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Survival of Lac Insects (*Kerria lacca* Kerr.) on *Cajanus cajan* (L) Millsp.

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A B S T R A C T

Growth and health of plants depends on the health substrate or soil, while growth and survival of phytophagous insects depends on the health of its host plants. Similarly, growth and survival of phloem feeders, including Lac insects (*Kerria lacca*) depends on the quality and quantity of phloem sap access to it. *K lacca* produce natural resin which is a cash crop and commodity of export. Commercially, *K lacca* is reared on the naturally standing trees of *Butea monosperma*, *Zizyphus mauritiana* and *Schleicheria oleosa*. But when it is reared on cultivated annual shrubby hosts like *Cajanus cajan*, soil health matters. In this context, a field experiment was conducted by rearing *K. lacca* on *C. cajan* grown on different substrates. The data reveals that the substrate combination of *Kapu* (45kg) + FYM (20kg) + *T. viride* was the best as it produced highest lac (288.33g) and seed yield (838.33g) of *C. cajan*. The survival percent of Lac insect from brood lac inoculation to the harvest of lac crop was highest (41.83%) on *C. cajan* grown on *Kapu* (45kg) + FYM (20kg) + *T. viride*. The study indicates the substrate play a major role in the growth of the host plants and survival of *K. lacca.*

Introduction

Survival and growth of insects depends on the availability of food (McGuinness, 1987; Gogi *et al.*, 2012) and protection in an ecosystem (Yumamura *et al.*, 2006; Ayers *et al.*, 2009), quality (Gimnig *et al.*, 2002) and quantity (Okech *et al.*, 2007)) of the food is essential for the growth and survival of insects. Among herbivores, there are foliage feeders (Douglas, 2003), phloem feeders (Kehr, 2006), stem borer (Heinrichs *et al.*, 2004) fruit borers (Thomas, 1992) and even root feeders (Potter *et al.*, 2002).

Lac is secreted by *Kerria lacca* Kerr. (Colton, 1984) while feeding on the phloem sap (Kehr. 2006; Kaushik *et al.*, 2012) of the host range of over 250 plants (Roonwal *et al.*, 1958; Sharma). Commercially *K. lacca* is reared on *Butea monosperma* (Sharma et al, 2015) *Zizyphus mauritiana* (Shah *et al.*, 2014,

Namdev, 2015), *Schleicheria oleosa* (Roonwal, 1962), *Flemingia semialata* (Kumar and Rani, 2019) as well as *Cajanus cajan* (Thomas, 2003). Phloem is an important mediator of whole plant communication (Ruiz-Mrdrano *et al.,* 2001). Plant nutrients are translocated through phloem tissue as phloem sap (Douglas, 2003). The quality and quantity of phloem sap influences the growth and survival of phloem feeders (Cisneros and Godfrey, 1999) like *K. lacca* (Mengel and Kirkby, 1987). Lac production is also dependent on the phloem sap accessible to Lac insect. Earlier workers have studied Lac production in context to Lac basal application of nutrients (Namdev *et al.,* 2016, Shah *et al.,* 2014) and foliar application (Sharma *et al.,* 2015; Gurjar *et al.,* 2016; Ghugal *et al.,* 2016). Thus, the present field trial conducted to study the survival of Lac insect on *C. cajan* growth on different combination of substrate treated with soil microbes.

Materials and Methods

Survival of *K. lacca* on *C. cajan* grown on different substrate combination was evaluated in field trial in Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh. The field trial was conducted from June 2018 to June 2019.The topography of the experimental area was fairly uniform. The Randomised Block Design experiment had seven treatments and three replications of *C. cajan* variety TJT- 501. Plant to plant and row to row spacing was 6ft x 6ft. There were three plants per replication in of each of the seven treatments (Table 1).

Weather: The weather of Jabalpur region is typically Sub humid, characterised by hot dry summer and cool dry winter. It is favourable for the growth and development of *C. cajan*. The total rainfall received during the crop season was 1162.90 mm, in 58 rainy days from July 2018 to last week of May 2019. The mean daily temperature ranged from minimum 4.80^{0}C to 24.90^{0}C and maximum 28.5 to 41.80 ^{0}C during the year 2018-2019.

