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Research Article

Effects of different thermal stress on life cycle of *Chrysoperla carnea* (Neuroptera: Chrysopidae) under laboratory condition Quetta Balochistan

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Abstract

The *Chrysoperla carnea* (Stephens) often recognized as an aphid lion which is a member of the Chrysopidae family of the Neuroptera order. It can be found in almost every habitat on the planet. An experiment was carried out in the laboratory to examine the biological and reproduction of the green lacewing under the optimal temperature regimes. The results revealed that a highest hatching % of *C. carnea* eggs (98.2 ± 0.9) was found on 29^{0}C and lowest mean hatching % of eggs was observed in 31^{0}C (84.89 ± 1.22). A minimum larval developmental day of *Chrysoperla carnea* (8.6 ± 0.01) was recorded at 31^{0}C, while maximum larval developmental days *C. carnea* (18.00 ± 1.01) was recorded at 19^{0}C. Similarly, a maximum larval motality % was observed (35.25± 1.00) at 19C and minimum larval mortality % (27.00± 2.04) was found on 27^{0}C. The lowest adult emergence % of *Chrysoperla carnea* pupae (74.00 ± 1.09) was noticed on 19^{0}C and highest adult emergence % of *Chrysoperla carnea* pupae (88.00 ± 0.6) was observed in 27^{0}C. The maximum pupation time of *Chrysoperla carnea* was observed in 19^{0}C (11.10 ± 0.34 days) and the minimum pupation time of *Chrysoperla carnea* was noticed on 31^{0}C (5.00 ± 0.88). The maximum adults fecundity of *Chrysoperla carnea* was observed on 27^{0}C (634.00 ± 0.88), and minimum fecundity of *Chrysoperla carnea* (393.33 ± 0.55) was found on 19^{0}C.

Keywords: Effects; Different Thermal Stress; Life Cycyle; Green Lacewin

Introduction

Green lacewing (*Chrysoperla carnea* (Stephens)), often recognized as an aphid-lion, it is a member of the Chrysopidae family of the Neuroptera order. It exists in most environments around the world. *Chrysoperla carnea* larvae considered as a rapacious predator of the uncovered eggs, tiny beetle maggots, and other lepidopterous pest. Jassids, Aphids, Whitefly, Thrips, Mealy Bugs, Scales and Mites are among the slow-moving, soft-

bodied arthropods it eats [1]. It can be farmed on a large scale in the laboratory and used to control vegetable pests. Because of its resistance to an extensive assortment of the environmental settings, this is used for an essential biological control agent. *Chryosperla carnea* has a high relative frequency of occurrence in several agricultural environments [2]. Ridgway and Murphey [3] found that it has a wide prey range and good hunting abilities, as well as a high resistance to a variety of pesticides Bigler, (1984). The breeding program has been designed to support in the production of high quantity of eggs and larvae's in order to discharge *Chryosperla carnea* in large quantities [4]. To start using *Chryosperla carnea's* biological program, a cost-effective and biologically efficient mass rearing program must be developed [5].

Their carnivorous tendencies make them important for agriculture. All of the larvae are predators, while others are aquatic and feed on jassids, coccids, psyllids, aphids, mites, and other terrestrial insects etc. In the tropics, it is unusual for a large group of aphids to feed on them without at least some neuropterous larvae. A larva may eat up to 500 aphids in its lifetime, and they are undoubtedly important in the control of various homopteran pests [6]. Chryosperla carneas are a varied group of predators, with more than 1200 species reported throughout the world, *Chrysoperla mediterranea* (Hölzel), , *Chrysoperla mediterranea* (Hölzel), *Chrysoperla rufilabris* (Burmeister), *Chrysoperla oculasinaata*, Chrysall *C. johnsoni*, *Chrysopa nigricornis*, *C. adamsi*,*C. plorabunda* (Fitch), *C. downesi*, *C. formosa* (Brauer),*Chrysoperla carnea*, *C. nigricornis* (Smith), *C. comanche*, *C. mohave*, *C. lacciperda* (Kimmins), *C. scelestes* (Banks)) are numerous manufacturers and suppliers sell *Chrysoperla* spp., particularly *Chrysoperla rufilabris* and *Chryosperla carneas* commercially to manage insect pests [7].

