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Comparison of the Newly Introduced Rearing Methods of Cotton Stainer, *Dysdercus koenigii* (Hemiptera: Pyrrhocoridae) With Classical Methods

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A B S T R A C T

Cotton stainer, *Dysdercus koenigii* (Hemiptera: Pyrrhocoridae) was reared using four rearing methods including cylindrical perforated plastic bottles (CPPB), cotton seeds in soil (CSS), cotton seeds without soil (CSWS) and integrated rearing technique (IRT) at Lab. maintained temperature 28±2°C along with 70±5% R.H. Data were recorded on five various parameters including number of egg batches, eggs batch,$^{-1}$ shortest longevity, longest longevity and transformed %mortality. Our results based compared their mean difference among the highly significant values to the least significant values of the recorded all parameters. In IRT method, cotton stainer laid significantly higher number of egg batches (6.0) as compared to CSS (2.67), CSWS (2.33) and statistically similar to CPPB (1.33). In IRT, cotton stainer laid maximum (56.00 eggs batch^{-1}). It was significantly higher (24.67) in CSS and statistically similar with difference of (21.67) and (3.33) CSWS and CPPB, respectively. The shortest longevity value (2.33 days) of cotton stainer was observed in CSS method. It was significantly different from the CPPB (8.0 days) and IRT (6.34 days) while, at par with CSWS (2.34 days). Longest longevity was recorded (23.67 days) in CPPB which was significantly (5.34 days) higher in CSS, at par recorded in CSWS (2.34 days) and IRT (1.34 days). Lowest transformed %mortality of was (51.81%) achieved in CPPB. It was recorded (37.62%) and (22.81%) significantly higher in CSS and CSWS respectively. However, it was at par with IRT (8.27%). On the basis of our findings, it was concluded that cotton stainer had shown extreme love for water in all stages for development. The cylindrical perforated plastic bottles had the highest water retention capacity than the other rearing techniques and they sucked the moist seed sap in the same behavior as from the cotton bolls in field. IRT proved most suitable for cotton stainer rearing followed by CPPB.

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SIAS designed the study with the help of MR and IRK. SASS helped in collection and rearing of cotton stainer. IRK analyzed the data with the help of ZH. SIASS wrote the article. THM and RIR helped in preparation of the article.

Key words:
Cotton stainer, *Dysdercus koenigii*, rearing methods, cylindrical perforated plastic bottles, moist cotton seed, integrated rearing technique, fecundity, biocontrol agent.

INTRODUCTION

The cotton having a share of 1.4% in GDP and 6.7% in agriculture value addition is an important source of raw material to the textile industry in Pakistan (PES, 2014). But the cotton is attacked by many chewing and sucking insects (Saeed *et al.,* 2007) causing about 20-40% loss annually (Ahmad, 1999). Recently, cotton stainer, *Dysdercus koenigii* Fabricius (Hemiptera: Pyrrhocoridae), which is commonly known as red cotton bug, has resurged as a destructive pest in cotton zone of Pakistan (Ahmad and Mohammad, 1983; Ashfaq *et al.*, 2011; Jaleel *et al.,* 2013). Both nymphs and adults of the genus *Dysdercus* have strong proboscis, a needle like stylet meant for piercing and sucking of plant sap (Elzinga, 1997; Wilson *et al.,* 2004; Shah, 2014). *Dysdercus* spp. feed on emerging cotton bolls and mature cotton seeds and transmits cotton staining fungus, *Nematospora gossypii* that develops on immature lint and seed (Ahmad and Khan, 1980; Ahmad and Schaefer, 1987; Yasuda, 1992). *Dysdercus koenigii* may insert its proboscis into the soft tissues of cotton bolls and causes an abnormal out-growth (warts) on the inner surface of carpel walls and lesions on the lint (Shah, 2014). This kind of damage on the inner walls of carpels is observed when locules are removed after dissecting the bolls (Wilson *et al*., 2004).

A major reason for increased population of the cotton stainer is its faster egg development (Venugopal *et al*., 1994) and wide spread growing of transgenic cotton that have reduced insecticides application, whereas, before the introduction of transgenic varieties, cotton stainer was not even a minor pest of cotton in Pakistan (Shah, 2014). Due to large scale adoption of *Bt* cotton in the sub-continent sucking pest pressure especially that of *D. koenigii* increased very much especially during 2011 and 2012 (Ashfaq *et al.,* 2011). Cotton stainer caused

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severe losses in 2011 onwards exhibiting bad opening of bolls with stained lint in major cotton growing areas of Pakistan (Shah, 2014).

