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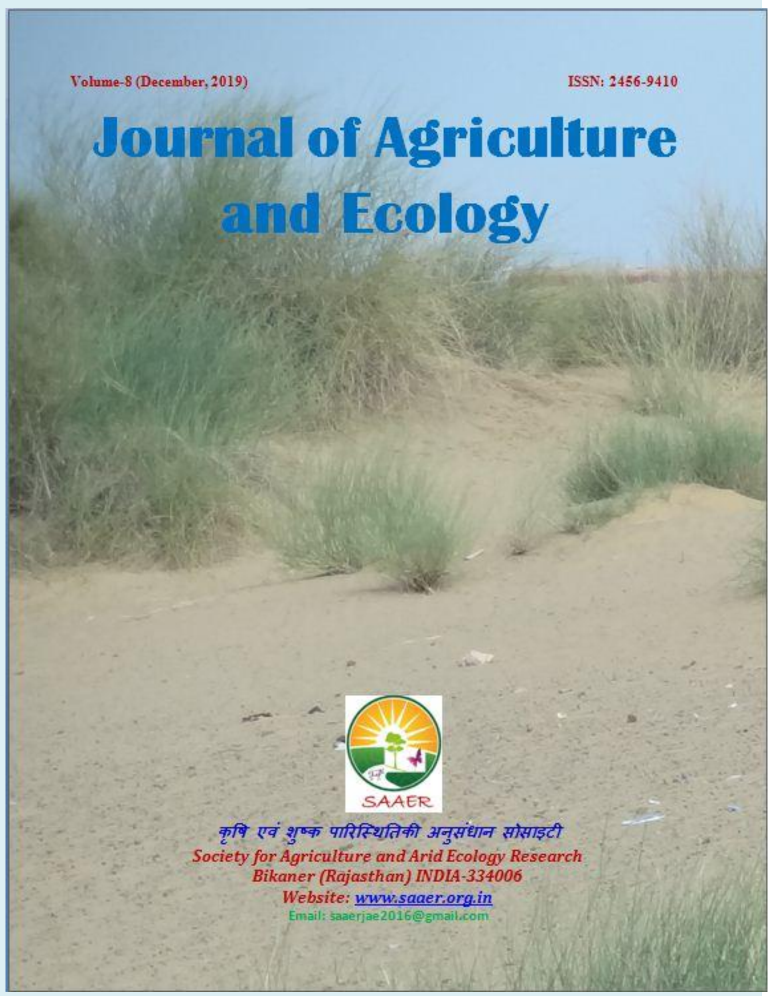

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Do bottom-up effects (resistance) of different accessions of Indian cherry (*Cordia myxa* L.) help against tingid bug [*Dictyla cheriani* (Drake)] attack?

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Abstract

The tingid bug, *Dictyla cheriani* (Drake) [Hemiptera: Tingidae] is an important pest of Indian cherry (*Cordia myxa* L.), leading to significant losses in yield in the hot arid agro-climate of India. The objectives of this study were to identify and categorize sources of resistance in India cherry accessions to *D. cheriani* from the arid region of India. We found that: three accessions were resistant; 3 accessions were moderately resistant; 2 accessions were moderately susceptible; 2 accessions were susceptible and three accessions were highly susceptible to *D. cheriani* infestation. Free amino acid had positive correlation with infestation, whereas phenols, tannin, alkaloid and flavonoid contents had significant negative correlation with infestation. The infestation had significant negative correlation with leaf length and width. Phenols and flavonoid contents explained (96.9 and 96.1%, respectively) of the total variation in bug infestation and in bug density per leaf. One principal component was extracted explaining cumulative variation of 90.07% in infestation. The flavonoid, alkaloid, tannins, phenols content, roughness and hairyness were the novel antibiosis and antixenotic characters found in Indian cherry accessions, which were resistant to *D. cheriani*. Growers can adopt potential accessions of Indian cherry (*Cordia myxa* L.) as identified for resistance (AHCM-22-1, AHCM-25 and AHCM-34 accessions) with minimal financial investment for obtaining higher yields. The bio-physical traits linked to resistance of Indian cherry against *D. cheriani* could be used as marker traits in plant breeding programmes to develop resistant cultivars.

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Introduction

Cordia myxa (syn. *C. domestica* Roth and *C. sebestena* Forssk.), commonly known as Indian cherry (folk names: *clammy chery*, *franrant manjack*, *lasora*, *shelu*, *cinnanakkeru*, *bahubara*, *chokri*, *lahsoda*, *lahsua*, *gonda*, *gondi*) belongs to the family Boraginaceae. It is matures in about 50 to 60 years when its girth height is about 1 to 1.5 m. Its main trunk is generally straight and cylindrical, attaining a height of nearly 3 to 4

m. The branches spread in all directions which its crown can be trained into a beautiful inverted dome like an umbrella. The leaves are broad, ovate, alternate and stalked with the spread being 7 to 15 cm x 5 to 10 cm. The fruit of *lasoda* start fruiting in April-May and it is a kind of a drupe, light pale to brown or even pink in color. This tree is a multi-use species distributed in hot arid and semi-arid regions of India. *Cordia myxa* originated in North-Western part of the country (Stewart and Brandis 1992; Sivalingum et al. 2012; Samadia & Haldhar 2017) and distributed in warmer regions of Myanmar, Afghanistan, China, Africa, Australia *etc*. and more recently also in America. The origin of this tree is also seemingly from eastern India. In India, it is found mostly in the northern part and is abundantly distributed and naturally growing in the north-western region (Pareek & Sharma 2009). All parts of ''*lasoda*'' plant are used for various purposes (Yadav & Goel 2006). Immature fruits can be used as vegetable or for making pickles. Mature fruits of *lasoda* are nutritionally very rich in carbohydrates (12.2 g/100 g of edible portion), total ash (2.13%), vitamins and minerals. with the fruit also have high medicinal value and can be used as an anti-helmenthic, diuretic, demulcent, expectorant and anti-tumorigenic and also for preparing of Ayurvedic medicines. Leaves are consumed as fodder by goat, sheep and cattle (Ouedraogo-Kone et al. 2006) as well as used for making of *pattal* (trays/plates).

