Research Article

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ALLELOPATHIC EFFECTS OF *CLERODENDRUM INERME* GAERTN. AND *CLERODENDRUM VISCOSUM* VENT. ON TWO CROP PLANTS *CICER ARIETINUM* L. AND *LATHYRUS SATIVUS* L.

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ABSTRACT

Allelopathic effects of aqueous leaf extracts of *Clerodendrum inerme* Gaertn. and *Clerodendrum viscosum* Vent. are investigated in *Cicer arietinum* L. and *Lathyrus sativus* L. using various parameters related to germination (germination frequency, mean germination time, speed of germination and germination rate index), seedling growth (radical and plumule length) and cytological (mitotic index and aberration frequency) consequences. The germination frequency is unaffected, although the speed of germination is delayed in comparison to controls. The seedling growth, however, is significantly affected and the inhibition is pronounced with an increase in duration of treatment. The crop species, *Cicer* and *Lathyrus* show reduced mitotic activity following treatment with *Clerodendrum* extracts. Among the two *Clerodendrum* species, *C. viscosum* exhibits stronger allelopathic and antimitotic effect than *C. inerme*. Both the *Clerodendrum* extracts manifest its genotoxic potential in *Lathyrus* only; while, mitotic aberrations are not observed in *Cicer*.

Keywords: Allelopathy, Leaf aqueous extracts, *Clerodendrum inerme*, *C. viscosum*, *Cicer* and *Lathyrus*.

INTRODUCTION

Allelopathy is a biological phenomenon in which biochemicals (allelochemicals) of one organism influences the growth, development, reproduction and survival of the other organism in a beneficial or detrimental way[1]. Allelopathy plays an important role in various ecosystems leading to a wide array of interactions between plant species[2]. The allelochemicals in plants are usually secondary metabolites[3] present in different amounts in different plant parts. Studies on allelopathy show tremendous potential in agricultural research[4] and community studies[5]. In recent years, the applied allelopathic research turns its focus to figure out the effects of weeds on crops, weeds on weeds, crops on weeds and crops on crops[6, 7, 8].

Clerodendrum viscosum Vent. (syn. *C. infortunatum* Linn.) and *C. inerme* Gaertn. (Family: Verbenaceae[9]; however, a recent phylogenetic classification includes these species in family Lamiaceae[10]) are common weeds of South Bengal found to grow near the crop fields and fallow areas. *C. viscosum* is an erect shrub possessing large leaves; while, *C. inerme* is a much branched, straggling, evergreen shrub. They grow luxuriantly during rainy season and increase their biomass rapidly. Both the species are reported for their phytochemical contents[11, 12,] and their potential use in various bioactivities and therapeutic uses[13, 14, 15]. A number of reports highlight the allelopathic effect of *Clerodendrum* spp. in mustard, wheat, fenugreek, mung bean, lettuce, gram and different weeds by inhibiting germination and seedling growth[16, 17, 18]. Studies indicate that the soil samples taken from the rhizosphere of *C. viscosum* and *C. trichotomum* can retard the germination, root and shoot growth of a number of weed species[19, 20].

The present study deals with the allelopathic effects of aqueous leaf extract of *C. viscosum* and *C. inerme* on two rabi crops namely, *Cicer arietinum* L. and *Lathyrus sativus* L. (Family: Fabaceae) with an objective for agronomic improvement. The study consider parameters like, germination frequency, mean germination time, speed of germination, germination rate index, seedling growth including radical and plumule length, mitotic index and mitotic aberrations.

MATERIALS AND METHODS

Plant material and seed source

Fresh leaves of *C. inerme* Gaertn. and *C. viscosum* Vent. were collected from Kalyani University campus (22.9750^0 N and 88.4344^0 E, 9.75 m above sea level), Kalyani, West Bengal during the month January 2016 and 2017. The voucher specimens were deposited in the herbarium of Department of Botany, Kalyani University, West Bengal. Seed samples of *Cicer arietinum*; var. Anuradha (chickpea) and *Lathyrus sativus*; var. Ratan (grass pea) were procured from Pulses and Oil seeds Research Station at Berhampore, Murshidabad –742101, West Bengal, India.

