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Cotton stainer, *Dysdercus koenigii* (Heteroptera: Pyrrhocoridae) eggs laying preference and its ecto-parasite, *Hemipteroseius* spp levels of parasitism on it

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Mass rearing,Different mediums, Eggs batches, Mortality

Abstract

Cotton is one of the important and main cash crop of Pakistan as listed in top four crops i.e. wheat, rice, sugarcane and maize. Its contribution is 1.4% in GDP and 6.7% in agriculture value addition. Insect pests are causing a key role in term of qualitative and quantitative losses. In 2010, cotton stainer was thought to be a minor insect pest in Pakistan, while, currently it becomes the most prominent among the sucking insects with piercing sucking mouthparts as causing serious economic losses in the cotton growing areas of Pakistan. Many control tactics were to be studied including biological and chemical. But keeping the drawbacks of insecticides, a biological control is to be highly recommended control tool. The newly introduced predator the *Antilochus coqueberti* (Heteroptera: Pyrrhocoridae) is being reared in the Central Cotton Research Institute (CCRI), Multan against the cotton stainer. This predator, repaid mass rearing in the laboratory completely depends on its natural host because; we don't find the literatures on its artificial diets rearing. In this context, our study is based on efficient and repaid mass rearing of cotton stainer in the lab. It will help in availability of food for its potential and voracious predator alternatively; it will promote the biological control. We provided different water levels to 12grm. of cotton leaves i.e. (0ml, 6ml, 8ml, and 10ml) and food-water petri-dishes. All these treatments were installed collectively in plastic cage measured (0.60m X 0.40m X 0.50m) and ten (10) pairs of cotton stainer were released over the treatment in single replication out of four (4). Our study revealed that cotton stainer mostly preferred wet leaves sprayed with 10ml water for eggs laying as it laid total of twenty-eight (28) egg batches below the wet leaves on soil. Mean no. of eggs were recorded (99) with 86.0% hatching. Although, highest no. of eggs/batch was recorded (252) below the food-water petri-dishes on moist soil followed by (181) below the wet leaves sprayed with 10ml water on moist soil. Lowest no. of eggs/batch was recorded (18) below the food-water petri-dishes on moist soil followed by (22) same with 10ml water. On the basis of our findings, it is concluded that cotton stainer efficient and most rapid mass rearing will be achieved if it will be provided 10ml water to cotton leaves as a best eggs laying medium.

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1 Introduction

The cotton crop contribution in GDP is 1.4% and 6.7% in agriculture value addition as the textile industry based on it. Cotton crop covered total of 2806 thousand hectares area in 2013-14 with 2.5% less than last year's area (2879 thousand hectares). Total of 12.8 million bales were produced during the period 2013-14. While, target was 14.1 million bales, which showed decline of 9.2% against the target and 2.0% over the last year production of 13.0 million bales (PES, 2014). There were several factors involved in reduction of cotton crop production in which insect pests are very important (Dhaka and Pareek, 2007). According to Gahukar, 2006 insect pests' are playing main role in deterioration lint quality and reduced 10-40% crop production.

Cotton stainer has been reported the most voracious insect pests of cotton in the major cotton growing ares of Pakistan in 2011 (Jaleel *et al*., 2013). Before the 2010, the red seed bug was imagined a minor pest in Pakistan, however, this potential insect with piercing sucking mouthparts (specialized stylet) has now become a more dangerous pest of cotton (ASPR-CCRI, 2014). In 2013, it was considered that major cause of cotton staining is actually the red seed bug (Anonymous, 2013). Shah, 2014 concluded that cotton stainer had successfully developed during the last three years in Pakistan, while, 2011 they were noted very nominal. But recently it has become an important insect pest of cotton. having a key role in the facts and figures of the country's economy. This insect has caused substantial economic losses in the cotton growing areas of Pakistan.