(National Tree Seed Project 1999), reported that damage caused by insect on *C. africana* seeds was reported as 45%, 30% and 20% from tested location Sekoru, Arjo and Wondo Genet places, respectively from Africa. Tibebu (2002) studied pre-dispersal insect seed predators on seeds of these two tree species, 10% and found 20% of *C. africana* seeds collected from Sekoru, and 8-10% of seeds collected from Denbi, Jimma and Arjo places. Few studies were made on the bug, *Dictyla cheriani* infestation on *Cordia sp.* (India) and bug, *D. monotropidia* infestation on *C. verbenacea* (Brazil) by Daniel et al. (2008).

The tingid bug, *D. cheriani* (Drake) [Hemiptera: Tingidae] is a major pest of Indian cherry in India and its outbreaks cause substantial crop losses to growers. The lace bugs sucked the sap from newly emerging leaves and young branches, which led to the leaves turned yellow and suppression of growth of the tree through drying of leaves and young branches. The maximum incidence of tingid bug is usually observed in October in two different phenotypic characters (51.67% on bold & 76.67% on small seeded plants) and minimum was in January (11.67% on bold & 21.67% on small seeded plants). The number of this lace bug ranged between (0.5 to 8.8 on bold & 4.5 to 25.97 on small seeded plants) nymphs and adults per leaves (Haldhar & Singh 2014).

Many integrated pest management (IPM) strategies have been utilized to develop sustainable pest management programmes which must show more resilience and less reliance on synthetic pesticides (Lin 2011; Tooker et al. 2012; Bustos-Segura et al. 2017; Haldhar et al. 2017; Muthusamy et al. 2017; Gassmann 2017; Haldhar et al. 2018 b & c). Plants and insects have been living together for more than 350 million years. During co-evolution, both have evolved some strategies to by-pass the defense systems of each other. To counter attack by herbivores, plants have innate ability to build-up specialized morphological structures or produce secondary metabolites and proteins that have toxic, repellent, and/or antinutitional effects on the herbivores (Usha & Jyothsna 2010; War et al. 2011). The genotypic variation of the plant may influence the distribution and damage levels of herbivores referred to as associational resistance or susceptibility (Barbosa et al. 2009; Haldhar et al. 2018d). The bottom-up effects in the crop plant is an economical and environment-friendly method of insect management. The farmer friendly and cost effectiveness for pest control are the attractive and beneficial features of bottom-up effect method. Plants confront the herbivores both directly and indirectly by affecting host plant preference or survival and reproductive success (direct defense), and through other species such as natural enemies of the insect pests (indirect defense) (Arimura et al. 2009; Haldhar et al. 2015c). Direct defenses are mediated by physical barriers of plant such as hairs, trichomes, thorns, spines, and thicker leaves that affect the herbivore's biology by mechanical protecting of the surface of plants. It also involved the production of toxic chemicals such as terpenoids, alkaloids, anthocyanins, phenols, quinones *etc.* that either kill or retard the growth of herbivores (Hanley et al. 2007). Indirect defenses against insects are mediated by the release of a blend of volatiles that specifically attract natural enemies of the herbivores. It may also provide food (e.g., extra floral nectar) and shelters to the natural enemies which enhance the effectiveness of the natural enemies (Arimura et al. 2009).

Cultivation of accession of *lasoda* plant resistant to *D. cheriani* is a major component of integrated pest management programs. Therefore, Indian cherry was first taken as case study in the present investigation. The progress for development of Indian cherry cultivars resistant to *D. cheriani* is very limited worldwide due to the inadequate information of resistant source in the accessions against pest infestations. Therefore, the present study was designed to identify various morphological (antixenotic mechanism) and biochemical (allelochemical compounds) leaf traits of Indian cherry accession associated with resistance against *D. cheriani* in terms of quantum of leaf infestation and density of bug per leaf under field conditions.

Materials and Methods

To conduct the study, a survey was done at different locations of Rajasthan and accessions of *C. myxa* were collected. During the survey, trees were selected randomly for the following phenotypic characters *viz.* tree spread and height, leaf and fruit characters. From each location, fifteen fully ripened fruits from a single tree were collected, labeled and brought to the experimental farm of ICAR-Central Institute for Arid Horticulture (ICAR-CIAH), Bikaner, Rajasthan (Sivalingam et al. 2012) (Table 1).

Table 1. Details of different accessions of Indian cherry, *Cordia myxa* and collection sites

Accessions	Village/District/State	Collection site
AHCM-01	Bhojka/Jaisalmer/Rajasthan	Forest area
AHCM-02	Asotra/Barmer/Rajasthan	Forest area
AHCM-03	Asotra/Barmer/Rajasthan	Farmers' field
AHCM-04	Asotra/Barmer/Rajasthan	Farmers' field
AHCM-06	Asotra/Barmer/Rajasthan	Farmers' field
AHCM-07	Asotra/Barmer/Rajasthan	Farmers' field
AHCM-08	Khuship/Barmer/Rajasthan	Farmers' field
AHCM-09	Siwana/Barmer/Rajasthan	Farmers' field
AHCM-11	Sibana/Barmer/Rajasthan	Farmers' field
AHCM-14	Bheswara/Jalore/Rajasthan	Farmers' field
AHCM-16	Ahor/Jalore/Rajasthan	Farmers' field
AHCM-22-1	Khamnor/Rajsmand/Rajasthan	Farmers' field
AHCM-23	Sadri/Pali/Rajasthan	Farmers' field
AHCM-24	Bilada/Jodhpur/Rajasthan	Farmers' field
AHCM-25	Surpura/Jodhpur/Rajasthan	Farmers' field
AHCM-26	Surpura/Jodhpur/Rajasthan	Farmers' field
AHCM-29	Mathania/Jodhpur/Rajasthan	Farmers' field
AHCM-30	Mathania/Jodhpur/Rajasthan	Farmers' field
AHCM-31	Bikaner/Bikaner/Rajasthan	Farmers' field
AHCM-32	Bikaner/Bikaner/Rajasthan	Farmers' field
AHCM-33	Bikaner/Bikaner/Rajasthan	Farmers' field
AHCM-34	Bikaner/Bikaner/Rajasthan	Farmers' field