Some work on the bionomics and life cycle of *D. koenigii* has been reported in India (Kamble, 1971) but information on these aspects of the pest was insufficient under environmental conditions of Pakistan. Insecticides and bio-control agents play important role and were normally more effective on early weaker growth stages of insect pests. Environmentally safe plant protection is a major challenge for sustainable cotton production because, producing high yield products had always been an important part of farming. Chemical control is an essential and sometimes unavoidable component to achieve high yield but, it is important to know and understand the drawbacks of continued insecticide applications. About 90% of the farmers use chemical insecticides (Prayogo *et al*., 2005). It is estimated that in Pakistan, farmers spend US$300 million on pesticides annually, of which more than 80% is being used on cotton, especially for bollworms (Rao, 2007). However, we could minimize the indiscriminate use of insecticides with alternate, environmentally safe control measures technique (Rafiq *et al.,* 2014).

For basic and applied studies like screening of most suitable insecticides, insecticides monitoring on regular basis against insect pests, promotion of biological control of predators and parasitoids, all needs its mass culture comprising different developmental stages. Since large scale rearing of *D. koenigii* was practically difficult therefore, we conducted experiments on different rearing techniques under laboratory conditions to find out the most suitable, easiest and economical rearing method for sufficient rearing of the pest.

MATERIALS AND METHODS

Mass culturing in the Lab.

Adults of cotton stainer, *Dysdercus koenigii* were collected during November, 2013 from cotton fields of Multan District and were kept at biological control laboratory. Plastic made general mass rearing cages measuring 30x18x28 inches (LxWxD) and having three aerations holes (4x6 inches each) covered with fine mesh were used to rear cotton stainer. About one inch layer of field soil with minute quantity of sand was spread on floor of cages to provide natural substrate. Insecticide free delinted seed of *Bt* cotton variety CIM-599 in a plastic petri-dishes with filter paper discs underneath were offered as a food along with fresh reproductive plant parts taken from field and green house. Lab. temperature was maintained at 28±2ºC along with 70±5% R.H. and 11:13 hours (L:D) to get eggs of the pest. Fifth instar nymphs were collected from the bulk and kept in separate general mass rearing cages for further development. Upon maturity to adult stage, ten adult pairs randomly selected for each replication while each treatment was replicated three times.

Rearing techniques

Selected cotton stainer pairs were released in the plastic experimental rearing cages measuring 15x9x15 inches (L x W x D) having two aerations holes (3x3 inches each) covered with fine mesh. A substratum used was one inch thick layer of field-soil mixed with minute quantity of sand and spread on the floor of cages. The following treatments were installed in respective cages after releases to evaluate mean number of egg batches, eggs batch,$^{-1}$ shortest longevity, longest longevity and %mortality for comparison of different classical methods with new introduced rearing technique.

Cylindrical perforated plastic bottles (CPPB)

Nine CPPB (three for each replication) of three inches length with 1.90 inches diameter while, twenty-four holes drilled to each plastic bottle homogeneously all around and stuffed with wet surgical cotton wool. Delinted cotton seeds were inserted randomly in twelve holes while rests were left empty for moisture purpose (Fig. 1). Each perforated bottle was placed inverted in plastic petri-dishes (2 inches diameter). Few dried cotton leaves were kept near each perforated bottle like (Fig. 4) for egg laying purpose. Daily 10ml water was evenly sprayed twice daily (morning and evening hours) over the perforated bottles and soil of the cages including the dried leaves. Seeds were replaced with three days intervals.

Cotton seed in soil (CSS)

Nine plastic petri-dishes were used each having a filter paper disc inside with field soil above and 12 moist delinted cotton seeds on the top (Fig. 2). Each experimental cage had three petri-dishes whereas, no dried leaves were provided and daily 10 ml water was evenly sprayed twice a day (morning and evening hours) over the petri-dishes and soil of the cages. Seeds were replaced with three days intervals.

Cotton seed without soil (CSWS)

This set included nine plastic petri-dishes (three for each replication) filled with moist cotton wool having a filter paper disc above and 12 delinted cotton seeds on the top (Fig. 3). No dried leaves were provided and daily 10 ml water was evenly sprayed over the petri-dishes and soil of the cages twice a day. Seeds were replaced with three days intervals.