Red cotton bug has already been declared one of the key and the most serious pest of cotton crop in other parts of the world too but, basically it was originated in South-East Asian's countries. According to Ahmad and Khan, 1980; Ahmad and Schaefer, 1987; Yasuda, 1992 cotton stainer, *Dysdercus koenigii* (Heteroptera: Pyrrhocoridae) feeds on immature and mature bolls. Sprenkel (2000) is of the view that it's both adult and nymphal stages feed on seed inside the boll and produced the stains on the lint. It attacks on flower buds and small immature bolls with matures. It insert the stylet inside the bolls, reach to the seed, thus, caused reduction in size and finally the fruiting body may abort and drop to the ground. The other hosts of *D. koenigii* are hollyhock (Kamble, 1971) and plants of family Bombacaceae (Kohno and Ngan, 2004). *D. koenigii* is active for damages almost the year and passes winter in the adult stage. Eggs can hatch in week period, while single female can lying about 100-130 light pale yellow eggs beneath the plant residues. Cotton stainer has total of five instars while, the life cycle completes in about 21-35 days period (Sprenkel, 2000).

In Pakistan, the cultivation of genetically modified cotton is now very common and it has successfully reduced insecticidal sprays against the bollworms. But the other side, many of secondary sucking insects of cotton is developing like the cotton stainer (Shah, 2014). According to (Greene and Turnipseed, 1996) they also mentioned that reduction in insecticides application, has allowed minor pest complex to become a major pest group of the crop. However, insect fast and efficient suppression with insecticides is the fact. It can control a heavy infestation with fast fruitful results as compared to biological, cultural and other control tactics. However, it has too many other serious disadvantages like, increase environmental pollution which badly impact on human and animal life. It creates the insecticides resistance problems and interrupt with human food chain (Carson, 1962) which are the two main and serious problems encountered (Brown, 1971). Ample evidence exists concerning the carcinogenic threat related to the use of pesticides. These major types of chronic health effects of pesticides include neurological effects, respiratory and reproductive effects, and cancer. There is some evidence that pesticides can cause sensory disturbances as well as cognitive effects such as memory loss, language problems, and learning impairment (Hart and Pimentel, 2002). Biological agents such as predators, parasites and parasitoids are adversely affected by pesticides (Pimentel *et al.*, 1993a).

In the current scenario, transgenic cotton crop technology has successfully control bollworms of cotton crop but, if we look other side of the coin, it has encouraged most of secondly insect pests. Alternatively, farmer's communities have adopted again intensive application of insecticides which will lead insect resistance, health hazards and environmental pollution etc. Keeping in the view the importance of cotton crop, impacts of genetically engineered cotton crop on sucking arthropods, drawbacks of indiscriminate applications of insecticides and merits of biological control, the current study was conducted for

the promotion of biological control of cotton stainer as its potential predator, *Antilochus coquebertii* (Heteroptera: Pyrrhocoridae) is being reared in the entomological lab. of Central Cotton Research Institute (CCRI), Multan on cotton stainer (ASPR-CCRI, 2014). The main objective of this study was to enhance the lab. production of cotton stainer for efficient mass rearing of its predator. During the studies, mite infestation on cotton stainer adults had also been reported. Banerjee and Dutta, (1980) mentioned that mite is important in biological control for cotton stainer. *Hemipteroseius indicus* is a mite and it infestation had seen with cotton stainer. It was seen with the posterior portion of the red cotton bug which sucks the haemo-lymph (Menon *et al*., 2011). Banerjee and Dutta, (1980) had keenly observed association of *H. indicus* with cotton stainer. While, we attempted on comparison of mite infestation levels with cotton stainer male and female while, some basic studies are in progress yet.

2 Material and Methods

The planned study was conducted in the "cotton stainer rearing and biological control lab." of Central Cotton Research Institute (CCRI), Multan in 2015.