Preliminary screening of accessions of *C. myxa* (2013-14)

For preliminary screening, twenty two accessions of *C. myxa* were collected from Rajasthan during May–July 2001 and established at the field gene bank at experimental farm of ICAR-CIAH, Bikaner (at 28°06'N latitude, 73°21'E longitude and altitude of 234.84m above sea level) for conservation, evaluation and screening for resistance (Table 1). The planting material was established with three replicates for each accession following a randomized block design (RBD). The plants were maintained at experimental farm with a spacing of 6x6 m area of plant to plant distance and drip irrigation system. All the recommended agronomical practices (e.g. weeding, fertilization, hoeing, *etc*.) were performed equally in each experimental plant. Twenty leaves were randomly selected in each of 3 replicates and average incidence of infestation was calculated as the percent of leaves infested with *D. cheriani* during 2013-14. Average number of lace bugs per leaves was calculated on the basis of observations recorded on ten randomly selected leaves of whole plant with 3 replications. The resistance category was mentioned as resistant: 11-20%, moderately resistant: 21-30%, moderately susceptible: 31-40%, susceptible: 41-50% and highly susceptible: >50% of all accessions of Indian cherry against *D. cheriani* infestation.

Final screening of selected accessions of *C. myxa* during 2014-15 and 2015-16

Thirteen selected accessions from preliminary screening of Indian cherry *viz.*, AHCM-14, AHCM-16, AHCM-09, AHCM-01, AHCM-30, AHCM-34, AHCM-22-1, AHCM-26, AHCM-33, AHCM-31, AHCM-24, AHCM-08 and AHCM-25 were used for experimentation from 2014-15 to 2015-16 following a RBD, with three blocks for each genotype and each block representing a replication. Agronomical practices were followed as described above.

Allelochemical content as leaf traits of the re-evaluated accessions

For estimation of allelochemical content, five fresh leaves of each accession were selected, cut in to small pieces and dried. The biochemical contents in dried leaf such as phenols (Malik & Singh 1980), tannins (Schanderl 1970), free amino acid (Lee & Takahashi 1966) and flavonoid were determined by colorimetric aluminium chloride method (Nabavi et al. 2008).

Antixenotic leaf mechanism of the re-evaluated accessions

Ten fresh leaves of each of the thirteen accessions were used to record the biophysical traits such as leaf length, leaf width, leaf size, leaf roughness and leaf hairyness. Leaf length and width were measured at ten different positions of each leaf using Digital Vernier Caliper (MITU-TOYO, 300 mm, 0.01 mm reading capacity). Leaf size, leaf roughness and leaf hairyness was visually recorded in the field as well as laboratory.

Statistical analysis

Transformations (angular and squire root transformed value) were used to achieve normality in the data before analysis. Untransformed means were also incorporated in the analysis and presented in all tables. The data on percentage bug infestation and bug density per leaf and biochemical leaf traits were analyzed through one-way ANOVA using SPSS 16 software (O'Connor 2000). Correlations between biophysical and biochemical leaf traits and tingid bug parameters (percent leaf infestation and bug density per leaf) were determined using correlation analysis and backward stepwise multiple regression analysis at the 95% significance level.

Results

Preliminary screening of accessions of *C. myxa*

The significant differences were found in percentage leaf infestation and bug density per leaf among the tested accessions of *C. myxa* during preliminary screening. The bug density per leaf had a significant positive correlation with percentage bug infestation ($r = 0.965$; $P < 0.01$). Based on Kaiser Normalization method, the accessions of *C. myxa* categorized as AHCM-22-1, AHCM-25 and AHCM-34 were found to be resistant; AHCM-14, AHCM-30, AHCM-31, AHCM-03 and AHCM-11 were found moderately resistant; whereas AHCM-16, AHCM-09, AHCM-29 and AHCM-04 were found to be moderately susceptible; AHCM-33, AHCM-08 AHCM-07 and AHCM-06 susceptible and AHCM-01, AHCM-26, AHCM-24and

AHCM-32 highly susceptible accessions. The bug density was recorded highest in AHCM-01 (25.90 bugs/ leaf) which was at par with AHCM-26 (24.77 bugs/ leaf) and AHCM-24 (23.70 bugs/ leaf). The minimum bug density was observed in AHCM-22-1 (4.27 bugs/ leaf) followed by AHCM-25 (4.50 bugs/ leaf) and AHCM-34 (5.53 bugs/ leaf). The per cent leaf infestation was the highest in AHCM-32 (68.51 %) and the lowest in AHCM-22-1 (12.26 %) followed by AHCM-25 (14.25 %). The leaf infestations ranged from 12.26 to 68.51 % and were significantly lower in resistant accessions and higher in susceptible accessions (Table 2).

Table 2. *D. cheriani* density and per cent infestation on different accessions of Indian cherry, *Cordia myxa* during preliminary screening trials (2013-14)

Accessions	Bug density/ leaf	Bug infestation (%)	Resistance category
AHCM-14	15.40^{def}	28.41 (32.17)^{f}	MR
AHCM-16	14.97^{de}	34.80 (36.13)^{gh}	MS
AHCM-09	17.63^{gh}	37.68 (37.85)^{ghi}	MS
AHCM-01	25.90^{l}	67.85 (55.46)^{m}	HS
AHCM-30	13.50^{d}	25.43 (30.25)^{ef}	MR
AHCM-34	5.53^{ab}	17.62 (24.79)^{bc}	R
AHCM-22-1	4.27^{a}	12.26 (20.45)^{a}	R
AHCM-26	24.77^{kl}	56.77 (48.88)^{l}	HS
AHCM-33	22.97^{jk}	46.47 (42.96)^{k}	S
AHCM-31	10.53^{c}	23.56 (29.00)^{de}	MR
AHCM-24	23.70^{kl}	53.51 (46.99)^{l}	HS
AHCM-08	20.90^{ii}	43.50 (41.25)^{jk}	S
AHCM-25	4.50^{ab}	14.25 (22.15)^{ab}	R
AHCM-23	6.57^{b}	18.27 (25.28)^{cd}	R
AHCM-02	22.70^{jk}	66.34 (54.54)^{m}	HS
AHCM-07	19.43^{hi}	43.78 (41.41)^{jk}	S
AHCM-29	17.60^{fgh}	33.55 (35.38)^{g}	MS
AHCM-03	14.90^{de}	27.89 (31.85)^{ef}	MR
AHCM-32	24.03^{kl}	68.51 (55.86)^{m}	HS
AHCM-04	16.87^{efg}	39.03 (38.63)^{hi}	MS
AHCM-11	14.53^{d}	26.03 (30.65)^{ef}	MR
AHCM-06	20.93^{ii}	45.22 (42.24)^{jk}	S
Mean $\pm$ SD	16.04 $\pm$ 6.77	36.86 $\pm$ 17.14	
SEm $\pm$	0.77	1.01	
LSD (P = 0.05)	2.20	2.89	