The *Chrysoperla carnea* is an example of a non -predatory species in adulthood; while, the predatory phase considered as a larval stage and some species became predator in the adult stage [8]. Tauber *et al*. [9] reported that green lacewing larvae are generalist predators, sometimes known as aphid lions, which have been seen as devour among hundred to six-hundred aphids aphid every day. Pollen, nectar, and honeydew aphids are the only foods available to adults. Length antennae with beautiful gold or copper eyes, pale green, 12-20 mm long. Their bodies are delicate, and their pale green wings are large and transparent. These adults are energetic riders, especially at night and at night, with typical flying flies [10]. These adults bear a high desire to fly, and they can fly up to 3- 4 hours every first two nights after emergence or before laying eggs on the fifth day. The eggs are oval in shape and are laid singly at the ends / ends of a long silky stalk that resembles a small cattail emerging from the leaves of a plant. They are pale green in colour and turn grey after 2-3 days. The larvae are energetic, which have thrice instars along with dark brown or grey in colour, look like crocodiles with well-developed legs and huge clamps for sucking prey bodily fluids, hatch after 6-7 days. The larvae ranging in size such as (≤ 1 mm or up to 6 or 8 mm) till third mature instar develop the round shaped silky cocoons, like parchment and pupate within them, generally in hidden locations in the plant. Zhu *et al*. [11] reported that emerging adults take 8 to 10 days, however there may be more generations with similar traits.

According to Adane and Gautam [12], the survival rate and development of *Chrysoperla carnea* is prejudiced by the relative humidity, temperature, food quantity, food quality and operating time. Birch [13] reported that temperature influencing plays an important role in the eco-friendly features, which insect species evolve. As a result, any economic examination of the link between

temperature and development is crucial. *C carnea* is a significant predator that is commercially accessible in many places across the world for use in agro - ecosystems to control the population of various insect pests. *Chrysoperla carnea* biology is influenced by a variety of biotic and abiotic variables. The host species, the stage of development at which it will be eaten as a host prey and crop at which the host bait *Chrysoperla carnea* are all biotic variables. On the biology of *Chrysoperla carnea*, there is a vast quantity of information accessible; here, some of the most relevant sources are examined.

Materials and Methods

Insect rearing

Green lacewing,*Chrysoperla carnea* were collected from the field of different vegetables, crops grown in the vicinity of Quetta. Initially, the cultures were started in the biological laboratory of Entomology, Directorate of Plant protection, Agriculture Research Institute (ARI), Quetta. Eggs of Angoumois grain moth *Sitotroga cerealla* were used as diet for the rearing of green lacewing under 25°C laboratory temperature. Seven different temperature ranges (19, 21, 23, 25, 27, 29, 31 °C) were used to find out the best rearing temperature for the performance of *Chrysoperla carnea*. Temperature 25 °C was used as a control group.

Host angoumois grain moth, *Sitrotoga cerealla*

Angoumois grain moth (*Sitotroga cerealla*) was cultured at a temperature of 28 ± 1 °C with 65 ± 5%relative humidity. Rice grains were used for the culture of Angoumois grain moth in the glass jars (capacity of 5 kg). The top of the jar was closed with a muslin cloth. Collected female moths were allowed to lay eggs outside the muslin cloth on the top of the glass jar and the eggs of which were providedto the larval instars of green lacewing as a fresh food.

Feeding adults predator (*Chrysoperla carnea)*

Transparent plastic cages were used for adults of *Chrysoperla carnea*, the top of cage was covered with black muslin cloth as medium for egg laying. Artificial diet was provided to the adults *Chrysoperla carnea*for the survival and reproduction. The ingredients of artificial dietwere water, sugar, yeast, and honey in a 3:1:1 ratio (6: 2: 2: 1). The prepared artificial diet was changed after each two days.