Preparation of plant aqueous extracts

Fresh leaf samples were collected washed with deionised water to remove the dirt and surface dried to get rid of excess water using blotting paper. Leaves (5g) were cut into small pieces and extracted with deionised water (1:10 w/v) by maceration for 24h at room temperature (30^{0}C± 1^{0}C) following gentle shaking. The crude extracts were filtered through a Whatman filter paper (No.1) to remove the leaf debris and the filtrates (aqueous extracts) were used in the study.

Germination and growth inhibitory assay

Seeds were surface sterilized (4% sodium hypochlorite solution for 10 min), washed repeated times and dried with filter papers to remove the surface moisture. The non-imbibed seeds were soaked in leaf extracts for 6h, 12h, 24h, and 48h. Control sets were maintained each time following treatment of seeds in deionised water. The experiments were set up in 6cm Petri dishes lined with moist (5ml water) filter papers. The experiments (3 replicates in each case with 20 seeds) were carried out under laboratory conditions (light period-14h, dark 10h; temp. 30 $\pm 1^0$C). Germination of seeds (bursting of seed coat and emergence of radical) was ascertained after every 6h up to 48h. The root (radicle) and shoot (plumule) lengths of all the seedlings were measured on a millimeter graph paper after 5th day (time from placing the seeds in Petri plates).

Germination parameters like, germination percentage (GP), mean germination time (MGT), germination rate index (GRI), and co-efficient of velocity of germination (CVG) were calculated by following the methods of ISTA[21]. The following formulae were used for determining different parameters:

a. Germination percentage (GP) = No. of seed germinated/ total no. of seeds x 100
b. Mean germination time[22] (MGT) = $\sum$ N. d/ $\sum$ N,

Where, N = No. of seeds germinated on day d

c. Germination Rate Index[23] (GRI) = No. of germinated seeds/ days of first count + …. + No. of germinated seeds/ days of final count.
d. Coefficient of velocity of germination[24] (CVG) = $N_1+N_2+\ldots. +N_n/100 \times N_1T_1+N_2T_2+\ldots.+N_nT_n$ where N_1 = number of germinated seeds on time T_1, N_2 = number of germinated seeds on time T_2, N_n = number of germinated seeds on time T_n.

Assessment of Mitotic activity

The control and treated roots (1-2 mm) of *Cicer* and *Lathyrus* were cut and fixed in acetic alcohol (1:3; v/v) for 24 h and preserved in 70% ethanol under refrigeration (16^0C). The roots were stained in 2% orcein: 1(N) HCl mixture (9:1) following gentle warming in a spirit lamp. The tips were cut and squashed in 45% acetic acid in a glass slide and observed under light microscope (Leitz Laborlux S.). Mitotic index (MI) was calculated from the formula:

Mitotic index = number of dividing cells/ number of cells observed ×100.

For each treatment, 3 replicates were maintained and the results obtained were statistically analyzed. The incidence of chromosome aberrations was calculated by expressing the number of aberrant cells as a percentage of total dividing cells for each treatment. Photomicrographs were taken from temporary squash preparations using Leica EC3 scientific camera.

RESULTS

Effect of plant extracts on germination parameters

Leaf extracts of *C. viscosum* and *C. inerme* do not show any effect on germination frequency of either of the studied plant species; however, delay in germination is evident (Table 1). Germination parameters like germination rate index (GRI) and coefficient of velocity of germination (CVG) are found to decrease; while, mean germination time (MGT) show higher values compared to controls following treatments with *Clerodendrum* extracts (Table 1). The extent of germination delay is minimal in *Lathyrus* compared to *Cicer*. However, treatment with *C. viscosum* extract on *Cicer* shows marked delay in germination compared to *C. inerme*. Retardation in germination increases with an increase in presoaking time of the seeds. The highest retardation is observed in *Cicer* following 48h presoaking with *C. viscosum* extract compared to control (GRI- 12.0/ 22.4; CVG- 59.2± 2.9/ 98.2± 0.89; MGT- 1.7± 0.01/1.0± 0.01).

