Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2023; 11(6): 115-119

Received: 02-10-2023
Accepted: 07-11-2023

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Study of the relative natural resistance in *Populus deltoides* Bartr. ex Marsh clones against poplar defoliator, *Clostera cupreata* Butler (Lepidoptera: Notodontidae)

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DOI: https://doi.org/10.22271/j.ento.2023.v11.i6b.9267

Abstract
Poplar, *Populus deltoides* is very susceptible to defoliator *Clostera cupreata* which causing about 50% tree infestation and economic growth loss nearly 66% every year. Resistance against the herbivore in plants govern by the genetic makeup of the tree species or clones, stands out major resources for managing the herbivore insect. Targeting the genetic variation and superiority of the clones against herbivore insect, present study was conducted. Forty-seven clones were studied for their superiority against leaf consumption by *C. cupreata* insect pest. Study was experimented through two methods 'choice' and 'no-choice' of leaf feeding. The results revealed that six clones developed by the Forest Research Institute, Dehradun (FRI-PD-FS-19, FRI-PD-AM-44, FRI-PD-AM-112, FRI-PD-FS-194, FRI-PD-FS-13, and FRI-PD-AM-96) along with two clones, WSL-22 and WSL-18, developed by ITC Seedlings Ltd, exhibited the highest resistance against the poplar defoliator C. cupreata. The feeding data from both the 'choice' and 'no-choice' experiments showed a positive correlation. Therefore, these clones are recommended for plantation in farm forestry in northwestern India to enhance the growth of poplar trees.

Keywords: Poplar, *Populus deltoides*, cottonwood, defoliator, relative resistant, clone, *Clostera cupreata*

Introduction
Populus deltoides Bartr. ex Marsh, commonly known as poplar and belonging to the Salicaceae family, stands as one of the fastest-growing species in forestry. The versatile nature of poplar species allows sustainable biomass production, catering to various applications such as bioenergy, bioproducts, plywood, boards, artificial limbs, native fodder, fuelwood, charcoal, and raw materials for industries like pulp and paper, match manufacturing, pencil production, and packaging cases, etc. (Kumar and Singh, 2012; Gangoo *et al*., 1997; Kumar *et al*., 2022) [18, 20, 12]. Moreover, these species contribute significantly to soil and water conservation efforts (Barontini *et al*., 2014, Maissupova *et al*., 2017) [6, 25].

Forest Research Institute in Dehradun, India introduced various clones of exotic *P. deltoides* and some of the clones extensively planted by farmers andgained widespread acceptance (Kaul and Sharma, 1982; Kumar *et al*. 2021) [16, 21]. Subsequently, numerous clones underwent rigorous monitoring in nurseries and field plantations across northern Indian states. The promising clones are widely cultivating by the farmers of Punjab, Haryana, Himachal Pradesh, Uttaranchal and Uttar Pradesh provinces on their farm lands as bund plantation, block plantation and in agroforestry. Poplar cultivation spans approximately 2.7 Lakh hectares within the agro-forestry system and holds significant importance and economic value in Indian farm forestry yielding returns of about Rs. 1.5 L/ha/year for farmers (Kumar *et al*. (1999) [20].

However, *Populus spp.* exhibits high susceptibility to insect pests, constituting major biotic factors that limit productivity in plantations worldwide. In India, 164 insect pests have been recorded to be associated with *Populus* spp., while 65 insect species found to be hostile specifically to *P.deltoides* (Ahmad and Faisal, 2012, Kumar *et al*., 2022) [1, 18]. Nonetheless, only a few insect pests have been documented as serious threats capable of reducing growth and increasing tree mortality (Dickmann and Stuart, 1983; Singh and Singh, 1986; Coley *et al*., 2005; Kumar *et al*., 2022) [11, 8, 34, 18].

Populus spp. is known for its rapid growth, making it particularly sensitive to insect defoliators. For instance, the *Chrysomela scripta* beetle (Coleoptera: Chrysomelidae) is considered a major and highly destructive defoliator of *Populus* in North America (Harrell *et al*., 1982; Coley *et al*., 2005) [14, 8]. Similarly, in China, *Apochemia cineraria* and *Lymantria dispar* (Lepidoptera: Lymantriidae) are noted as the most serious defoliators, causing up to 40% growth loss (Hu *et al*., 2001). In India, *Clostera cupreata* and *C. fulgurita* (Lepidoptera: Notodontidae) have been reported as the most serious defoliators, leading to infestations over 50% of trees and widespread defoliation across more than 1100 hectares in northwestern India. More than 50% defoliation results in a reduction of tree growth by upto 66% (Lohani, 1976; Chaturvedi, 1981; Singh *et al*., 1983; Gao *et al*. (1985) Ahmad *et al*., 2001; Singh *et al*., 2004; Kumar *et al*., 2022a) [24, 35, 7, 3, 18, 32].