2.1. Mass Rearing of Cotton Stainer

Cotton stainer were reared in plastic cages measured (0.60m X 0.40m X 0.50m) with three aeration holes (0.30m X 0.30m) at 28±2^{0}C temperature and 65±5% R.H. Floor of the cages were covered with 0.04m layer of soil with minute amount of sand as a natural substrate. Filter paper disc inside the plastic petri-dish (0.05m diameter) and fuzzy cotton seed above the filter paper were offered as a food. Cotton wool inside the petri-dish and filter paper disc above the cotton wool, daily 2-5ml water was sprayed over the filter paper, were offered as a water and moisture requirement. In each cage, four (4) petri-dishes of food and four (4) of water were installed. Dry leaves (8-12 grm.) with twigs of cotton (small heaps) were also kept in the cages for efficient eggs-laying medium. Additionally, 5-8ml water was daily sprayed over the soil of cages and the cotton leaves.

2.2. Collection of 5th Instar Nymphs

A bulk of 5th instar nymphs were collected from mass culture of cotton stainer and were released in transparent glass jars measured (0.15m X 0.15m X 0.20m) with 0.12m diameter. In each glass jar, two (2) petri-dishes of food and one (1) of water were kept.

2.3. Experimental Cages

Four (4) same sized of mass culture, prepared for experiment with same procedure mentioned above for cotton stainer mass culture. Each cage was represented a replication whereas, all treatments i.e. 0ml, 6ml, 8ml, 10ml and 3 of each food-water petri-dishes were collectively installed in a single cage (replication). These collectively installed five (5) treatments in a single cage were replicated four (4) times. In each cage, treatment first, 0ml water was sprayed over the 8-12grm. leaves, in treatment 2nd 6ml, intreatment 3rd 8ml and treatment 4th 10ml water on cotton leaves while, treatment 5th were the food and water petri-dishes. In treatment 5th, each food petri-dish had 2grm. of fuzzy seed, daily 1ml water was sprayed over the seed while, 2-3ml water to each water petri-dish. Food was replaced with three (days) interval. Ten (10) pairs of newly terminated 5th instar stage (adults) were released in each cage (replication) over the five (5) treatments.

2.4 Collection and incubation of eggs

Eggs were collected with the help of fine small brush from the respective treatments below the wet leaves and petri-dishes on moist soil. Daily morning and afternoon, each treatment (mediums) were thoroughly checked for cotton stainer eggs. Glass petri-dish (0.05m diameter) was filled with 12grm. dry sterilized soil while, 2ml water was sprayed over the soil and left 5-10 minutes. Moist soil of the petri-dish pressed with the help of thumb. Eggs were placed inside the petri-dishes and keptin incubator at 30ºC and 50±5% R.H.

2.5. Study on Ecto-Paraistes

Expired adults were daily collected from mass rearing cages and kept as a single individual in glass petri-dish (0.05m diameter). Five (5) samples, i.e. $N_{1,}$ N_2, N_3, N_4 and $N_5$for male and female separately whereas, each sample had further four (4) sub-samples i.e. $N_{1\text{-}5} = n_1 + n_2 + n_3 + n_4$ and each sub-samples was replicated twenty (20) times i.e. $n_{1\text{-}4} = r_1 + r_2 + r_3 \ldots\ldots r_{20}$. All sub-samples replications were inspected under the stereoscope for ecto-parasites infestation on cotton stainer male and female. Means were calculated by the following equations:

1. $n_{1-4} = r_1 + r_2 + r_3 \ldots\ldots\ldots\ldots\ldots\ldots r_{20} / 20$

2. $N_{1-5} = n_1 + n_2 + n_3 + n_4 / 5$

3 Results

Cotton stainer mostly preferred wet leaves sprayed with 10ml water for egg-laying as compared with rest of all provided mediums. It laid total of twenty-eight (28) egg batches recorded below the wet leaves on soil while, mean no. of eggs were (99)with86.0%hatching (Figure 1).

The second most preferable medium for cotton stainer was the food-water petri-dishes. It laid total of (9) egg batches below the petri-dishes on moist soil. Mean no. of eggs/batch was 87 recorded with 92 %hatching (Figure 1).

