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International Journal of Current Research
Vol. 4, Issue, 04, pp.032-040, April, 2012

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ALIEN INVASIVE LEGUMES AND ALLELOPATHY: A CASE STUDY AT GANGETIC WEST BENGAL, INDIA

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ARTICLE INFO

Article History:
Received 25th January, 2012
Received in revised form
25th February, 2012
Accepted 27th March, 2012
Published online 30th April, 2012

Key words:
Allelopathy,
Biological invasion,
Lathyrus sativus L.,
Legumes,
Leucaena leucocephala.

ABSTRACT

Alien legumes exert major influence in modulation of sustainable agriculture and crop productivity in diverse ways. An investigation was carried out in inventorizing alien legume plants and their invasive nature in districts of Nadia, Hooghly, Howrah, 24 pgs (N) and Kolkata in Gangetic West Bengal during 2005-2011. As many as 40 alien species were documented, of which 14 species are highly invasive in nature and the tree *Leucaena leucocephala* was the worst invasive in nature. About 40% of plants have been used by local people as food and fodder, 20% in medicinal, and rest 40% as biomass fuel, manuring and in other diverse economic and commercial purposes. Allelopathic effect of an invasive legume on crop legume was ascertained by studying germination, growth traits and root-tip mitotic activity of *Lathyrus sativus* L., treated with leaf aqueous extracts of *Leucaena leucocephala* in different concentrations (0, 10, 20, 30 and 40%). A dose-dependent reduction in germination and plant dry weight was accompanied with significant ($P<0.05$) decrease in mitotic index and enhancement of mitotic abnormalities in *Lathyrus* plants. While 10% extract dose had no significant effect, concentrations of 20% onwards were found highly inhibitory to growth, and induced chromotoxicity in *Lathyrus* genomes.

INTRODUCTION

Invasive alien species are colonizer species that have established populations outside their native distributional ranges and that have potential to spread and affect native ecosystems or local human-mediated systems (Mooney *et al*., 2005). Biological invasions by alien species are widely recognized second worst threat to native biodiversity and impose high costs to agriculture, forestry, and aquatic ecosystems with far greater magnitude in developing worlds including south Asian nations than those in developed countries (Barua *et al*., 2000; Afrin *et al*., 2010; Singh *et al*., 2010). About 18% of the Indian flora is composed of alien species (Mandal, 2011), of which legumes constitute a major part along with other angiospermic species. The status of alien legume plants are still uncertain, because these group of plants contain common edible crops, commercial crops and under-utilized species, and is considered second largest economically important family after daisies (Dixon and Sumner, 2003). The state of West Bengal is located between 85° 50′ and 89° 50′ E and 21° 38′ and 27° 10′ N, and one of the densely populated but the biodiversity rich states in India. The lower Indo-Gangetic basin constitutes fertile hub for diverse types of flora and fauna, covering districts of Nadia, Hooghly, Howrah, and 24 pgs, dotted with numerous wetlands, forest covers and vast agricultural lands. Bhagirathi is the major river and with Jalangi, Churni, Ichhamati, Rupnarayan and some small rivers constitute the riverine and floodplain ('Baor') systems in this basin. The tropic of cancer passes through the upper part (Nadia district) of this basin. The climate of this basin region is tropical monsoon with three distinct seasons-summer (March-early June), rainy (June-September) and winter (October –February), and mean annual rainfall ca, 1800mm. While maximum summer temperature may sore to 43 °C, winter is extremely chilled with night temperature may plummet to 2-3 °C. Although some reports are available regarding the floral diversity of this region in wetland and forest areas (Das and Lahiri, 1990; Bala and Mukherjee, 2010), no investigation was carried out to document the legume plants, alien in this basin. As invasive species have huge ecological impacts and preference over native species in native ecology due to their faster rate of growth, documentation of alien plants is necessary. More specifically, as legumes are main source of plant protein in our daily meal and diverse economic activities in this region but at the same time contained many compounds (like non-protein amino acids), potential to participate in invasion, their roles as invasive species on native biodiversity of this basin region need to be assessed. Allelopathy, the chemical inhibition of one plant species by another, represents a form of chemical warfare between plants competing for limited light, water, and nutrient resources (Bais *et al*., 2003). This biological phenomenon has been considered one of the most potent weapons for the successful invasion of many exotic plants