Effect of plant extracts on seedling growth

The effects of plant extracts on seedling growth are evaluated by measuring root and shoot length. Compared to controls, a decrease in root and shoot growth is observed with treatments (Figure 1). Maximum inhibition in root and shoot length is observed with *C. viscosum* extracts and the inhibition increases with the duration of treatment. The root growth of *Cicer* is marginally affected by *C. inerme* extracts; however, shoot growth is affected significantly with duration of treatment (Figure 1; A & B). *C. inerme* extracts exert inhibitory effect on root and shoot growth of *Lathyrus* and it is significant at 24h and 48h treatments (Figure 1; C & D). *C. viscosum* extracts significantly inhibit the root and shoot growth of *Cicer* and *Lathyrus* and the effect is proportional to duration of treatment. A comparison of root and shoot growth following treatments reveal that shoots are more affected than roots in both the crop species.

Evaluation of plant extracts on mitotic activity and cytotoxic effects

As compared to controls, plant extracts are found to decrease divisional frequency in both *C. arietinum* and *L. sativus* and the reduction is pronounced with an increase in duration of treatments (Table 2). Results demonstrate that *C. viscosum* exert relatively higher inhibition in mitotic index than *C. inerme*. Out of the two crop species, divisional frequency seems to be more affected in *Cicer* than *Lathyrus*. Maximum inhibition in *Cicer* is observed at 48h treatment with extract of *C. viscosum*. No mitotic aberrations are recorded in treated samples of *Cicer*. On the contrary, mitotic anomalies (Figure 2B-I) including normal dividing cells (2n=14; Figure 2A) are observed in *L. sativus*. The aberrations encountered in *Lathyrus* are clumping and stickiness of chromosomes, ring configuration of chromosomes (Figure 2B), paired fragments (Figure 2C), polyploid cell formation with fragments (Figure 2E), laggards (Figure 2F), bridges (Figure 2G), multipolarity (Figure 2H) and giant cells (Figure 2I) following treatments with both the leaf extracts. The percentage of mitotic abnormality enhances in *L.sativus* with duration of treatments, of which *C. viscosum* is more effective.

Table 1: Germination parameters in response to *Clerodendrum* extracts

Treatments		*Lathyrus sativus*				*Cicer arietinum*			
Duration (h)	**Extract type**	**GP (%)**	**GRI (%/day)**	**MGT (Day)**	**CVG**	**GP (%)**	**GRI (%/day)**	**MGT (Day)**	**CVG**
6	Control	100	20.7	1.1± 0.03	89.5± 2.2	100	21.0	1.1± 0.06	91.7± 5.0
	C.inerme	100	19.7	1.2± 0.02	84.5± 2.0	100	18.4	1.3± 0.15	79.6± 9.0
	C.viscosum	100	18.8	1.2± 0.02	80.4± 1.5	100	17.3	1.3± 0.15	77.9± 9.0
12	Control	100	22.0	1.0± 0.02	97.2± 2.0	100	22.5	1.0± 0.00	99.3± 0.7
	C.inerme	100	21.8	1.0± 0.02	95.8± 2.1	100	21.0	1.1± 0.06	91.7± 5.0
	C.viscosum	100	20.2	1.1± 0.02	86.6± 1.6	100	16.0	1.4± 0.09	70.6± 5.0
24	Control	100	22.5	1.0± 0.00	99.3± 0.7	100	22.2	1.0± 0.00	99.0± 1.0
	C.inerme	100	20.7	1.1± 0.03	89.5± 2.5	100	20.5	1.1± 0.04	88.4± 3.0
	C.viscosum	100	20.4	1.1± 0.03	87.8± 2.5	100	12.0	1.7± 0.03	59.2± 3.0
48	Control	100	22.0	1.0± 0.02	97.2± 2.0	100	22.4	1.0± 0.01	98.2± 1.0
	C.inerme	100	20.9	1.1± 0.06	91.1± 5.0	100	22.2	1.0± 0.02	97.9± 2.1
	C.viscosum	100	19.7	1.2± 0.08	85.1± 5.5	100	12.05	1.7± 0.01	58.9± 1.0

Table 2: Mitotic index and aberration frequency in the studied plant species following leaf extract treatments