Cotton stainer laid only single egg batch in each 6ml and 8ml treatments below the wet leaves on soil. Mean no. of eggs/batch and %hatching were 99, 91 with 8ml while, 54 and 81 with 6ml respectively (Figure 1).

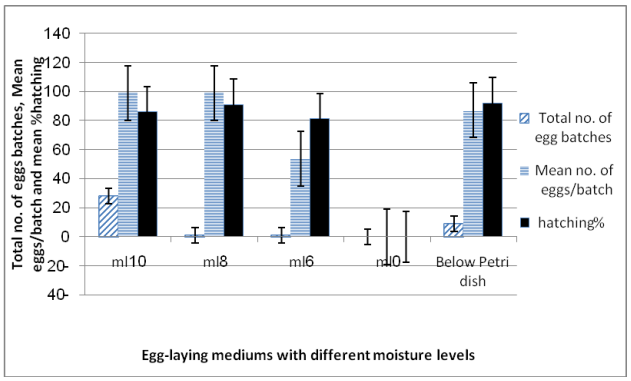

Figure 1: Shows Total No. of Egg Batches, Mean No of Eggs/batch and %Hatching of Cotton Stainer Provided Different Mediums for Egg Laying.

Highest no. of eggs/batch was recorded (252) below the food-water petri-dishes on moist soil followed by (181) below the wet leaves sprayed with 10ml water on moist soil. Lowest no. of eggs/batch was recorded (18) below the food-water petri-dishes on moist soil followed by (22) below the wet leaves sprayed with 10ml water (Figure 2).

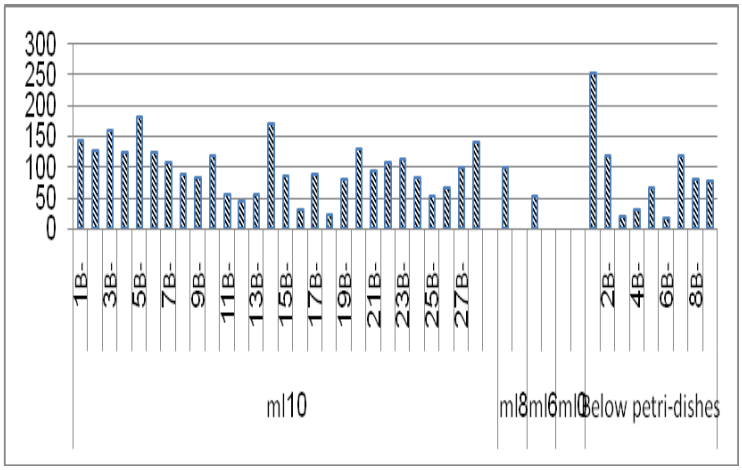

Figure 2: Shows Details of Egg Batches i.e. No. of Eggs/batch Laid by Cotton Stainerunder Different Provided Mediums.

Female cotton stainers were highly preferred by ecto-parasite, the *Hemipteroseius* spp as compared to male cotton stainer. On female cotton stainer, highest parasitism infestation was (22.3) at sample N-5 while, lowest (13.5) in sample N-1 whereas, on male it was highest recorded (17.8) at sample N-5 and lowest (6.5) at sample N-3 (Figure 3).

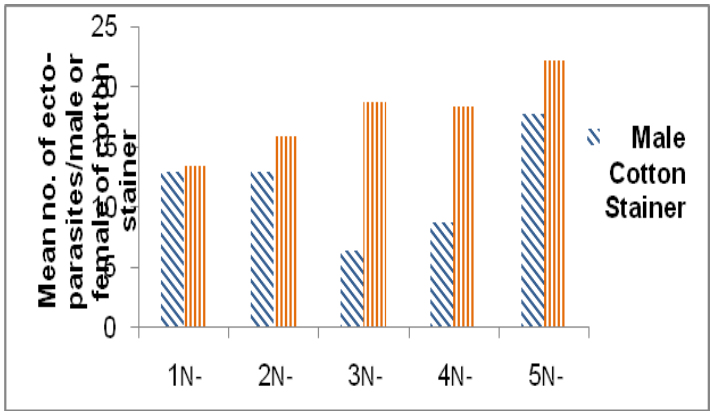

Figure 3: Shows Ecto-Parasite Infestation Levels on Male Verses Female at Five (5) Different Sample