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(Hierro and Callaway, 2003). Legumes are considered major component of different cropping systems due to their biological nitrogen fixation capacity. However, this group of plants often exhibits alleopathic interaction within them and are potential targets of non-leguminous alien weeds and large trees (Narwal, 2000; Manimegalai and Manikandan, 2012). The declining legume population and inhibition of their nitrogen fixation capacity due to alleopathic effect of invasive weed is a major cause of concern for grain and forage production (Halsall *et al*., 1995; Narwal, 2000). In the preset investigation, a case of allelopathic effect of invasive legume on germination, seedling growth and induction of cytotoxicity on a crop legume was studied considering *Leucaena leucocephala* (Lam.) de Wit as a donor plant. This evergreen tropical and sub-tropical legume is an aggressive tree in pastures, grasslands, along riverbanks and roadsides, and even agricultural fields of India (Sahoo *et al*., 2007), and has been enlisted one of the 100 worst invasive plants in the world (Lowe *et al*., 2000). It is introduced mainly for development of agro-forestry, and grows well in different parts of India including Gangetic basin of West Bengal. Although used as a multipurpose legume, its introduction often causes toxicity to other plants and even animals (Yeung *et al*., 2002). Among the crop legumes, grass pea or *Lathyrus sativus* L. was selected as the target species. This is an annual dual-purpose crop cultivated for both food as well as forage in different geographical regions, and is known for its remarkable hardiness against diverse types of biotic and abiotic stresses including salinity, heavy metals and drought (Talukdar, 2011a,b; Vaz patto *et al*., 2006). The crop is also an excellent cytogenetic material as utilized in recent years to develop desirable mutants and cytological tester stocks for trait mapping, stress perception and genomic research (Talukdar, 2009a, 2010a, 2011e, g, 2012). The crop is selected for the present study due to its high seed germinability, easy seedling growth, and handy cytological analysis which is ideal for rapid screening of alleopathic effect on legume plants at cytogenetic level. As *Leucaena* is a common and wide-spread perennial shrub in and around fields of legume cultivation, leaf allelopathic effect of this invasive tree has been tested, for the first time, on grass pea as target crop, considering growth traits and cytological parameters as bio-indicators of allelopathy and invasion. The objectives of the present study are, therefore, set to document the alien legume flora, their classification and use by local people in selected areas of Gangetic basin of West Bengal. To assess the allelopathic effect, growth traits parameters and mitotic activities along with chromotoxicity have been investigated on grass pea plants, treated with leaf aqueous extracts of *Leucaena leucocephala*.

MATERIALS AND METHODS

Study area

The present investigation was carried out by extensive field survey during the last seven years (2005-2011) in different intervals (March-June, September-January) in selected areas of Nadia (situated between 22˚52´30˝ N and 40˚05´40˝ N latitude and 88˚08´10˝ E and 88˚48´15˝E longitude), Hooghly (Latitude: 23 ˚ 01' 20"N -22 ˚ 39' 32"N, Longitude: 88 ˚ 30' 20"E-87 ˚ 30' 15"N), Howrah (22˚48′ N and 22˚12′ N latitudes and between 88°23′ E and 87°50′ E longitudes) and North 24 pgs (Latitude: 23°15'N-22°11'N, Longitude: 89°5'E - 88°20' E) districts and also in and around Kolkata (22° 34' N - 88° 24' E) (Fig. 1).

Collection of data and methods of inventory

Plant samples were collected either in flowering or in fruiting stage, and voucher specimens were deposited in departmental herbaria, R.P.M. College, Uttarpara, Hooghly. Invasive nature of alien species, enlisted by IUCN, was studied using techniques of Baider and Florens, (2011) and other recent works (Huang *et al*., 2009; Feng and Zhu, 2010), namely through a combination of random walks through the area along with a more quantitative sampling of the seedlings and larger woody plants (flowering or fruiting stage) in a series of square quadrats (1 × 1 m for seedlings and 10 × 10 m for tree). Frequency (F%) of particular plant species was calculated by dividing the number of quadrats in which a particular species occur with total number of quadrats laid down. The specimens were identified through extensive survey of available literatures, monographic works, and confirmed by IPNI data base (www.IPNI.org). Use of documented flora was tabulated through interviews of knowledgeable people like temple priests, village head, old experienced folk, medicinemen, farmers, teachers, etc. Gathered information was thoroughly cross-checked through structured questionnaires, and documented thereafter. Nativity of the species was tested from the available web literatures (Lowe *et al*., 2000).