*Values in parenthesis are angular-transformed; Resistant: 11-20%, moderately resistant: 21-30%, moderately susceptible: 31-40%, susceptible: 41-50% and highly susceptible: >50%; Degree of freedom for replication=2; treatment=21; error=42

Final screening of Indian cherry accessions

The final screening of Indian cherry accessions revealed different levels of the resistance among the accessions. The analysis showed 3 accessions (AHCM-22-1, AHCM-25 and AHCM-34) as resistant and AHCM-14, AHCM-30 and AHCM-31 as moderately resistant. While AHCM-16 and AHCM-09 were found as moderately susceptible and AHCM-33 and AHCM-08 as susceptible. Contradictory, AHCM-01 and AHCM-26 were found as highly susceptible accession (Table 3). The significant positive correlation ($r = 0.965$; $P < 0.01$) was observed between the percent leaf infestation and bug density per leaf. From the pooled data analysis of bug density per leaf in both seasons (4.18–25.86 bugs per leaf) it was found significantly lower in resistant accessions whereas higher in susceptible accessions of Indian cherry. The pooled data of leaf infestation in both seasons (12.22–67.90%) was showed significantly lower infestation in resistant accessions as comparatively higher in susceptible accessions. From the pooled data of both seasons, the per cent leaf infestation was recorded highest in AHCM-01 (67.90 %) and the lowest in AHCM-22-1 (12.22 %) followed by AHCM-25 (14.17 %) (Table 3, Fig 1 & Plate 1).

Table 3. *D. cheriani*density and per cent leaf infestation on different accessions of Indian cherry, *Cordia myxa* during final screening trials

Accessions	Bug/ leaf			Bug infestation (%)			Resistance category
	2014-15	2015-16	Pooled	2014-15	2015-16	Pooled	
AHCM-14	15.25 cd	15.63 de	15.44 cd	27.80 (31.78)* e	28.87 (32.47)* d	28.33 (32.13)* d	MR
AHCM-16	14.73 c	15.13 de	14.93 cd	34.26 (35.80) fg	35. 23 (36.39) ef	34.75 (36.10) ef	MS
AHCM-09	17.39 d	17.73 ef	17.56 d	37.20 (37.56) g	37.93 (37.97) f	37.57 (37.77) f	MS
AHCM-01	25.56 g	26.17 i	25.86g	67.49 (55.24) k	68.30 (55.74) k	67.90 (55.50) k	HS
AHCM-30	13.27 c	13.70 cd	13.48 c	25.15 (30.07) de	25.73 (30.45) d	25.44 (30.26) d	MR
AHCM-34	5.24 a	5.80 a	5.52 a	17.35 (24.58) b	17.80 (24.92) b	17.57 (24.75) b	R
AHCM-22-1	3.96 a	4.40 a	4.18 a	12.01 (20.22) a	12.43 (20.60) a	12.22 (20.41) a	R
AHCM-26	24.44 fg	24.70 hi	24.57 fg	56.41 (48.67) j	56.90 (48.96) j	56.66 (48.81) j	HS
AHCM-33	22.80 ef	23.13 gh	22.97 ef	46.15 (42.77) i	46.97 (43.24) h	46.56 (43.01) h	S
AHCM-31	10.36 b	10.60 b	10.48 b	23.30 (28.83) cd	24.03 (29.32) cd	23.67 (29.08) cd	MR
AHCM-24	23.47 fg	23.83 ghi	23.65 fg	53.27 (46.86) j	54.00 (47.28) ij	53.63 (47.07) ij	HS
AHCM-08	20.54 e	21.03 fg	20.79 e	43.06 (40.99) hi	43.80 (41.42) gh	43.43 (41.21) gh	S
AHCM-25	4.17 a	4.67 a	4.42 a	13.97	14.37	14.17	R

				(21.92) ^{ab}	(22.24) ^{ab}	(22.08) ^{ab}
Mean± SD	15.47± 7.78	15.89± 7.76	15.68± 7.77	35.19± 17.35	35.87± 17.42	35.53± 17.38
SEm±	0.787	1.020	0.869	1.002	1.170	1.050
LSD (P = 0.05)	2.31	3.00	2.55	2.94	3.44	3.09

*Values in parenthesis are angular-transformed; Resistant: 11-20%, moderately resistant: 21-30%, moderately susceptible: 31-40%, susceptible: 41-50% and highly susceptible: >50%; Degree of freedom for replication=2; treatment=12; error=24