4 Discussion

Verma *et al.,* (2013) reported pale yellow, oval, smooth surfaced eggs of cotton stainer. Jaleel *et al.,* (2013) reported creamy white eggs of cotton stainer which turns toyellowish orange before hatching. Bissdorf (2005) reported cotton stainer eggs in pale color. In our findings, we observed that newly laid eggs were creamy white but, turned orange buff near to hatch. Wilson *et al.,*(2008) reported that cotton stainer females laid eggs in shallow

depressions in the soil under debris or occasionally on the undersides of cotton leaves low in the canopy. Bissdorf (2005) stated that cotton stainer laid eggs in the soil, or under soil and plant debris, or in the cotton plant.

Figure 4: Mating was observed in Mass Rearing Cages. Female was just Ready for Eggs Laying

We provided different mediums for egg laying to cotton stainer. According to our results, cotton stainer preferred highest moist soil for eggs laying. We did not receive eggs in dry and open soil.

Figure 5: Female Cotton Stainer was Laying Eggs in Mass Culture.

They laid eggs below the wet leaves of cotton on moist soil and narrow spaces like between the food and water petri-dishes. We received eggs on all different water levels and food, water petri-dishes in batches (Cluster). During the observations, we did not receive egg singly while, Verma *et al.,* (2013) recorded cotton stainer eggs was singly or in small, loose clusters on the bottom of petri-plate. Wilson *et al.,* (2008) recorded eggs in batches. We recorded highest no. of eggs/batch (18-252) while, (Bhalerao, 1992; Jaleel *et al.,* 2013; Verma *et al.,* 2013 and Bissdorf ,2005) about 7-100 eggs per batch recorded. We recorded highest no. eggs per batch which may be due to different water levels provided on cotton leaves and soil, efficient water provided on fuzzy cotton seed and additionally 2-3ml daily water in the shape of moist cotton wool. Therefore, we highly recommend the rearing method of cotton stainer in lab. conditions with 10ml water sprayed over 8-12 grm. of cotton leaves and small twigs, daily water to fuzzy cotton seed and wet cotton wool on 28±2^{0}C and 65±5% R.H. Varma *et al.,*(2012) recorded average hatching percentage 87.33±3.61 while, we recorded average hatching percentage 70±2.0 on all eggs laid mediums.

Mites inhabiting insects show a great variety and unique types of associations like predatory, parasitic, commensalisms and phoretic. Hunter and Rossanio (1988) opined that insect-mite association may be opportunistic, possibly accidental. Among those, many of the predatory and parasitic mite species can be exploited judiciously for biological control against agri-horticultural and household pests as well as insects of medical importance.

Sarangi *et al.,* (2012) *Hemipteroseius indicus* were found abundantly on *D. koenigii* and its population was found throughout the year with seasonal and monthly fluctuation. Cotton stainer is often seen as infested with one Otopheidomenis mite *Hemipteroseius indicus*, (a new species is currently reported on this host from India is *H. vikrami*as reported byvMenon *et al.,*(2011)generally occurs on porter's body and is known to suck haemo-lymph causing weakening and in some cases even death. It was confirmed during the experiment that highest no. of parasites were recorded on female cotton stainer as compared with mail cotton stainer. Sarangi *et al.,*(2012) in most cases they were found under the wings. However, in case of heavy infestation (where population ranged > 100), the mites were found in other body region also, like,

dorsal surface of thorax as well as near the head; but they are never on the ventral surface. We recorded parasites on live individuals mostly on above the wings, head and abdomen regions.

Figure 6: View of Experimental Cage. Four of Each Food-Water Petri-dishes were Prominent.

Figure 7: Twenty Petri-dishes of cotton stainer which Represented n1= r1+r2+r3+…….r20.

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