Therefore, management of this insect pest is very essential but, application of pesticide in the forestry sector is very injurious to the environment, human beings and other beneficial organisms, also cost and labour intensive. The natural resistance of the clone can be targeted and manipulatedto manage the pest in a ecofriendly manner. It is proven that resistance against herbivoretothe pest varies amongst clones, hybrids, cultivars and species of the genus *Populus* (Defauce, 1976; Qin and Gae, 1985; Jodal, 1987; Ahmad,1993 and Singh and Singh, 1995) [2, 28, 15]. Unfavourable food availability may lead to deformed life stages and mortality in the insect herbivore. Variations also exist among poplar clonesin terms of feeding preference, feeding potential, leaf consumption by insect herbivore,growth, survival, pupal weight and insect life cycle (Augustin *et al*., 1994, Robison and Ruffa, 1994, Singh, 2000, Ahmad, 1993, Augustin *et al*., 1993) [5, 4, 2]. The selection of productive clones with different superior modes and mechanism of pest resistance will facilitate sustainable pest management in poplar plantation (Kennedy *et al*.1987; Libby 1982) [17, 23]. Therefore, present study was undertaken to selection the relatively resistant poplar clones against *C. cupreata* defoliator for its effective and eco-friendly pest management in poplar plantation under farm forestry.

Materials and Methods

Forty seven clones of *P. deltoides* were experimented for their relative resistance against *C. cupreata*, among these 23 clones belongs to Forest Research Institute, Dehradun, twelve clones belongs to ITC (formerly WIMCO) Seedlings Pvt Ltd. Developed, nine clones belongs to Uttarakhand State forest department and three clones belongs to Mississippi, USA. The plant material was taken from germplasm bank of Silviculture Division, FRI and laboratory experiment was executed at Forest Entomology Discipline, Forest Research Institute, Dehradun, Uttarakhand during 2020-21.

The larval insect culture of *C. cupreata* was mass multiplied in the laboratory for further experimental from the insect population collected from the poplar filed. Two type of experimentation was done in this study -1) choice feeding experiment; 2) no-choice experiment. Under choice experiment pieces of leaf of 3x3 cm^2 (9 cm^2) were made and placed in big container at same distance interval, and leaves of each clone were replicated nine times. Thereafter 4thintar larvae of *C. cupreata* (kept in starvation for six hours) were released in the container in ratio of 1:1(leaf and larval) for leaf feeding as per the methodology adopted by Kumar *et al*. (2022) [18]. Leaf consumption by the *C. cupreata* larvae was recorded after one hour of experiment. Consumed leaf in each leaf pieces were measured on graph sheet and feeding area was calculated. Recorded data was subjected to mean and standard deviation (SD) calculation using statistical software MS Excel.

Under no-choice experiment larval population of second instar was used from nuclear. Initially thirty larvae were reared on the individual clones in group under ventilated glass jars of size 30 cm in height and 15 cm diameter. As the larvae grown to 3rd instar stage, treatment was divided into three replications in each clone and ten larvae were grouped and placed on each replication. This treatment was conducted in petri plate of 90 mm up to pupation. The supplies leaf of the clones were weighed and measured individually (before and after feeding) of related clone. Leaf was changed initially at two days interval and laterevery day. In case of larval mortality, larva was replaced with same size healthy larva reared on the same clone. The experiment was conducted under laboratory at the photoperiod of16:8 (L:D), temperature regime of (28±2) °C and relative humidity of (80± 5)%.

Resistance and susceptibility analysis: On the basis of leaf area consumed by the larvae of *C. cupreata* weightage percentage of resistance and weightage percent of susceptibility was calculated as per method given by Ahmed (1993)

$$\text{Weightage \% of resistance (WPR)} = \frac{100 \times [Max.leafarea\ fed - leaf\ area\ fed\ of\ concernedclone}{[Max.leafarea\ fed - Min.leafarea\ fed]}$$

$$\text{Weightage \% of suceptibility (WPS)} = 100 - \text{WPS of concerned Clone}$$

Base on the results of weightage grouped into six different classes of resistance and susceptibility as follow:

1. $\text{Most tolerant clone} = x < (\bar{X} - SD)$
2. $\text{Tolerant clone} = (\bar{X} - SD) < x < \left(\bar{X} - \frac{SD}{2}\right)$
3. $\text{Modratoly tolerant clone} = \left(\bar{X}\frac{SD}{2}\right) < x < \bar{X}$
4. $\text{Moderately Suceptible} = (\bar{X}) > x > \left(\bar{X} + \frac{SD}{2}\right)$
5. $\text{Suceptible clome} = (\bar{X} + SD) > x > \left(\bar{X} + \frac{SD}{2}\right)$
6. $\text{Most suceptible clome} = x > (\bar{X} + SD)$

Where:

x=mean of consumed leaf area by larvae of a clone per day

$\bar{X}$ = Overall mean of consumed leaf area by larvae of all clones per day

SD= standard deviation

The results of both the experiments were correlated and correlation coefficient was calculated with following formula:

$$r = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum(xi - \bar{x})^2 \sum(y_i - \bar{y})^2}}$$

r= correlation coefficient

x_i = values of the x-variable in a sample

$\bar{x}$ = mean of the values of the x-variable

y_i = values of the y-variable in a sample

$\bar{y}$= mean of the values of the x-variable

Results

Result of leaf area feeding by the larvae of *C. cupreata* exhibit in Table 1 clearly shows that there was a difference in the leaf area consumption by the larvae. Under no choice condition consumption of poplar leaf area by *C. cupreata* was categorized based on the mean ($\bar{X}$) leaf area consumed by the larvae was i.e. 91.27 cm^2 (SEM=9.30; CD at 5%=34.21; p=4.44). In this experiment 47 clones were studied and based on the formula of Ahmed (1993) clones were categorized under six groups of relative susceptibility. Eight clones *viz.*FRI-PD-FS-19, FRI-PD-AM-44, WSL-22, FRI-PD-AM-112, WSL-18, FRI-PD-FS-194, FRI-PD-FS-13, and FRI-PD-AM-96 were exhibited to be the most resistant against *C. cupreata* leaf consumption. Six clones were exhibited to be moderately resistant *viz.* FRI-PD-FS-85, FRI-PD-FS-60, FRI-PD-FS-26, WSL-7, FRI-PD-AM-114, and L-62-84. Ten clones were exhibited to be least resistant *viz.*FRI-PD-FS-193, ST-238, FRI-PD-FS-46, FRI-PD-FS-38, FRI-PD-FS-149, Wimco-61, ST-92, FRI-PD-FS-57, UDAI, and L-7/15. Eleven clones were exhibited to be least resistant *viz.* FRI-PD-FS-78, FRI-PD-FS-67, FRI-PD-FS-179, WSL-12, Bahar, WSL-49, L-37/16, L-13/16, FRI-PD-FS-117, FRI-PD-FS-135, and L-8/15. Nine clones exhibited to be moderately susceptible *viz.*WSL-4, L-5/16, WSL-42, ST-153, Kranti, L3/15, and FRI-PD-FS-74. And three clones were exhibited to be most susceptible *viz*. L-64/16, WSL-45 and FRI-PD-FS-52.

The result of choice based experiment exhibited (table 1) significant variation in the leaf area consumption by *C. cupreata* larvae. The tread of results of leaf area consumption under 'choice' and 'no choice' condition experiments were exhibited positive correlation (0.452) between both.

Discussions

The relative resistance in plants against herbivore refers to the preference or less preference among the offered or food, in which two or more food types are simultaneously offered to herbivore. The experiments are widespread underway in relative resistance or feeding-preference study (Peterson and Renaud, 1989) [27] to find out the natural resistance against herbivore in the clonal material for sustainable and stable plantation (Nichole *et al*., 2010) [26]. The selection of productive clones with deferent modes and mechanisms of pest resistance facilitate the more stable and pest free plantation (Kennedy *et al* 1987; Libby, 1982) [17, 23]. We have also conducted this study based on feeding preference to screen the relatively resistant clone of poplar in which 47 cloned were offered at the same time and same environmental conditions to the larvae of *C. cupreata.* Based on the level (cm^2) of leaf consumption eight clones were exhibited to be most resistant category. The variation in the leaf consumption by the herbivore reflects that the genetic/biochemical mechanism which is responsible for preference or non preference. It is proven that leaf of the clone also negatively affects to the larval growth and negatively correlated with defoliation tolerance (Dannel and Kennetha 1994) [10].