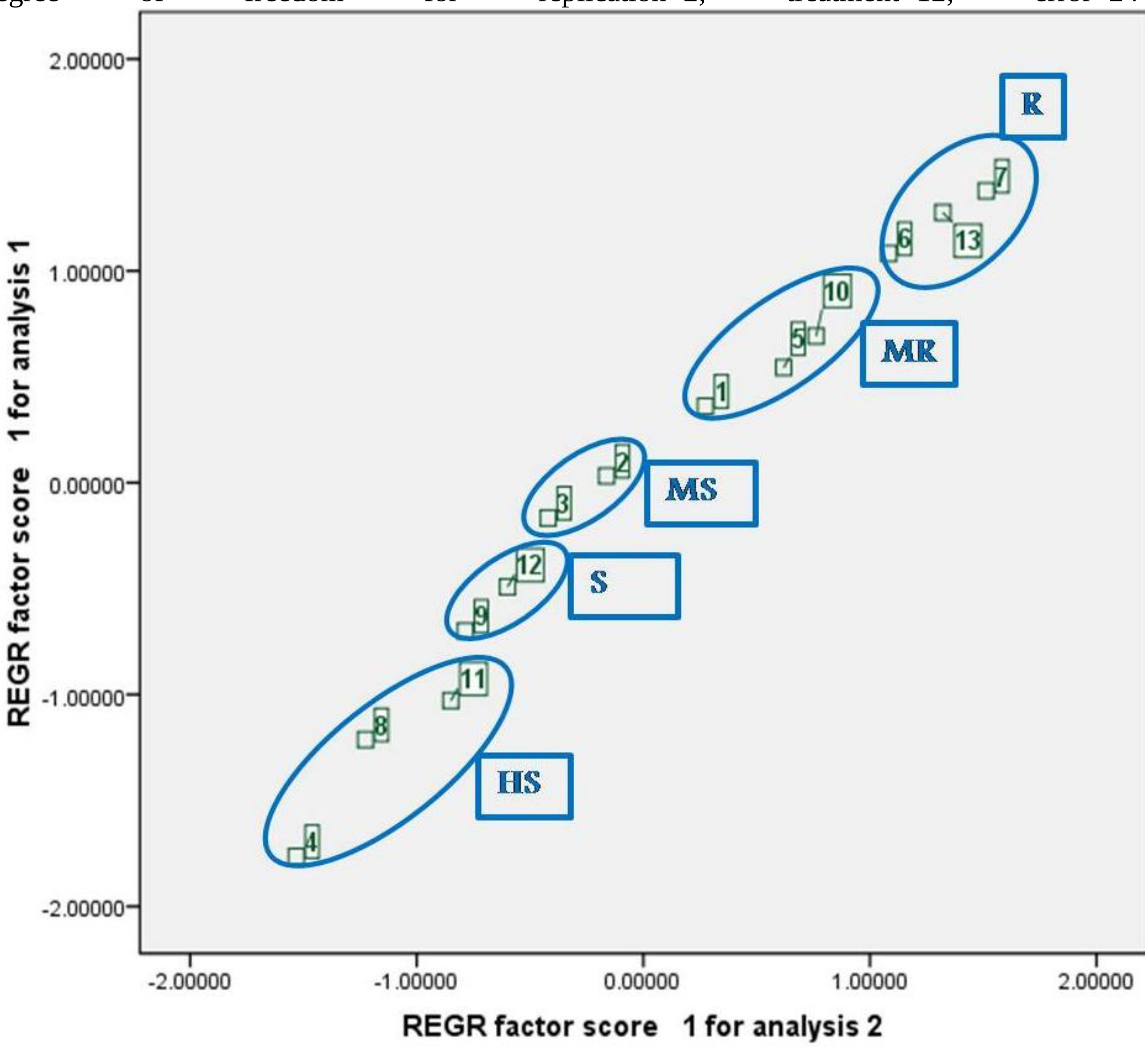

Fig. 1. Plot of PC showing clusters of Indian cherry, *Cordia mixa* accessions showing resistance to *Dictyla cheriani.*

Plate 1. Variation in germplasm accession fruits of Indian cherry, *Cordia myxa* during final screening trials

Allelochemical leaf traits of the re-evaluated Indian cherry accessions

Flavonoid content: The flavonid content of re-evaluated Indian cherry accessions studied in the present investigation was ranged from 2.38 to 5.43 mg/g, the maximum content was found in accession AHCM-22-1 (5.43 mg/g) (Table 4). The flavonoid content was significantly correlated to tannins content (0.961), phenols content (0.877) and total alkaloid content (0.979) of the accessions. However, the negative correlations were observed with percent bug infestation (-0.982), bug density per leaf (-0.977) and free amino acid (-0.965) (Table 6).

Tannins content: The tannins content of test accession was ranged from 14.49 to 22.38 mg/g, whereas the maximum was observed in AHCM-22-1 (22.38 mg/g) followed by AHCM-25 (20.40 mg/g) (Table 4). The significant positive correlations were observed between tannins content and flavonoid content (0.961) as well as phenols content (0.919) and total alkaloid content (0.982). Similarly the significant negative correlation was observed with percent bug infestation (-0.942), bug density per leaf (- 0.972) and free amino acid (-0.940) (Table 6).

Phenols content: The phenols content of the test accession was ranged from 11.31 to 18.44 mg/g, the highest phenol was found in AHCM-22-1 (18.44 mg/g) and the lowest was in AHCM-01 (11.31 mg/g) (Table 4). Similarly, the significant positive correlations were observed between phenols content and tannins content (0.919), flavonoid content (0.877) and total alkaloid content (0.873) of the accessions. However, the significant negative correlation was recorded with percent bug infestation (-0.865), bug density per leaf (-0.914) and free amino acid (-0.835) (Table 6).

Total alkaloids: The total alkaloid content in the accessions was estimated with range from 0.14 to 0.43%. The maximum percentage of alkaloid content was recorded in AHCM-22-1 whereas the minimum in AHCM-01 with a significantly higher values in resistant accession and lower in susceptible accession. The significant positive correlations were observed between total alkaloids and tannins content (0.982), flavonoid content (0.979) and phenols content (0.873). Similarly, the significantly negative correlation was observed with percent bug infestation (-0.976), bug density per leaf (-0.972) and free amino acid (- 0.948) (Table 6).

Free amino acid: The free amino acid ranged from 0.87 to 2.02 mg/g, the maximum was found in AHCM-01 followed by AHCM-08 and the minimum in AHCM-22-1 (Table 4). Significantly positive correlations were observed between free amino acid and percent bug infestation (0.955) and bug density per leaf (0.938). Significantly negative correlations was observed with tannins content (- 0.940), flavonoid content (-0.965), phenols content (-0.835) and total alkaloid content (-0.948) (Table 6).