Fig 1. Cylindrical perforated plastic bottle (CPPB) filled with moist cotton wool and single cotton seed **was** inserted to each twelve holes of the bottle.

Fig. 2. Cotton seeds in soil (CSS) *i.e.* a filter paper disc inside the petri-dish while, field soil above and 12 moist delinted cotton seeds on the top of the soil.

Integrative rearing technique (IRT)

In this treatment, cotton stainers were reared in combination of all above mentioned three treatments and were termed as Integrated Rearing Technique (IRT). Each experimental cage had a single perforated bottle, petri-dishes with and without soil (Fig. 4). Dried leaves were provided near the perforated bottle and daily 10ml water was evenly sprayed over perforated bottle, petri-dishes, soil of the cages and dried cotton leaves. Seeds were replaced with three days intervals.

Fig. 3. Cotton seed without soil (CSWS) *i.e.* filled with moist cotton wool having a filter paper disc above and 12 delinted cotton seeds on the top of the filter paper.

Fig. 4. View of Integrated Rearing Technique (IRT) method *i.e.* combination of CPPB, CSS and CSWS.

Statistical analysis

Experiment was planned and laid out using Completely Randomized Design (CRD). Data were recorded on five parameters including mean number of egg batches, mean eggs batch^{-1} mean shortest longevity, mean longest longevity, and mean %mortality. The %mortality was calculated by dividing the number of dead cotton stainers per replicate with total number of releases per replicate. Data collected on afore mentioned parameters were analyzed using Analysis of Variance ANOVA with Tukey's HSD (Honestly Significant Difference) post hoc test. The %mortality before the analysis was arcsine transformed in degrees using the following expression presented by Gomez and Gomez (1984).

$$\arcsin(\sqrt{percent\ mortality} \times (180/\pi)$$

However, before transformation all the 100 values were replaced by using the following formula,

$$(100-(1/4n))$$

where n is the number of insects released, whereas in our case $n=20$.

RESULTS

Table I shows comparison of egg batches and number of eggs/batch laid, longevity and mortality of cotton stainer using different rearing techniques.

Egg batches laid

In the IRT method, the cotton stainer laid (6.00) significantly higher mean number of egg batches as compared to CSS (2.67), without soil (2.33) and statistically similar to CPPB (1.33) (Table I). The coefficient of variation (CV) for the data on mean number of egg batches was 16.01% and P = 0.0069 while, in the CPPB method they laid statistically similar mean number of egg batches (1.34) as compared to CSS method and CSWS (1.0 egg batch).

Number of eggs batch^{-1} laid

In IRT method, cotton stainer laid 56.00 mean number of eggs batch^{-1}. It was significantly higher 24.67 mean number of eggs batch^{-1} than in CSS and statistically similar with difference of 21.67 and 3.33 cotton CSWS and CPPB methods respectively (Table I), having C.V. = 20.21% and P = 0.0186. In perforated bottle method they laid 52.67 eggs batch^{-1} with difference of 21.34 and 18.34 respectively from CSS and CSWS methods (statistically at par).

Longevity

The cotton stainer's mean shortest longevity 2.33 days was recorded in CSS method. A total of 10 pairs of the cotton stainer were released in each replication of the four rearing methods. The mean shortest longevity value of cotton stainer in CSS method was significantly different from the CPPB (8.0 days) and IRT (6.34 days) (Table I) having C.V.=37.16% and P = 0.0132; while the value was statistically at par with CSWS (2.34 days). The CPPB was statistically similar to IRT and CSWS method respectively with a difference of 1.66 and 5.66 mean numbers of days.

Longest longevity of cotton stainer was recorded 23.67 days in CPPB. It was significantly 5.34 days higher in CSS method while, statistically at par recorded in CSWS 2.34 days and IRT methods 1.34 days (Table I), having C.V.=5.39% and P = 0.0028. In IRT longest longevity of cotton stainer was 22.33 days reported. It was significantly 4.00 days higher from the treatment of CSS while statistically at par 1.00 days with CSWS method.

Mortality (%) of cotton stainer

Lowest transformed percent mortality of cotton stainer was 51.81 achieved in CPPB method. It was recorded 37.62% and 22.81% significantly higher in CSS and CSWS methods respectively than the perforated bottles (Table I), having C.V.= 10.16% and P = 0.0008. However, it was statistically at par with IRT method (8.27%). In IRT, cotton stainer transformed percent mortality was 60.08% recorded which is significantly 29.35% lower from cotton seed in soil 29.35% while, statistically similar 14.54% in CSWS treatment.

