instar to adult) of three spider species. |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **S.O.V**                  | **d.f**         | **S.S.**        | **M.S.**        | **F.Value**     | **Pr(>F)**      |
| Spider species             | 2               | 162             | 80.9            | 14.788          | 2.1e-06 ***     |
| Instars                    | 8               | 6052            | 756.5           | 138.296         | <2e-16 ***      |
| Spider species: Instars    | 16              | 670             | 41.9            | 7.654           | 9.82e-12 ***    |
| Residual                   | 108             | 591             | 5.5             |                 |                 |

NS= Non Significant at P>0.05; *=significant at P<0.05; **= highly significant at P<0.01.
There was significant difference for the number of days spent at each molting stage for *P. leucopalpis*. The 6th instars showed highly significant difference because of the higher number of days spent within the species and among the species while in case of 1st molting stage least number of days spent within the species.

In case of *P. oakleyi*, there was a significant difference for the number of days spent between 1st and 2nd instars but 2nd and 3rd instars were significantly different from each other. (Fig. 3).

### Mean number of days for maturation

Mean Number of days for maturation of three *Pardosa* spp. viz., *P. birmanica*, *P. leucopalpis* and *P. oakleyi* had 107.2 ± 6.996, 114.2 ± 5.97, 106.4 ± 7.391 respectively the *P. leucopalpis* had higher number of days for adult hood which is followed by *P. birmanica* and *P. birmanica* followed by *P. oakleyi*. (Fig. 4).

### Table 2. ANOVA for the total length of adult spider species.

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<th>M.S</th>
<th>F. Value</th>
<th>Pr(&gt;F)</th>
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</table>

*NS = Non significant at P>0.05; * = significant at P<0.05; **=highly significant at p<0.01.*

#### ANOVA for total length at adult stage

The interaction between adult spider species and their instar were significant for the total length of their body three spider species had significantly different body length. Male and female of these species were also significantly different in their body length (Table 2).

#### Fig. 1. Mating chamber used for *Pardosa* species.

#### Attainment of total length at adult stage

The male *P. birmanica* had attained the total length (5.25±0.692mm) at adult stage. The female *P. birmanica* had achieved the (7.42±0.706mm) length at adult stage the female *P. birmanica* had higher total length than the male *P. birmanica*. The male *P. leucopalpis* had got the length (7.62±0.692mm) at adult stage and female *P. leucopalpis* attained the total length (8.94±0.706mm) at adult stage. The female *P. leucopalpis* had higher total length as compared with the male. The male *P. oakleyi* had attained the total length of (4.7±1.555mm) and female had got the total length of (5.78±1.857 mm) within each species female had higher total length as compared to the male. *P. leucopalpis* had higher total length and followed by *P. birmanica* and *P. birmanica* followed by *P. oakleyi*.

#### Fig. 3. Mean (SEM) number of days spent at each instar level of *Pardosa* species.

There was a statistically significant difference between adult male and female of *P. birmanica* for total length. The adult female had a significantly longer total length as compared to male *P. birmanica* while adult male and female *P. leucopalpis* was statistically non-significant. The adult female of *P.*
leucopalpis had a non-significant had longer total length as compared to male. Similarly female P. oakleyi had a non-significant difference for total length between male and female. The adult female had a non significantly higher total length as compared to male (Fig.5).

Discussion
Number of instars

The present study shows that three selected Pardosa species viz., P.birmanica, P.leucopalpis and P.oakleyi were reared under standard laboratory conditions and found that they exhibited 8 instars in order to become adult. The present investigations are corroborating with the findings of Punzo and Farmer (2006) had investigated the life cycle of P.sierra under controlled laboratory conditions and observed 9 instars. The 9th instars were due to the difference of different species. The present investigations showed variation with the finding of Amalin & Pena (2003) for the developmental period. This may be due to different species and different laboratory conditions.

Methods of rearing

In the present study rearing was done by using the boxes which were packed with soil/ sand for shelter and these methods were same which was adopted by Dinter (2004).

Parental behaviour/maternal care

In case of parental behavior/maternal care the present investigation sported by the finding of Amalin & Pena (2003), reported the female remained near to the egg-mass during the period of spider-ling.

Diet

In the present study the balanced man made diet was used and major component comprised of cholesterol which is important for molting and same observation is described by the Foelix (1982) & Singh (1984).

Observation after incubation

In this work, after incubation the spider-ling climbed on the mother back and ride till they are able to move. These findings are supported by the Ender (1996) who also reported the same behavior.

Attainment of total body length at adult stage

In the present study the mail P. birmanice, P. leucopalpis and P. oakleyi has attained the mean total length 5.25±0.692 mm, 7.62±0.692 mm, 4.77±1.55mm respectively and females have 7.42±0.706, 8.94±0.706 and 5.78±1.857 mm respectively. These findings were also agreed with the findings of Ghafoor et al (2003) who determined the mean total length of these species, also indicating the higher size of females.
The present investigations also supported by the Barion and Litsinger (1995) who observed the mean total length of male and female at each instar level within the same range.

**Conclusion**

Summarizing all this it is recommended that man made Diet is suitable for *Pardosa* spider species under laboratory conditions. The spider species can be successfully reared supplemented with artificial food control conditions. Rearing of such important predatory species against serious crop pests could be beneficial for the insect pest susceptible plants.

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