Backward stepwise regression analysis indicated that phenols and flavonoid contents explained 96.9 % of the total variation in leaf infestation (Table 7). The maximum variation in leaf infestation was explained by phenols content (83.6%) followed by flavonoid (13.3%) and total tannins content (0.4%). The phenols and flavonoid contents explained 96.1 % of the total variation in bug density per leaf. The maximum variation in bug density per leaf was explained by phenols content (74.8%) followed by flavonoid content (21.3%), whereas the rest of the biochemical fruit traits explained < 2% variation in bug density (Table 7).

Table 4. Biochemical (allelochemical) leaf traits of different accessions of Indian cherry, *Cordia myxa*

Accessions	Flavonoid content** (mg/g)	Tannins content** (mg/g)	Phenols Content** (mg/g)	Total alkaloids** (%)	Free Amino Acid (mg/g) **
AHCM-14	4.20 ef	17.15 def	13.44 bc	0.29 e	1.44 c
AHCM-16	3.94 de	17.37 ef	13.85 cd	0.27 de	1.56 cd
AHCM-09	3.74 cde	16.36 bcde	13.48 bc	0.24 d	1.48 c
AHCM-01	2.38 a	14.49 a	11.31 a	0.14 a	2.02 f
AHCM-30	4.70 fg	18.47 fg	13.41 bc	0.34 f	1.15 b
AHCM-34	5.14 gh	20.03 gh	17.62 fg	0.36 f	1.01 ab
AHCM-22-1	5.43 h	22.38 i	18.44 g	0.43 g	0.87 a
AHCM-26	2.74 a	14.78 ab	12.41 abc	0.16 ab	1.89 ef
AHCM-33	3.29 bc	15.68 abcd	12.78 abc	0.19 bc	1.45 c
AHCM-31	4.66 f	19.18 gh	16.70 ef	0.34 f	1.14 b
AHCM-24	3.34 c	15.51 abc	11.99 ab	0.19 bc	1.73 de
AHCM-08	3.45 cd	16.39 cde	15.26 de	0.23 cd	1.76 e
AHCM-25	5.59 h	20.40 h	18.27 fg	0.37 f	0.97 ab
Mean± SD	4.05± 1.01	17.55± 2.39	14.53± 2.46	0.27± 0.09	1.42± 0.37
SEm±	0.172	0.539	0.567	0.015	0.063
LSD (P = 0.05)	0.51	1.58	1.67	0.044	0.184

**Analysis on dry weight (DW) basis

Degree of freedom for replication=2; treatment=12; error=24

Table 6. Correlation coefficient (r) between percent leaf infestation and bug density per leaf with different allelochemical and antixenotic leaf traits of different accessions of Indian cherry, *Cordia myxa*

	Bug infestation (%)	Bug/ leaf	TC	FC	PC	TAC	FAA	LL
Bug/ leaf	0.965**							
TC	-0.942**	-0.972**						
FC	-0.982**	-0.977**	0.961**					
PC	-0.865**	-0.914**	0.919**	0.877**				
TAC	-0.976**	-0.972**	0.982**	0.979**	0.873**			
FAA	0.955**	0.938**	-0.940**	-0.965**	-0.835**	-0.948**		
LL	-0.868**	-0.854**	0.832**	0.886**	0.645*	0.889**	-0.836**	
WL	-0.835**	-0.767**	0.752**	0.844**	0.597*	0.836**	-0.804**	0.903**

**Significant at P = 0.01 (two-tailed); * Significant at P = 0.05 (two-tailed)
FAA- free amino acid (mg/g), TC- tannins content (mg/g), PC- phenols content (mg/g), TAC- total alkaloid content (%), FC- flavonoid content (mg/g), LL-length of leaf & WL-width of leaf

Table 7. Backward stepwise regression models showing effect of different allelochemical and antixenotic leaf traits of Indian cherry on percentage leaf infestation and bug per leaf

Percent leaf infestation	R^2	Role of individual traits (%)
$Y=49.54- 0.76 X_1- 4.30 X_2 +0.29 X_3 -26.89 X_4 -0.22 X_5 - 0.78 X_6 =0.62 X^7$	98.10	00.60
$Y=62.50- 0.67 X_1- 4.02 X_2 -0.86 X_3 +0.86 X_4 -1.29 X_5 - 0.34 X_6$	97.50	00.10
$Y=62.18- 0.47 X_1- 4.83 X_2 -0.91 X_3 -4.04 X_4 -2.15 X_5$	97.40	00.00
$Y=54.39- 0.55 X_1- 4.05 X_2 -0.70 X_3 -8.00 X_4$	97.40	00.10
$Y=56.53- 0.50 X_1- 4.37 X_2 -0.91 X_3$	97.30	00.40
$Y=50.65- 0.79 X_1- 5.81 X_2$	96.90	13.30
$Y=57.69- 2.89 X_1$	83.60	83.60
Bug per leaf	R^2	Role of individual traits (%)
$Y= -18.95- 6.12 X_1+ 8.87 X_2 -274.63 X_3 +13.34 X_4 -0.34 X_5 +0.99 X_6 -1.86 X^7$	99.00	00.30
$Y=1.93- 5.66 X_1+ 7.01 X_2 -229.92 X_3 +11.61 X_4 +0.38 X_5 -1.72 X_6$	98.70	00.00
$Y=2.30- 4.74 X_1+ 7.07 X_2 -224.36 X_3 +12.58 X_4 -1.94 X_5$	98.70	00.50
$Y=47.81- 9.29 X_1+ 5.82 X_2 -201.20 X_3 -1.51 X_4$	98.20	01.70
$Y=101.42- 17.32 X_1+ 0.45 X_2 -0.26 X_3$	96.50	00.40
$Y=104.34- 16.61 X_1- 0.11 X_2$	96.10	21.30
$Y=124.46- 6.12 X_1$	74.80	74.80

X1- phenols content (mg/g), X2- flavonoid content (mg/g), X3- tannins content (mg/g), X4- total alkaloid content (%), X5- free amino acid (mg/g), X6-length of leaf & X7-width of leaf