We have used both 'choice' and 'no-choice' method of feeding in this study, and both the results of experiments have been correlated with each other. Many researchers have studied the clones of *P. deltoides* for their relative resistance using no choice method only. Ahmed (1993) has given this method of 'no choice' of leaf feeding and followed by Singh (2000) for the study of forty clones, Singh and Pandey (2000) [33] studied 80 clones, Sangha *et al.* (2011) [29] has also evaluated 13 poplar clones and Kumar *et al*. (2022) [18] have evaluated thirty-nine. Our result suggests that six clones viz. FRI-PD-FS-19, FRI-PD-AM-44, FRI-PD-AM-112, FRI-PD-FS-194, FRI-PD-FS-13, and FRI-PD-AM-96 developed by our institute FRI, Dehradun and two clones namely WSL-22, and WSL-18 developed by ITC Seedlings Ltd, were most resistant against poplar defoliator, *C. cupreata*. This result is also positively correlated with the results of choice feeding experiment, therefore, these superior clones may be opted by the farmers for plantation in their farm forestry.

Table 1: Relative leaf consumption of *Populus deltoides* clones by the *Clostera cupreata* larvae, measured under 'Choice' and 'No-choice' condition and their categorization in different resistance/ susceptible categories.

***Populus deltoides* clones**	**Parentage (Female x male) or origin**	**Leaf area consumption under**	
		No choice (cm^2)	**Choice (cm^2/ Hour)**
Most resistant (08)			
FRI-PD-AM-19	Not Known	45.44	0.6
FRI-PD-AM-44	Not Known	56.73	0.64
WSL-22	Not Known	58.7	0.88
FRI-PD-AM-112	Not Known	62.72	0.47
WSL-18	Not Known	67.98	1.5
FRI-PD-FS-194	D-121 X S7C2	68.2	0.69
FRI-PD-FS-13	3324 X 82-42-5	68.82	1.66
FRI-PD-AM-96	Not Known	72.6	0.51
Moderately resistant (06)			
FRI-PD-FS-85	S7C8 X 113324	75.17	0.9
FRI-PD-FS-60	S7C8 X 82-36-1	76.85	2.73
FRI-PD-FS-26	L-34/82 X S7C20	77.26	2.81
WSL-7	Not Known	81.4	1.01
FRI-PD-AM-114	Not Known	82.09	0.86
L-62-84	Open pollinated Bhimtal	83.25	2.76
Least resistant (10)			
FRI-PD-FS-193	3201 X S4C2	83.69	0.96
ST-238	Not Known	84.4	1.82

FRI-PD-FS-46	S7C8 X 3167	85.17	1.29
FRI-PD-FS-38	110702 X S7C15	85.23	1.1
FRI-PD-FS-149	S7C8 X S4C21	87.43	0.98
Wimco-61	Not Known	88.8	1.7
ST-92	Not Known	91.01	1.21
FRI-PD-FS-57	D-121 X 3167	92.36	1.02
UDAI	Not Known	92.48	1.18
L-7/15	S_7C_8xPIP-220	92.5	1.97
Least susceptible (11)			
FRI-PD-FS-78	ST-72 X 3167	95.49	1.51
FRI-PD-FS-67	Not Known	95.56	1.42
FRI-PD-FS-179	D-121 X S4C21	96.25	1.57
WSL-12	Not Known	97.43	1.32
Bahar	Not Known	99.25	1.6
WSL-49	Not Known	100.99	1.61
L-37/16	PIP204xS_5C_2	101.45	1.76
L-13/16	PIP204xPIP-213	101.62	0.41
FRI-PD-AM-117	Not Known	101.8	1.63
FRI-PD-AM-135	Not Known	101.96	1.72
L-8/15	S_6C_8x PIP-220	103.08	1.6
Moderately susceptible (09)			
WSL-4	Not Known	103.61	1.83
L-5/16	PIP204xPIP-214	104.23	1.89
WSL-42	Not Known	104.55	1.95
ST-153	Not Known	104.75	1.75
Kranti	Not Known	109.85	2.04
L-3/15	S_7C_8xPIP-101	110.03	1.24
FRI-PD-AM-11	Not Known	110.15	1.95
L-9/15	S_6C_8 xPIP-220	110.76	1.47
FRI-PD-FS-74	L-49 X G-3	111.6	1.93
Highly susceptible (03)			
L-64/16	PIP204x82-42-5	118.4	1.1
WSL-45	Not Known	118.69	2.27
FRI-PD-FS-52	D-171 X S7C15	127.09	2.55
Mean		78.48	2.23
SD		24.34	0.70
*UKSFD= Uttarakhand State Forest Department, India			

Acknowledgements
The authors are grateful to DG, ICFRE for providing financial support and Director FRI for providing necessary facilities for conducting this research work.

Conflict of interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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