Experiments procedure

In plastic petri plates, 150 fresh eggs of *Chrysoperla carnea*were randomly selected in each treatment with three replications (total 450 eggs) from the rearing culture and used on different temperatures (19, 21, 23, 25, 27, 29, 31°C) to observe the hatching % of eggs. After hatching % of eggs, eighty (80) young larvae of *Chrysoperla carnea* were collected from treated eggs with three replications (total 240) in each treatment and exposed to various treatment temperatures (19, 21, 23, 25, 27, 29, 31°C) to find out the larval period and larval mortality %. Fifty (50) one day old pupae of *Chrysoperla carnea* were collected from the treated larvae with three replications (total 150) and released on different treatment temperatures (19, 21, 23, 25, 27, 29, 31°C) to observe the pupal period and pupal mortality %. Ten (10) pairs of *Chrysoperla carnea* adults were collected from treated pupae and exposed on various treatment temperatures (19, 21, 23, 25, 27, 29, 31°C) to observe the male and female adult's longevity and reproduction (Fecundity and oviposition rates). The experiment was continued until the end of death of all males and females. Each treatment was replicated three times and experiment was designed in Randomized Complete Block Designed (RCBD) under laboratory conditions.

Results

A maximum hatching % of green lacewing eggs was found in 29°C temperature (98.22±1.09) followed by 27°C

temperature (97.33±0.23), 25°C temperature (96.00±2.0), 23°C temperature (93.33±0.92), 21°C temperature (90.33±1.92) and 19°C temperature (86.89±1.02), while a minimum hatching % of green lacewing eggs was found in 31°C (84.89±1.22). Statistically significant P< 0.05 difference was observed among control group 25°C, 23°C, 21°C, 19°C and 31°C temperature. However, statistically, no significant difference was noticed between 19°C and 31°C temperature. Similarly, statistically no significant differences were seen among 27°C, 29°C and in control group 25°C temperature (Table 1).

Table 1: Influence of different temperatures on the hatching % of green lacewing, *Chrysoperla carnea* eggs

Treatments	Mean of egg hatching (%)
19 °C	86.89±1.02d
21 °C	90.33±1.92c
23 °C	93.33±0.92b
25°C (Control)	96.00±2.0a
27 °C	97.33±0.23a
29 °C	98.22±1.09a
31°C	84.89±1.22d

Values (mean ± SE) in given column letters are significantly different by Tukey test (p<0.05)

A maximum larval periods (days) of green lacewing eggs were found in 19°C temperature (18.00±1.01) following by 21°C temperature (17.00±0.22), 23°C temperature (14.33±0.26), 25°C temperature (10.66±0.76), 27°C temperature (10.33±1.00) and 29°C temperatures (9.33±0.22), while a minimum larval periods of green lacewing was found in 31°C (8.6±0.01). Statistically, significant P< 0.05 difference was observed among control group 25°C, 23°C, 21°C, and 19°C temperature. However, statistically no significant difference was noticed between 19°C and 31°C temperature. Similarly, no statistically significant differences were seen among 27°C, 29°C and 31°C as compared with in control group 25°C temperature (Table 2).

Table 2: Influence of different temperatures on larval developmental period of green lacewing

Treatments	Mean of larval developmental period
19°C	18.00±1.01a
21°C	17.00±0.22a
23°C	14.33±0.26b
25°C (Control)	10.66±0.76c
27°C	10.33±1.00c
29°C	9.33±0.22c
31°C	8.6±0.01cd

Values (mean ± SE) in given column letters are significantly different by Tukey test (p<0.05)

A maximum adult emergence of green lacewing was found in 27 °C temperature (88.00±0.6) followed by 25°C temperature (85.00±0.5), 23°C temperature (83.66±0.4), 29°C temperature (82.00±0.55), 31°C temperature (79.00±0.45) and 21°C temperature (78.66±0.6), while a minimum hatching % of green lacewing eggs was found in 19°C (74.00±1.09). Statistically, significant P< 0.05 difference was observed among control group 25°C, 19°C, 21°C, 27°C, and 31°C temperatures. However, statistically no significant difference was

noticed between 23°C and 25°C temperature. Similarly, statistically no significant differences were seen between 21°C, and 31°C in temperature (Table 3).

Table 3: Influence of different temperatures on adults emergence % of green lacewing

Treatments	Adults emergence % Mean
19 °C	74.00±1.09d
21 °C	78.66±0.6c
23 °C	83.66±0.4b
25 °C (control)	85.00±0.5b
27 °C	88.00±0.6a
29 °C	82.00±0.55b
31°C	79.00±0.45c

Values (mean ± SE) in given column letters are significantly different by Turkey test ($p<0.05$)

A maximum larval mortality of green lacewing was found in 21°C temperature (36.10±1.2) followed by 19°C temperature (35.25±1.00), 23°C temperature (32.00±0.9), 31°C temperature (29.12±2.08c), 29°C temperature (29.08±2.01c) and 25°C temperature (28.70±2.00), while a minimum larval mortality was found in 27°C (27.00±2.04c). Statistically, significant P< 0.05 difference was observed among control group 25°C, 21°C, 23°C, temperature and 23°C. However, statistically no significant difference was noticed between 19°C and 21°C temperature. Similarly, statistically no significant differences were seen among 27°C, 29°C, 31°C. and control group 25°C temperature (Table 4).