Antixenotic mechanism leaf traits of the re-evaluated Indian cherry accessions

The length of leaf ranged from 9.49 to 14.62 cm, the maximum was found in AHCM-22-1 followed by AHCM-25 and the minimum in AHCM-01 (Table 5) and were significantly high length in resistant and minimum length in susceptible germplasm accession. Significantly positive correlation was observed between length of leaf and width of leaf (0.903). Significantly negative correlations was observed with percent bug infestation (- 0.868) and bug density per leaf (-0.854) (Table 6). The width of leaf ranged from 6.60 to 14.75, the maximum was recorded in AHCM-14 and the minimum in AHCM-26 with values significantly higher in resistant germplasm accession and lower in susceptible germplasm accession (Table 5). Significantly negative correlation was observed between width of leaf and percent bug infestation (-0.835) and bug density per leaf (-0.767) (Table 6).The resistant germplasm accession like AHCM-22-1, AHCM-25 and AHCM-34 having leaf size (very large leaf), leaf roughness (high rough) and leaf hairyness (high hairy) and being high in resistant and low in susceptible germplasm accession (Table 5). Backward stepwise regression analysis indicated that the width of leaf explained 0.6 % of the total variation in the leaf infestation and 0.3 % of the total variation in the bug per leaf (Table 7 & Plate 2).

Table 5. Morphological (Antixenotic) leaf traits of different accessions of Indian cherry, *Cordia myxa*

Accessions	Leaf length (cm)	Leaf width (cm)	Leaf size	Leaf roughness	Leaf hairyness
AHCM-14	14.36 ^{e}	14.75 ^{e}	Very large	Medium rough	Less hairy
AHCM-16	12.38 ^{cd}	8.54 ^{bc}	Medium	Soft	Less hairy
AHCM-09	10.51 ^{ab}	8.74 ^{bc}	Medium	Soft	Less hairy
AHCM-01	9.49 ^{a}	7.27 ^{ab}	Small	Soft	Less hairy
AHCM-30	14.56 ^{e}	14.70 ^{e}	Very large	Less rough	Medium hairy
AHCM-34	13.81 ^{de}	12.89 ^{d}	Large	High rough	High hairy
AHCM-22-1	14.62 ^{e}	12.88 ^{d}	Large	High rough	High hairy
AHCM-26	10.56 ^{ab}	6.60 ^{a}	Very small	High soft	Less hairy
AHCM-33	9.85 ^{ab}	8.56 ^{bc}	Small	Soft	Less hairy
AHCM-31	14.10 ^{e}	12.74 ^{d}	Large	Medium rough	Medium hairy
AHCM-24	11.47 ^{bc}	9.60 ^{c}	Small	High soft	Less hairy
AHCM-08	9.52 ^{a}	9.34 ^{c}	Medium	Less rough	Medium hairy
AHCM-25	14.59 ^{e}	13.66 ^{de}	Large	High rough	High hairy
Mean± SD	12.29± 2.12	10.79± 2.88			
SEm±	0.567	0.513			
LSD (P = 0.05)	1.67	1.51			

Degree of freedom for replication=2; treatment=12; error=24

Plate 2. Variation in leaf hardiness of germplasm accession of Indian cherry, *Cordia myxa* during final screening trials

Based on Kaiser Normalization method

Based upon the above mentioned morphological and biochemical characters individually, it was impossible to group them as variables because they were not in agreement with each other. Therefore, principal component analysis (PCA) was performed to achieve parsimony and reduce the dimensionality in the samples. It was done by excluding the smallest number of components that accounted for most of the variation in the original multivariate data. Taking into consideration, nine parameters *viz*., leaf infestation, bug density per leaf, flavonoid content, total alkaloid content, tannins content, free amino acid, phenols content, length of leaf and width of leaf PCA was performed. One principal component (PC) was extracted with eigen value ≥1.0, after varimax rotation with Kaiser normalization procedure which converged into three iterations. The extraction communalities for all the variables tested were ≥ 0.5 indicating that the variables were well represented by the extracted PC which explained a cumulative variation of 90.07 %. PC had the loadings for flavonoid content (0.99), tannins content (0.97), total alkaloid (0.99), phenols content (0.88), free amino acid (-0.96), leaf length (0.90) and leaf width (0.86) (Table 8).

Table 8. Component loadings of parameters for resistance against *D. cheriani* in Indian cherry,*Cordia myxa*

S. No.	Parameters	Principal component
1	Leaf infestation (%)	-0.98
2	Bug density per leaf	-0.98
3	Tannins content	0.97
4	Flavonoid content	0.99
5	Phenols content	0.88
6	Total alkaloid	0.99
7	Free amino acid	-0.96
8	Leaf length	0.90
9	Leaf width	0.86

Extraction Method: Principal Component Analysis; One component extracted.

Discussion

Plant defense strategies against insect herbivores may involve the synthesis of a plethora of biologically active compounds (allelochemicals) which are phylogenetically conserved in specific plant families or genera (Mithofer and Boland 2012). Furthermore, host quality also depends on differences between the genotypes of the plant, including differences in morphological traits, nutrient contents, and the concentration of secondary compounds (Haldhar et al. 2013b; Cartea et al. 2014; Haldhar et al. 2015a; Haldhar et al. 2017; Haldhar et al. 2018a). In the present study, a variable level of resistance and susceptibility was shown by accessions of Indian cherry against tingid bug. To our knowledge, published literature particularly focused on the resistance attributes in accessions of Indian cherry against tingid bug is not known till time. However, numerous studies on different crops have shown that accessions of the same species could significantly differ in their resistance to insect-pests (Wet & Botha 2007; Moslem et al. 2011; War et al. 2012; Haldhar et al. 2013a, Cartea et al. 2014; Haldhar et al. 2015a; Haldhar et al. 2016 a & b) which was influenced by morphological and biochemical traits of plants. Similar to our findings, Gogi et al. (2010) and Haldhar et al. (2015b) observed lower fruit infestation and larval densities on resistant genotypes as compared to susceptible genotypes of other crops.