Substrate: The seedlings of *C. cajan* was transplanted in used polypropylene bags (PPB) of size 93cm x 61cm filled with substrate (Patent appl.no.201921005340A). There were three types or combination of substrates used for the present field experiment. Substrate- S_1 consisting of a mixture of 45 kg river bed basin soil (*Kapu*) and 20kg well rotten Farmyard manure (FYM) + *Trichoderma viride*), Substrate-S_2 consisted45kg (FYM) + *Trichoderma viride*) and Substrate-S_3 was 65 kg (Table 3) river bed basin soil (*Kapu*) + *Trichoderma viride*). The *Kapu* and FYM in the above ratio was thoroughly mixed with the help of a spade to obtain a homogenised substrate.

The physio-chemical property of the substrate is mentioned in the table (3). The substrate was gradually filled into the PPB with help of a *tasala* followed by constant shaking the bag to ensure proper settlement and compactness of the substrate in it. The 65 kg substrate filled PPB attains a dimension of 46 cm height and 125 cm circumference. The PPB was filled with substrate on the designated spot in the layout of the experiment for clarity.

Nursery raising of sapling

Nursery of *C. cajan* was raised in the month of May 2018 in the substrate (*Kapu* + FYM) filled perforated polythene bag (18 x 16 cm) by sowing seeds treated with *T. viride*. Polythene bags were irrigated at weekly intervals. The seedlings were sprayed with insecticides to prevent insect pest incidence. The growing tips of the seedlings were nipped at 10-12 days interval till its transplantation.

Transplantation of *C. cajan* saplings

C. cajan seedling on attaining a height varying from 1.5 feet to 2 feet were transported to the main field. Each of the 63 seedlings were placed at the base of substrate filled PPB.

The polythene bag of the *C. cajan* seedling was carefully removed without disturbing its root system of the seedling. The seedling with substrate base was carefully transplanted in the PPB and pressed tightly from all corners, followed by watering. The transplantation was done in the evening hours of 15thAugust 2018.

Brood Lac inoculation (BLI)

Rangeeni brood Lac purchased from M/s Adarsh Lac Samiti, Jamankhari village, Tehsil Barghat, District Seoni, Madhya Pradesh, on 03.11.2018. The brood Lac was sorted for predators free and good quality before inoculation on *C. cajan*. Brood Lac stick weighing 15 g was tied at the base of each *C. cajan*in the PPB on with the help of a twine as per the treatments scheme of the experiment.

Lac insect count

Live Lac insects were counted per 2.5 cm^2 (2.5cm length and 1.0cm width) space marked on the Lac insect settled branches of *C. cajan*.

Marking of slot

Usually by 30 days of BLI, majority of the nymph of *K. lacca* leaves the brood Lac and settles on the succulent branches of the host plant. Lac insect inserts its stylets into the phloem tissue and becomes sedentary. Thus, thirty days after BLI, branches with good Lac insect settlement were randomly selected for marking of slot. A slot of 2.5cm^2 (1cm x 2.5cm) was marked on the bark of the branch bearing good settlement of the Lac insects. Three such slots were made on each plant. Each slot was designated as S_1, S_2, and S_3. Stretching a thread between the index fingers of both the hands, the insects settled adjacent to the boundaries of the slot was carefully removed to make the slot clearly differentiate from the rest of the Lac insect settlement on the branch.

Digital recording

The crawlers of Lac insect are initially about 0.5 mm in size. It is difficult to count from a small area of 2.5cm^2 slot. And if the size of the slot is increased, there will be again difficulty in counting more insects per slot. Therefore the Lac insect settlement within the slot was digitally photographed with the help of a Digital Single Lens Reflex (DSLR) camera fitted with 100 mm micro lens by settling it in manual mode with ISO 400 and shutter speed of 4.5 to 6. Several pictures of the slot was taken for clarity, finally the best click is selected.

Digital counting

The selected sets of best digital images from the DSLR camera were transferred to the Laptop, with the help of memory card reader. The images were opened in the "Paint 3D" programme of the Microsoft Office 10 (Plate). After enlarging the image on the screen of the Laptop, the "Brush tool" on the Tool bar of the Paint 3D programme was selected. This was followed by selecting the thickness point of the Calligraphy pen from 1 x to 18 x as well as the contrast colour of the Brush tool.

Placing the cursor on the individual Lac insect within the slot on the image displayed on the computer screen, when a left click of the mouse is done, a dot of the selected thickness and colour appears on the image of the selected insect in the screen. The process was

followed till all the Lac insects in the slot had a dot on it. All the dots were then counted and recorded, followed by saving the image in a designated folder after renaming it, for retrieval in future (Patent appl.no.201921007852A).

Frequency of Lac insect count: Counting of Lac insects within the slots was done on 65th, 95th, 125th, 155thand 185thday after BLI.