Table 4: Influence of different temperatures on larval mortality (%) of green lacewing

Treatment	Mean larval mortality %
19°C	35.25±1.00a
21°C	36.10±1.2a
23°C	32.00±0.9b
25°C (control)	28.70±2.00c
27°C	27.00±2.04c
29°C	29.08±2.01c
31°C	29.12±2.08c

Values (mean ± SE) in given column letters are significantly different by Tukey test ($p<0.05$)

A maximum timing of pupation (days) of green lacewing eggs was found in 19°C temperature (11.10±0.34) followed by 21°C temperature (11.00±0.30), 23°C temperature (8.6±0.46), 25°C temperature (7.6±0.56), 27°C temperature (6.66±0.76c) and 29°C temperature (5.66±0.77c), while a minimum timing of pupation days of green lacewing was found in 31°C (5.00±0.88c). Statistically, significant P< 0.05 difference was observed among control group 25°C, 21°C, 23°C, temperature and 23°C. However, statistically no significant difference was noticed between 19°C and 21°C temperature. Similarly, statistically no significant differences were seen among 27°C, 29°C, 31°C. and control group 25°C temperature (Table 5).

A maximum fecundity of green lacewing eggs was found in 27°C temperature (634.00±0.88) followed by 25°C temperature (529.33±0.78), 29°C temperature (520.33±0.43), 31°C temperature (485.33±0.11), 23°C temperature (454.66±0.77) and 21°C temperature (408.66±0.55), while a minimum fecundity rate of green lacewing was found in 19°C (393.33±0.55). Statistically, significant P< 0.05 difference

was observed among all given treatments and control group (Table 6).

Table 5: Influence of different temperatures on pupal period of green lacewing

Treatments	Pupal Period Mean
19°C	11.10±0.34a
21°C	11.00±0.30a
23°C	8.60±0.46b
25°C (controll)	7.60±0.56bc
27°C	6.66±0.76c
29°C	5.66±0.77c
31°C	5.00±0.88c

Values (mean ± SE) in given column letters are significantly different by Tukey test ($p<0.05$)

Table 6: Influence of different temperatures on fecundity of green lacewing adult

Treatments	Fecundity Mean
19°C	393.33±0.55f
21°C	408.66±0.55g
23°C	454.66±0.77e
25°C (control)	529.33±0.78b
27°C	634.00±0.88a
29°C	520.33±0.43c
31°C	485.33±0.11d

Values (mean ± SE) in given column letters are significantly different by Tukey test ($p<0.05$)

A maximum oviposition rate of green lacewing was found in 27°C temperature (10.17±0.87) followed by 31°C temperature (10.04±0.65), 19°C temperature (9.42±1.00), 25°C temperature (8.67±0.98), 29°C temperature (8.62±0.87b) and 21°C temperature (8.54±2.78b), while a minimum oviposition rate of green lacewing eggs was found in 23°C (7.97±1.87). Statistically, significant P< 0.05 difference was observed among control group 25°C, 27°C and 31°C temperature. However, statistically no significant difference was noticed between 19°C, 21°C, 23°C, 25°C and 29°Ctemperatures (Table 7).

Table 7: Influence of different temperatures on oviposition rate of green lacewing adult

Treatments	Oviposition rate Mean
19°C	9.42±1.00b
21°C	8.54±2.78b
23°C	7.97±1.87cb
25°C (control)	8.67±0.98b
27°C	10.17±0.87a
29°C	8.62±0.87b
31°C	10.04±0.65a

Values (mean ± SE) in given column letters are significantly different by Tukey test ($p<0.05$)

A maximum of male adults longevity of green lacewing eggs was found in 27°C temperature (44.27±1.99) followed by 25°C temperature (40.26±1.98), 31°C temperature (39.36±2.01), 29°C temperature (38.00±1.33), 23°C temperature (32.00±2.00) and 21°C temperature (32.00±1.32), while a minimum survival of green lacewing eggs was found in 19°C (31.33±1.02). Statistically significant P< 0.05 difference was observed among control group 25°C, 23°C, 21°C, 19°C and 31°C temperature. However, statistically no significant

difference was noticed between 19°C and 31°C temperature. Similarly, statistically no significant differences were seen among 27°C, 29°C and in control group 25°C temperature (Table 8).