DISCUSSION

Lab. temperature and humidity

Muthupandi *et al.* (2014) reared and maintained *D. cingulatus* adults under lab. conditions of 31±10°C, 75±5% R.H. and 11:13 hours (L:D). Jaleel *et al.* (2013) reared and maintained adults of *D. koenigii* at 28±2°C, 70±5% R.H. and 11:13 hours (L:D). Varma and Patel (2012) reared *D. koenigii* at an average room temperature of 25.50±7.36°C and average relative humidity of 55.19±21.36%. Sahayaraj and Jeeva (2012) maintained *D. cingulatus* in the laboratory at 28±2°C and 70±5% R.H. Sahayaraj and Ilyaraja (2008) were collected *D. cingulatus* adults and nymphs from cotton fields and were maintained under laboratory conditions 27±2°C, 70±5% R.H. and 11:13 (L:D).

Similarly, we maintained our lab temperature at 28±2ºC, R.H. at 70±5% and 11:13 hours (L:D). Cotton stainers are reported to be sensitive to changes in temperature and humidity (Jaleel *et al*., 2013). It was re-confirmed and keenly observed in rearing process that cotton strainers are very much sensitive to changes in temperature and humidity. Very few of them were survived for egg laying in general mass rearing cages when brought a bulk of cotton stainer adults from field to lab. Fluctuations in the temperature and humidity adversely affected its population. Low mortality of cotton stainer was observed in mass reared from eggs laid of field collected population of cotton stainer in general mass rearing cages. Our results revealed that highest number of eggs and egg batches were found in the IRT

Table I.- Eggs batch,-1 shortest longevity, longest longevity and %mortality of different rearing techniques.

Parameters	Rearing methods				Overall M±SE
	CPPB	CSS	CSWS	IRT	
Egg batches	4.67±0.33ab	3.33±0.33b	3.67±0.33b	6.00±0.58a	4.42±0.36
Eggs batche^{-1}	52.67±5.55ab	31.33±4.67b	34.33±3.28ab	56.00±6.66a	43.58±3.95
Longevity shortest	10.33±1.45a	2.33±1.20b	4.67±0.88ab	8.67±1.86a	6.5±1.12
Longest	23.67±0.67a	18.33±0.88b	21.33±0.67ab	22.33±0.33a	21.42±0.66
Mortality (%)	51.81±2.63c	89.43±0.00a	74.62±7.41ab	60.08±1.92bc	68.98±4.66

Lettering is done using Tukey's HSD test at 5% level of significance.
CPPB, cylindrical perforated plastic bottle; CSS, cotton seed in soil; CSWS, cotton seed without soil; IRT, integrated rearing technique.
Eggs batch,$^{-1}$ shortest longevity, longest longevity and %mortality of different rearing techniques.

Table II.- Eggs batch,-1 shortest longevity, longest longevity and %mortality of different rearing techniques.

Parameters	Rearing methods			
	CPPB	CSS	CSWS	IRT
Egg batches	4–5	3–4	3–4	5–7
Eggs batch^{-1}	44–63	24–40	24–40	47–69
Shortest longevity	8–13	0–4	3–6	5–11
Longest longevity	23–25	17–20	17–20	22–23
Mortality (%)	47.87–56.79	89.43–89.43	89.43–89.43	56.79–63.44

followed by CPPB; while highest survival period of cotton stainer was found in CPPB followed by IRT at 28±2^{o}C along with 70±5% R.H. and 11:13 h (L:D).

Mass rearing of cotton stainer in the lab.

Muthupandi *et al.* (2014) reared *D. cingulatus* in plastic containers of 20 inches diameter on moist *Gossypium arboretum* L. seed. Jaleel *et al.* (2013) reared *D. Koenigii* in plastic chamber 4x4 inches on soaked fuzzy cottonseeds of MNH-886 (*Bt*). The plastic chambers were half filled with sterilized soil as natural medium for oviposition. Filter paper was placed on the soil to maintain moderate moisture level in the plastic chamber. Nymphs were collected from the field and confined to plastic jars with filter paper on the bottom and mouth closed with muslin cloth. Fresh seeds of okra were provided at two days interval until adult emergence (Verma *et al.,* 2013). Sahayaraj and Jeeva (2012) reared *D. cingulatus* on water soaked cotton seeds and fresh cotton leaves in plastic container. Sontakke *et al.* (2013) were reared *D. cingulatus* on moist cotton seed in glass bottles covered its mouth with muslin cloth and rubber bands. Sahayaraj and Ilyaraja (2008) reared *D. cingulatus* in plastic containers 20x10x15 inches on soaked cotton seeds.