Emergence of male Lac insects: The date of emergence of adult male Lac insects as well as duration of its presence on the Lac insect settlement was recorded.

Flower removal: Flower removal was carried out in the respective treatments at flower initiation phase (i.e.90 DAT).

Pod removal: Pod removal from the *C. cajan* in the specific treatments of the experiment, was done at pod initiation stages. (i.e.120DAT). Young pods were removed before grain formation filling stage during the growth season.

Data analysis: Data of the parameters were analysed. The significance among different treatment means was judged by critical difference (C.D) at 5% level of significance for comparison, for which the marginal means of each treatment was considered. The following formula was used for various estimations.

Standard error of mean SEm± = $\sqrt{\frac{EMS}{r}}$

Critical difference (C.D.) = SEm± x √2 x t 0.05

where, EMS = error mean sum of square, t = 't' value at 5 % level at error d.f., r = number of replications, SEm ± = standard error of any treatment mean, CD = Critical difference.

Results and Discussion

Mean live Lac insects (MNL) per 2.5cm^2 on 65th day after BLI (07.01.2019)

On 65th day of BLI the MNL per 2.5 cm^2 of branches was maximum (211.61) in T5 (*C.cajan* grown on S_3 with Lac insects and hand picking of mature pods), while it was minimum (147.00) (Table 2) in T1 (*C. cajan* grown on S_1 with Lac insects and hand picking of mature pods). At 65th day of BLI, plants were in its growth stage, there was no flower bud initiation at this stage of the crop. Though T5 was significantly highest over T1, but was at par with rest of the treatments.

Counting of live Lac insects in a unit space of 2.5cm^2 (2.5cm long and 1.0cm width) space on the Lac insect settled branches is considered as a standard as suggested by earlier workers viz., Vajpayee *et al.,* (2019) Khobragade, (2010), Rathore, (2011), Patel, (2013), Bhalerao, (2013), Shah *et al.,* (2014), Sharma *et al.,* (2015), Gurjar, (2016), Ghugal *et al.,* (2016), Namdev *et al.,* (2015), Jhanghel (2014), Mohanta *et al.,* (2014), Kalahal *et al.,* (2017), Sharma *et al.,* (2018).

However, Hazarika *et al.,* (2018) has reported Lac insect count from 1cm^2. Counting of larvae insects of size varying from 0.2 to 0.5mm within a small space of 1 cm is extremely difficult in field condition; while from 2.5cm^2 is quite comfortable.

MNL per 2.5cm^2 on 95th day after BLI

On 95th day of BLI the MNL per 2.5 cm^2 of branches was again maximum (155.33) in T5 but it was minimum (119.78) in T3 (*C. cajan* grown on S_1 with Lac insects and removal of flowers). It was significantly highest in T5 over T3. However, rest of the treatments was at par with each other.

MNL per 2.5cm^2 on 125th day after BLI

On 125th day of BLI the MNL per 2.5 cm^2 of branches continuedto be maximum (143.39) in T5 but it was minimum (104.00) in T2 (*C.cajan* grown on S_2 with Lac insects and hand picking of mature pods) on 125th day of BLI. It was significantly highest in T5 over T3. However, rest of the treatments was at par with each other.

MNL per 2.5cm^2 on 155th day after BLI

On 155th day of BLI the MNL per 2.5 cm^2 was highest (109.67) in T5 and lowest (81.72) in T2.It was significantly highest in T5 over T2. However, rest of the treatments it was at par with each other.

MNL per 2.5cm^2 on 185th day after BLI

On 185th day of BLI the MNL per 2.5 cm^2 of branches was highest (71.72) in T6 (*C. cajan* grown on S_1 with Lac insects and removal of young pods) and lowest (51.39) in T2 (*C.cajan* grown on S_2 with Lac insects and picking of mature pods). There was significant difference in the MNL per 2.5 cm^2 in all treatments over T2.

The MNL in different treatments is reported to vary from 60 to 95.80 (Patel, 2013), 28.13 to 40.53 (Jhanghel, 2013), 51.35 to 64.08 (Namdev, 2014), 38.31 to 43.37 (Gurjar, 2016), 37.05 to 39.34 (Kumar *et al.,* 2017) and 57.48 to 64.08 (Shah *et al.,* 2018). During different growth period the MNL varied in the previous studies as there was a variation in the host, season, Lac insect strain, location and treatments in their studies. However, these studies indicate a universal decline in the trend of the MNL per 2.5cm^2 during its growth phases in comparison to that occurred at BLI. Thus the MNL per 2.5cm^2 continuously decreased from 65th days of BLI to 185thdayof BLI, irrespectively of the treatments except T4 (with no Lac insect) the mean percent of MNL at 185th day over 65th day of BLI was highest (41.83%) in T1 while it was least (31.72%) in T2. Lac insects were protected from parasites and predators by the application of contact insecticides. Thus, the loss of the Lac insect may be due to its natural death.