Table 8: Influence of different temperatures on male adult longevity of green lacewing adult

Treatments	Male adults Mean
19°C	31.33±1.02d
21°C	32.10±1.32d
23°C	32.00±2.00d
25°C (controll)	40.16±1.98b
27°C	44.27±1.99a
29°C	38.28±1.33c
31°C	39.36±2.01c

Values (mean ± SE) in given column letters are significantly different by Tukey test ($p<0.05$)

A maximum survival rate of female of green lacewing was found in 27°C temperature (62.66±0.23) following by 25°C temperature (61.00±0.22), 29°C temperature (60.33±2.33), 23°C temperature (57.00±0.28), 31°C temperature (48.33±0.76) and 21°C temperature (46.00±0.98e), while a minimum of green lacewing was found in 19°C (43.33±1.99f). Statistically, significant $P< 0.05$ difference was observed among control group 25°C, 23°C, 21°C, 19°C and 31°C temperature. However, statistically no significant difference was noticed between 19°C and 31°C temperature. Similarly, statistically no significant differences were seen among 27°C, 29°C and in control group 25°C temperatures (Table 9).

Table 9: Influence of different temperatures on survival of female

Treatments	Female adult Mean
19°C	43.33±1.99f
21°C	46.00±0.98e
23°C	57.00±0.28c
25°C (control)	61.00±0.22a
27°C	62.66±0.23a
29°C	60.33±2.33b
31°C	48.33±0.76d

Values (mean ± SE) in given column letters are significantly different by Turkey test ($p<0.05$)

Discussion

Present results indicated that a maximum hatching % of green lacewing eggs was found in 29°C temperature, 27°C temperature and 25°C temperature, while a minimum hatching % of green lacewing eggs was found in 31°C (Table 1). Similarly result also was found by Pathan et al 2016. Who reported that above 30 °C and below 23°C temperature have negative impact on the hatching % of green lacewings and their harsh impact can take long time for embryonic hatching. A maximum larval, pupal and adults longevity periods (days) of green lacewing eggs was found in 19°C and 21°C temperature, however a minimum larval, pupal and adults longevity periods (days) green lacewing was found in 31°C. Josain and Sonia **[14]** reported that incubation period was 4 days at 25 °C, while Afzal and Khan **[15]** reported that incubation period of *C.carnea* eggs was 4.8 ± 0.4 days under laboratory conditions. The insect passes through three larval instars before transforming into pupa. The