The allelochemical compounds of leaf were significantly differ among the tested accessions of Indian cherry. The flavonoid content was positively correlated to tannins, phenols and total alkaloid content. The negative correlations were observed with percent bug infestation, bug density per leaf and free amino acid. This study also agreement with the finding of different workers (Simmonds & Stevenson, 2001; Renwick et al., 2001; Simmonds 2003; Treutter 2006). The tannins content of tested germplasm was the maximum in AHCM-22-1 followed by AHCM-25 and the minimum was found in AHCM-01. The negative correlation was observed with percent bug infestation, bug density per leaf and free amino acid and positive correlations with flavonoid, phenols and total alkaloid content. Tannins had a strong deleterious effect on phytophagous insects and affected the insect growth and development (Barbehenn & Peter Constabel 2011). Tannins were astringent (mouth puckering) bitter polyphenols and acted as feeding deterrents to many insect-pests. In addition, tannins also chelated the metal ions, thereby reducing their bioavailability to herbivores. When ingested, tannins reduced the digestibility of the proteins thereby decreased the nutritive value of plants and plant parts to herbivores (Bernays 1981; Grayer et al. 1994) The phenols content of the test germplasm was the maximum in AHCM-22-1 and the minimum in AHCM-01. The positive correlations were observed between phenols content and tannins, flavonoid and total alkaloid content and negative correlation with percent bug infestation, bug density per leaf and free amino acid. Phenols acted as a defensive mechanism not only against herbivores, but also against microorganisms and competing plants to various abiotic factors. Qualitative and quantitative alterations in phenols and elevation in activities of oxidative enzyme in response to insect attack was a general phenomenon (Barakat et al.

2010; War et al. 2011). Lignin, a phenolic heteropolymer played a central role in plant defense against insects and pathogens (Barakat et al. 2010). Phenols played an important role in cyclic reduction of reactive oxygen species (ROS) such as superoxide anion and hydroxide radicals, H2O2 and singlet oxygen, which turn activated a cascade of reactions leading to the activation of defensive enzymes (Maffei et al. 2007). Similar trend was also observed in different previous study (Simmonds 2003; Johnson et al. 2009; Shivashankar et al. 2015).

Plant structural traits such as leaf surface wax, thorns or trichomes, and cell wall thickness and lignifications formed the first physical barrier to feeding by the insect (Agrawal et al. 2009; Haldhar et al. 2015c). In present study, the antixenotic mechanisms of leaf traits were significantly different among the tested germplasm accessions of Indian cherry. The maximum length of leaf was found in AHCM-22-1 followed by AHCM-25 and minimum in AHCM-01 and being high length in resistant and the minimum length in susceptible germplasm. The positive correlation was observed between length of leaf and width of leaf and negative correlations with percent bug infestation and bug density per leaf. The maximum width of leaf was recorded in AHCM-14 and the minimum in AHCM-26. The negative correlation was observed between width of leaf and percent bug infestation and bug density per leaf. The resistant germplasm of Indian cherry (AHCM-22-1, AHCM-25 and AHCM-34) had the maximum leaf size, high rough and high hairy. In these findings, biophysical traits were also found significantly different among tested genotypes (Gogi et al. 2010). The first line of plant defense against insect pests is the erection of a physical barrier either through the formation of a waxy cuticle (Hanley et al. 2007) and/or the development of spines, setae, and trichomes (Sharma et al. 2009). Structural defenses include morphological and anatomical traits that confer a fitness advantage to the plant by directly deterring the herbivores from feeding (Agrawal et al. 2009) and range from prominent protrubances on a plant to microscopic changes in cell wall thickness as a result of lignification and suberization. Structural traits such as spines and thorns, trichomes, toughened or hardened leaves, incorporation of granular minerals into plant tissues and divaricated branching played a leading role in plant protection against herbivory (He et al. 2011). Chamarthi et al. (2011) reported that leaf glossiness, plumule and leaf sheath pigmentation were responsible for shoot fly *Atherigona soccata* (Rondani) resistance in sorghum *Sorghum bicolor* (L.) (Moench).

Based on Kaiser Normalization method, one principal component (PC) was extracted explaining cumulative variation of 90.07% in tingid bug infestation. PC had the loadings for flavonoid content (0.99), tannins content (0.97), total alkaloid (0.99), phenols content (0.88), free amino acid (-0.96), leaf length (0.90) and leaf width (0.86). The PC was plotted showed five discrete classes of germplasm accession which grouped into resistant (R), moderately resistant (MR) and moderately susceptible (MS), susceptible (S) and highly susceptible (HS) as depicted in Fig. 1. According to Gogi et al., (2010) maximum variation in fruit infestation was explained by tannin and flavonoid contents whereas, rest of the biochemical fruit traits explained <0.2% variation in the fruit infestation. Haldhar et al. (2015a) found that two principal components (PCs) were extracted explaining cumulative variation of 90% in melon fruit fly infestation and length of ovary pubescence, rind thickness, flavonoid content, ascorbic acid, free amino acid, tannins content, and phenols content were the reliable variables for characterization of resistance. Ridge gourd varieties/genotypes AHRG-57, Pusa Nasdar and AHRG-29 were classified as resistant to *B. cucurbitae* and these could be used in future breeding program as resistant sources. Prasad et al. (2015) observed two principal components (PCs) were extracted explaining cumulative variation of 76.2%. Seed weight, grain hardness, oviposition, adult emergence, median development period and grain weight loss were the reliable variables for characterization of resistance to *S. oryzae*. The sorghum lines EC 24, EC 22, PEC 8, PEC 7, EP 78, EP 57, AKR 354 were classified as resistant to *S. oryzae*.

Conclusion

Thus, from the foregoing account, it could be argued that reduction in tingid bug infestations on resistant accessions could be due to phenotypic (biophysical) characters and antibiosis (allelochemicals). Indian cherry accessions AHCM-22-1 (12.22%), AHCM-25 (14.17%) and AHCM-34 (17.57%) were classified as resistant to *D. cheriani* and these could be used in future breeding program as resistant sources. Certain biochemical traits (e.g. flavonoid, tannins, phenols content and total alkaloid) and biophysical traits (e.g. length and width of leaf, leaf roughness and leaf hairyness) were linked to resistance of Indian cherry against *D. cheriani* and therefore, could be used as marker traits in plant breeding programmes to select resistant accessions.

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