Survival of Lac insects at different treatments

We have to understand that among many of the deciding factors for the Lac crop productivity, the percent survival of Lac insects from the BLI to harvest is one of the important factors. It is because Lac is produced by female Lac insects. Now, there are again different substrates that can play a vital role in Lac production. The emergence and presence of male Lac insects, in the Lac ecosystem is one sub factor while another sub factor is the loss in the number of Lac insects before and after emergence of the male Lac insects. This matter though be discussed later, but the data must be looked with that perspective.

The percent survival of Lac insects is reported here is in three phases of Lac insect growth after BLI. The duration between 65th and 125th day of BLI is usually larval growth and pupal period of the *Baishakhi* crop of *Rangeeni* Lac insects. Male emergence and mating takes place between 125thand 155th day of BLI while the major Lac secretion phase is after mating of female Lac insect i.e., 155thday onwards in case of *Baishakhi* crop of *Rangeeni* Lac. Between 65th to 95th day after BLI, the maximum survival of Lac insects was 86.24 percent in T_1 (*C. cajan* grown on S_1 with Lac insects and hand picking of mature pods) followed by 84.03 percent in T7(*C. cajan* grown on S_1 with Lac insects and only one picking of mature pods followed by removal of flowers).

Table.1 The details of the treatments and notations used are as below

Treatment number	Treatment details
T_1	*C. cajan* grown on S_1 with Lac insects with hand picking of pods
T_2	*C.cajan*grown on S_2 with Lac insects with hand picking of pods
T_3	*C.cajan* grown on S_1 with Lac insects and removal of flowers
T_4	*C.cajan* grown on S_1 without Lac insects with hand picking pods
T_5	*C.cajan* grown on S_3 with Lac insects with hand picking pods
T_6	*C. cajan* grown on S_1 with Lac insects and removal of young pods
T_7	*C. cajan* grown on S_1 with Lac insects and only one hand picking of mature pods followed by removal of flowers

Table.2 Mean number of live Lac insects settled per 2.5cm^2 on the *C. cajan* branches under different treatments

Treatments	Mean no. of Lac insects settlement per 2.5cm^2 on days after BLI					
	65 Days	95 Days	125 Days	155 Days	185 Days	Survival %
T_1-*C. cajan* grown on S_1 with Lac insects and hand picking of mature pods	**147.00** (12.07)	**126.78** (11.22)	**107.28** (10.37)	**86.33** (9.30)	**61.50** (7.87)	41.83
T_2- *C.cajan* grown on S_2 with Lac insects and hand picking of mature pods	**162.00** (12.70)	**123.11** (11.11)	**104.00** (10.20)	**81.72** (9.05)	**51.39** (7.20)	31.72
T_3- *C.cajan* grown on S_1 with Lac insects and removal of flowers	**163.72** (12.73)	**119.78** (10.90)	**106.39** (10.28)	**87.00** (9.31)	**62.89** (7.94)	38.41
T_4- *C.cajan* grown on S_1 without Lac insects and hand picking of mature pods	**0.00** (0.71)	**0.00** (0.71)	**0.00** (0.71)	**0.00** (0.71)	**0.00** (0.71)	0.00
T_5- *C.cajan* grown on S_3 with Lac insects and hand picking of mature pods	**211.61** (14.55)	**155.33** (12.47)	**143.39** (11.98)	**109.67** (10.49)	**71.61** (8.49)	33.84
T_6- *C. cajan* grown on S_1 with Lac insects and removal of young pods	**184.89** (13.45)	**150.28** (12.20)	**130.78** (11.40)	**102.06** (10.10)	**71.72** (8.49)	38.79
T_7- *C. cajan* grown on S_1 with Lac insectsand only one hand picking of mature pods followed by removal of flowers	**151.72** (12.27)	**127.50** (11.26)	**116.67** (10.77)	**90.28** (9.49)	**63.44** (7.97)	41.81
SE(m)±	**0.63**	**0.50**	**0.44**	**0.34**	**0.20**	
CD at 5%	**1.94**	**1.56**	**1.38**	**1.05**	**0.62**	