Treatments		*Lathyrus sativus*				*Cicer arietinum*			
Duration (h)	**Extract type**	**No. of cells observed**	**No. of dividing cells**	**Mitotic index (%)**	**Aberration frequency (%)**	**No. of cells observed**	**No. of dividing cells**	**Mitotic index (%)**	**Aberration frequency (%)**
6	Control	1720	310	18.0	0	1552	225	14.5	-
	C. inerme	1824	246	13.5	1.6	1380	174	12.6	-
	C.viscosum	1640	188	11.5	2.8	1350	120	8.8	-
12	Control	1510	270	17.8	0	1654	217	13.1	-
	C. inerme	1580	216	13.7	1.6	1950	244	12.5	-
	C.viscosum	1330	150	11.3	4.6	1615	146	9.0	-
24	Control	1326	242	18.3	0	1042	146	14.0	-
	C. inerme	2112	276	13.1	3.0	891	87	9.7	-
	C.viscosum	1966	198	10.0	5.1	1592	104	6.5	-
48	Control	1752	332	19.0	0	1220	165	13.5	-
	C. inerme	2120	260	12.3	3.5	1492	126	8.4	-
	C.viscosum	2096	192	9.2	5.3	1020	58	5.68	-
*CD at 5% level				3.18	3.84			2.22	

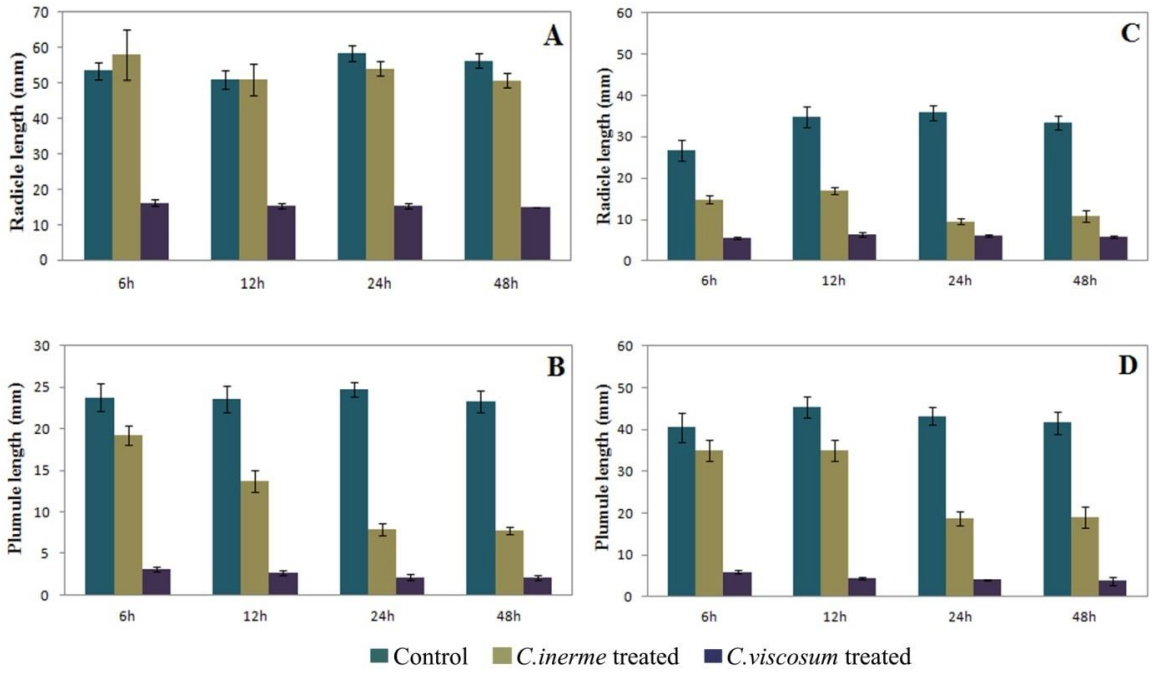

Figure 1: Seedling length (A and C -radical; B and D -plumule) in *Cicer arietinum* (A and B) and *Lathyrus sativus* (C and D) after 5 days following treatments