We reared *D. koenigii* in plastic cages measuring 30x18x28 inches (LxWxD) having three aeration holes (4x6 inches each) covered with fine mesh on delinted moist cotton seed and fresh reproductive parts of cotton plant in a general mass rearing; while for experimental purpose, they were reared in 15x9x15 inches (LxWxD) having two aeration holes (3x3 inches each) covered with fine mesh called the plastic experimental rearing cages. About one inch ticked layer of field-soil was spread over the cage floor with minute quantity of raw sand in both the general mass and experimental rearing cages. In plastic experimental rearing cages (Figs. 1, 2, 3) moist seed of CIM-599 (*Bt*) were provided in different mediums. Cotton seed used for stock feed is an important alternate source of food for cotton strainers (Wilson *et al.,* 2008). On the basis of above mentioned results, we therefore recommend that the most efficient commercial mass rearing methods for *D. koenigii* are the IRT and CPPB.

Fecundity

Dysdercus cingulatus lays eggs singly or in small, loose clusters on the bottom of petri-dish (Verma *et al*., 2013). *D. koenigii* laid eggs singly or in clusters in sand, soil, or on plants (Jaleel *et al*., 2013). In our findings, *D. koenigii* preferred to lay eggs in the moist soil under plants debris or a narrow hide moist place. Based upon our results, we will strongly recommended that for commercial rearing of *D. koenigii* moist soil and plant debris for cotton stainer egg laying medium are the best one. A single female may lay 10-50 eggs, scattered on the provided substrate (Ahmad and Mohammad, 1983). Fecundity of female ranged between 55-56 eggs per female (Verma *et al*., 2013). *D. koenigii* laid eggs varied from 39-183 (Jaleel *et al*., 2013). The fecundity of red cotton bug was 115 eggs per female (Verma and Patel 2012). Likewise, we found in our study the ranges of eggs per batch as 12-105, 19-91, 13-77 and 18-62 with respective average of 55.0, 52.0, 31.0 and 34.0 eggs per batch respectively in IRT, CPPB, CSS and CSWS rearing methods,. We collected eggs mostly from the moist soil and under moist dried leaves in the rearing cages.

Longest and shortest longevity

Survival period of *D. koenigii* adults was 20.85±6.12 days (Jaleel *et al.,* 2013). *D. cingulatus* longevity of adults was 20-24 with an average of 21.6±1.81 days (Varma *et al.*, 2013). The average longevity of *D. koenigii* was 22.33±1.44 days. Whereas, the cotton stainer adults survived for 25, 23, 22 and 20 days, respectively in the CPPB, the IRT, the CSWS and the CSS rearing methods.

Mode of damage

Cotton stainer had been declared as one of the most destructive cotton pest in other parts of the world (Sprenkel, 2000). Most of its nymphal stages as well as its adults feed on the seeds of cotton bolls. Shah (2014) in his study had observed very keenly its mode of damage. Both nymphs and adults of this insect have a very strong proboscis. They insert and used proboscis up and down and sucked the sap seeds from inside the cotton boll. We found as cotton strainers efficiently responded in the perforated bottles method because they inserted proboscis with up and down movement in the holes of perforated bottles and sucked sufficient sap from the seed in seed inserted holes and moisture from wool of empty holes of the perforated bottles.

CONCLUSION

On the basis of above mentioned findings, it was concluded that cotton stainers extremely love moisture (mesophylic). The perforated bottles had the highest moisture retention capacity than the other rearing techniques and cotton stainer suck the cell sap from the moist cotton seed in the same behavior when it insert its proboscis into cotton boll in the field, reached to the seed, sucked the sap from the seed with frequent upward and downward moment of the proboscis. It means that provision of same feeding behavior can greatly matter in the nourishment of insect especially the cotton stainer. We therefore, highly recommend the IRT and CPPB methods for rearing of the cotton stainers particularly for commercial rearing of the insect on biological control aspects.

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