Figure in parenthesis are transformed value $\sqrt{x+0.5}$

Table.3 Physico-chemical properties of the substrate (65kg) filled in Poly propylene bag (PPB)

Parameters	Value (g/65kg substrate)	Method used
Available N	136.15	Alkaline permanganate method (Subbiah and Asija,1956)
Available P_2O_5	45	Calorimeter method (Olsen et al.,1954
Available K_2O	304	Flame Photometer method (Chapman and Pratt, 1961)

Table.4 Percent of loss in the MNL Lac insects per 2.5cm^2

Treatments	Percent of loss in MNL per 2.5 cm^2 at different periods after BLI			
	65th to 95th Day	65th to 125th Day	65th to 155th Day	65th to 185th Day
T_1- *C. cajan* grown on S_1 with Lac insects and hand picking of mature pods	13.76	27.02	41.27	58.16
T_2- *C.cajan* grown on S_2 with Lac insects and hand picking of mature pods	24.01	35.80	49.55	68.28
T_3- *C.cajan* grown on S_1 with Lac insects and removal of flowers	26.84	35.02	46.86	61.59
T_4- *C.cajan* grown on S_1 without Lac insects and hand picking of mature pods	-	-	-	-
T_5- *C.cajan* grown on S_3 with Lac insects and hand picking of mature pods	26.59	32.24	48.18	66.16
T_6- *C. cajan* grown on S_1 with Lac insects and removal of young pods	18.72	29.27	44.80	61.21
T_7- *C. cajan* grown on S_1 with Lac insectsand only one hand picking of mature pods followed by removal of flowers	15.96	23.11	40.50	58.18

The lowest percent (73.40%) survival of Lac insect during this period was in T5 (*C.cajan* grown on S_3 with Lac insects with hand picking of mature pods). In comparison to the MNLbetween65th and 95th day after BLI the maximum survival of Lac insects between the 65th and 125th day period was 76.89 and 72.97 percent in T7 (*C. cajan* grown on S_1 with Lac insects and only one hand picking of mature pods followed by removal of flowers) and T1 (*C. cajan* grown on S_1 with Lac insects and hand picking of mature pods) respectively. The lowest percent (64.19%) survival of Lac insects during this period was in T2 (*C.cajan* grown on S_2 with Lac insects and hand picking of mature pods).

However between 65th to 155thday the mean percent survival of Lac insects varied from 50.44 to 59.50 percent. Similar, the mean survival percent of Lac insects between 65th and 185th day insects was still higher ranging from 31.72 to 41.83 percent.

The percent loss of MNL during 65thto 185th day period was lowest (58.16 and 58.18%) in T1 and T7 while highest loss of 68.27 and 66.15 percent was in T2 and T5 respectively.

Loss of Lac insect during its growth stages is common and reported by previous workers viz., Vajpayee, (2019), Khobragade, (2010), Patel, (2013), Jhanghel, (2013), Bhalerao, (2013), Namdev, (2014), Ghugal, (2015), Gurjar, (2016), Sahu, (2016) and Shah *et al.*, (2018). The percent loss of Lac insect from BLI to maturity or harvest varies and depends on the various conditions. The mean percent Lac insect loss ranged from 7.62 to 16.83 percent (Khobragade, 2010), while according to Patel, (2013) it ranged from 69.14 to 74.52 percent (Table 4).

In conclusion the lac insects are phloem feeders and they continuously feed on stem of the host crop. Therefore the host plant to be more vigorous in growth and healthy. So in present study used different substrate combination with bio-culture for the growth of host plant as well as Lac insects and its survival. It was found the treatment T1 (*C. cajan* grown on S_1 with Lac insects with hand picking of pods) was the best for *C. cajan* grain yield and Lac production also because of the combination of substrate was *Kapu* and FYM ratio 1:1 with *T. viride*. But in Treatment 3 (*C.cajan* grown on S_1 with Lac insects and removal of flowers), the100 lac cell weight, raw Lac yield and Fuel wood was more in comparatively rest of the treatments. *C. cajan* can be successfully grown on substrate treated with soil microbes in PPB.*K. lacca* influence the plant growth in comparison to *C. cajan* grown on S2 (FYM only) T2 to that S1 (Kapu and FYM) T1, there was an increase in plant height, thicknesses of stem, primary branches, secondary branches and these growth indicators showed positive effects on Lac insects survival. Maximum settlement of *K. lacca* was on secondary branches.

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