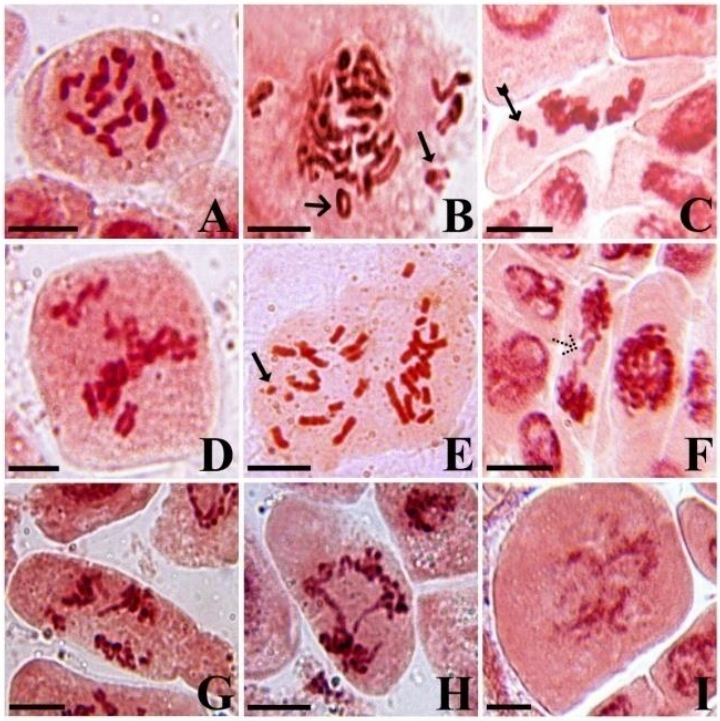

Figure 2: A –I. Mitotic configurations (A –E, metaphase; F –H, anaphase; I, resting cells) showing normal (A) and aberration (B-I). **A.** Normal, **B.** Ring configurations (→), **C.** Paired fragments (>—>), **D.** Groupings, **E.** Polyploid cell with fragments, **F.** Laggards (¯>), **G.** Multipolarity, **H.** Bridge with multipolarity, **I.** Giant cell. Scale bar = 10µm.

DISCUSSION

The study demonstrates that the two *Clerodendrum* species exert their effects on germinability and seedling growth of *Cicer* and *Lathyrus*. Similar studies documenting allelopathic effects are reported in other crop species including *Cicer* following treatments with aqueous leaf extracts of *C. viscosum*[16, 17, 18]. *C. viscosum* can also inhibit the germination and seedling growth of a number of common weeds[19, 20]. *C. trichotomum* has shown promise in weed control by inhibiting seed germination of *Eichhornia crassipes*[25]. Contrary to earlier observations, present study demonstrates that the germination frequency of *Cicer* and *Lathyrus* is unaffected by *Clerodendrum* extracts; however, the treatments slow down the speed of germination. Hence, results indicate that the allelopathic effect on germinability of these seeds by *Clerodendrum* extracts is minimal. This is may be due to different treatment conditions of the extracts to the seeds or due to the selectivity of allelochemicals in the extracts against these two crops.

The seedling growth of these two crops is however substantially inhibited by the extracts. The higher growth inhibition corroborates with the duration of treatment time indicating increase in time allow allelochemicals to permeate and give more time to interact with cellular metabolites that influences growth. These observations are in agreement with the earlier studies[17, 26, 27]. However, the divergent effects studied in germination and seedling growth point towards the existence of at least two types of allelochemicals in the extracts which merits the identification of these compounds in future research.

In treatments, mitotic indices of are significantly lower than the controls. Deviation from normal mitotic activity is considered as a measure for cytotoxicity in living organisms. The reduction in mitotic index might be explained as hindrance before the onset of prophase, the arrest of one or more mitotic phases or slowing down the cell cycle progression during mitosis. Studies on *Allium* root tip cells and mouse bone narrow cells exhibit decreased mitotic activity with *Clerodendrum* extracts[28]. The antimitotic activity of root tip cells was exhibited by a number of plant extracts[29, 30]. Results suggested the possible presence of cytotoxic substances in the *Clerodendrum* leaf extracts.

Among the two crop species, *Lathyrus* manifests aberration in mitotic cells. The chromosomal aberrations encountered in *Lathyrus* show breakage, spindle aberrations and cellular metabolic defects. The absence of mitotic aberrations in *Cicer* may be due to its specificity against these extracts and/or smaller chromosome size. Whereas, the chromosomal aberrations induced in *Lathyrus* may be the consequence of biochemical components present in *Clerodendrum* extracts.

CONCLUSION

The work emphasizes the allelopathic potential of aqueous leaf extracts of *C. viscosum* and *C. inerme*, studied in *C. arietinum* and *L. sativus*. Differential response of the species under study is noted vis-à-vis plant extract(s). The study highlights the necessity for further investigation on the identification of allelochemicals as well as its allelopathic potentiality in other crop species for agronomic management.

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