average duration of the first, second and third instar were 3.6 ± 0.07, 3.4± 0.11 and 4.9 ± 0.10 days at 24±1°C respectively. The result indicates that with increasing temperature developmental duration for different instars of *C. carnea* were significantly decrease. Previous workers reported different developmental duration for different instars. Afzal *et al*. [15] reported the average duration of the first, second and third instar were 3.2±0.49, 2.8 0.20 and 6.9 0.49 days respectively, while Khan *et al*. [16] reported that developmental duration of first, second and third instar were 2.46±0.05, 4.36±0.10 and 5.91±0.19 days when feeding on aphid. A maximum larval periods (days)of green lacewing eggs was found in 19°C temperature (18.00±1.01) following by 21°C temperature (17.00±0.22), 23°C temperature (14.33±0.26), 25°C temperature (10.66±0.76), 27°C temperature (10.33±1.00) and 29°C temperature (9.33±0.22), while a minimum larval periods of green lacewing was found in 31°C (8.6±0.01) days, respectively which are significantly different from each other at three temperatures (Table 2). These different values found for *C. carnea* larvae developmental time may be due to differences in the environmental condition under which the experiments were carried out and the capacity of each species to utilize a given type of prey. The larvae completed two moult during the active feeding period and passed the last moult with in the coccon. A maximum timing of pupation days of green lacewing eggs was found in 19°C temperature (11.10±0.34) following by 21°C temperature (11.00±0.30), 23°C temperature (8.6±0.46), 25°C temperature (7.6±0.56), 27°C temperature (6.66±0.76c) and 29°C temperature (5.66±0.77c), while a minimum timing of pupation days of green lacewing was found in 31°C (5.00±0.88c). a statistically significant P< 0.05 difference was observed among control group 25°C, 21°C, 23°C, temperature and 23°C. However, no statistically significant difference was noticed between 19°C and 21°C temperature. Similarly, no statistically significant differences were seen among 27°C, 29°C, 31°C. and control group 25°C temperature (Table 5), which are significantly different from each other. A maximum adult emergence of green lacewing was found in 27°C temperature (88.00±0.6) following by 25°C temperature (85.00±0.5), 23°C temperature (83.66±0.4), 29°C temperature (82.00±0.55), 31°C temperature (79.00±0.45) and 21°C temperature (78.66±0.6), while a minimum hatching % of green lacewing eggs was found in 19°C (74.00±1.09). a statistically significant P< 0.05 difference was observed among control group 25°C, 19°C, 21°C, 27°C, and 31°C temperature. However, no statistically significant difference was noticed between 23°C and 25°C temperature. Similarly, no statistically significant differences were seen between 21°C, and 31°C in temperature. The (Table 3) present results are supporting with the results of Khan *et al*. [16]. Who reported that temperature has significant effect on the developmental duration, adults emergence and survival rate of immature stages of *C. carnea*. A maximum fecundity of green lacewing eggs was found in 27°C temperature (634.00±0.88) following by 25°C temperature (529.33±0.78), 29°C temperature (520.33±0.43c), 31°C temperature (485.33±0.11d), 23°C temperature (454.66±0.77e) and 21°C temperature (408.66±0.55), while a minimum fecundity rate of green lacewing was found in 19°C (393.33±0.55). a statistically significant P< 0.05 difference was observed among all given treatments and control group (Table 6) same results also were found on fecundity of *Spodoptera litura* indicating that both low and high temperature can have adverse effects on adult reproductions. A maximum male and female adults longevity of green lacewing was found in 27°C temperature, 29°C temperature and

25°C temperature, but a minimum of green lacewing male and female adults longevity was found in 19°C (Table 8 & 9). Similerly, results also were noticed by Khan *et al*. **[16]** and Pathan *et al.* **[17]**, they reported that cold temperature reduces the adults longevity of green lacewing due to increasing reactive oxygen species (ROS). Temperature changes can also have profound effects on many other aspects of the biology of insects such as body size **[18]**, immunity **[19]**, feeding ability and feeding rate, fitness, mating **[20]**, metabolism **[21]**, and respiratory metabolism **[22]**. In future studies, these factors should also be taken into account to more clearly compare the effects of temperature on insects. In addition to temperature there are many abiotic and biotic environmental factors that can affect the population dynamics of insects, such as host plant **[23]**, and fertilizer and pesticide **[24]** application. However, differences in the developmental stages and reproduction of green lacewing exposed to these factors may differ when examined either under artificial laboratory conditions or under natural field conditions. In the future, it will be necessary to conduct more studies under laboratory condition and green houses in natural condition to learn more about the green lacewing population.

Conclusion

In present study different temperature was used to find the effects of thermal stress on the life parameters and reproduction of green lacewing under laboratory condition. Results revealed that variation of temperature have negative and positive influence on the developmental stages of green lacewing and their reproductions. We observed that 25^0C, 27^0C and 29^0C temperatures is favourable for the survival of green lacewing and increasing more generations, whereas 19^0C, 21^0C and 31^0C was found harsh temperature on the survival of mature and immature stages of green lacewing and its reproduction. So we concluded that 27^0C±2 temperature is best temperature for the rearing of green lacewing under laboratory condition. This consequence will be helpful in the field of scientific community and extension workers.

Authors' contributions

Conceived and designed the experiments: M Sharif, A Ali & BK Solangi, Performed the experiments: MA Tariq, R Shah, MJK Bazai, Analyzed the data: MA Tariq & F Ahmed, Contributed materials/ analysis/ tools: BK Solangi, MJK Bazai, R Shah, Fahim Ahmed, Wrote the paper: M Sharif, Mkhan & A Ali.

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