Preparation of aqueous leaf extracts

Leaves of *Leucaena leucocephala* plants were collected district-wise from infested areas. Collected samples were carefully cleaned, shade dried for 96 h, ground, and stored. The extracts were prepared by soaking 40 g of crushed mass in sterilized distilled water for 24 h at room temperature (24-28 °C), filtered through Whatman No. 1 filter paper, made up to final volume of 100ml (40% w/v). The extract was considered as stock solution (kept at 5 °C until used).The solution was further diluted with appropriate amounts of distilled water to get strength of 30%, 20% and 10% (v/v). The protocol and dose of extracts were standardized by preliminary experiments on survival of *Lathyrus sativus* plants.

Seed germination and measurement of seedling growth parameters

Fresh and healthy seeds of grass pea (*Lathyrus sativus* L.) cv. 'BioR-231' were surface sterilized in 70% ethanol for 2 min, rinsed twice in deionized water and then placed on water-moistened filter papers in 9cm diameter Petridishes in an incubator at 25 °C with 12 h light following the protocol as reported earlier (Talukdar, 2011d). Germination was tested in five treatments, namely 0 (control), 10, 20, 30 and 40% extract concentrations in three replicates with the layout of completely randomized block design at department of botany, R.P.M. College, Uttarpara (22° 40'E, 88° 20' N), Hooghly, West Bengal, India during last week of October, 2009-2010. Instead of extract, 10 ml of distilled water were used to soak filter papers for the control treatment. After 5 d, the germination rate was recorded in each treatment considering emergence of both plumule and radicle, and expressed as percentage. For seedling stage study, germinated seedlings were immediately transferred to twelve inches earthen pots containing farm land soil (6.5 Kg, sandy-loam, pH 7.2) during first week of November 2010. Before that, soil was passed through 2-mm sieve to discard non-soil particles and then drenched with 100 ml of leaf extracts of different concentrations in separate sets. The soil drenched with 100 ml of sterile distilled water was served as control. Transferred seedlings were thinned to one per pot after emergence and the pots were then kept under control condition (temperature day/night 20 °C /27 °C, humidity of 70%, photon-flux density of 200µmol m^{-2} sec^{-1} and 14 h photoperiod) for 30 d (onset of flowering) in randomized block design with three replications per treatment (10 seedlings per treatment). Treatment commenced on eight-day old seedlings with the test solutions on alternate days and with tap water on the other days. For measurement of growth parameters, lengths of both root and shoot (cm $plant^{-1}$) were measured at harvest (30 d old plant). Dry masses (g $plant^{-1}$) of the shoot and root were obtained after drying the samples at 60 °C for 48 h. Leaf injury level was ascertained with a scale of 0-4 standardized for grass pea (Talukdar 2011d, f) considering 0: no injury (without any damage), 1: mild injury, indicated by small area (approximately1/5) of leaflet apex and margin turning brownish yellow, 2: moderate injury, indicated by ½ of the leaflet turning whitish-yellow, 3: severe injury, when over 80% of total leaflet area turned whitish yellow and very thin, and 4: extreme injury, when leaflets became necrotic, crinkled, and finally fell off. Leaflets, borne on first formed primary branches, were considered for visual scorings of the trait.

Measurement of mitotic activity and abnormalities

For cytogenetic assay of allelotoxicity induced by *Leucaena* extracts on mitosis, seeds of grass pea plants were sterilized and placed for germination in similar conditions as described above, except the facts that the seeds were at first allowed to germinate in Petri dishes lined with two sheets of germination paper pre-moistened with 5 mL of distilled water, and after germination, were transferred to Petri dishes moistened with 10 mL of different concentrations of aqueous extracts (10 to 40%) for 18 h. Root tips (2-3 mm) were cut and washed thoroughly with distilled water. After washing, the root-tips were fixed in a freshly prepared mixture of absolute ethanol and propionic acid (2:1v/v) for overnight following the procedure of Talukdar (2010b, c). To determine the mitotic index and the presence of chromosomal aberrations, root tips were stained and hydrolyzed with 9:1 aceto-orcein (Hi-media, 2%) and HCl (1 N) and kept for 45 min, and subsequently squashed in a drop of 45% acetic acid. Three replicates were performed for each treatment and scoring was determined from 5 roots of each replicate. The mitotic index was calculated as the percentage of dividing cells among the examined total cells; at least 3000 cells were analyzed per treatment. Chromosomal abnormalities were calculated as the percentage in relation to examined total cells. The chromosomal abnormalities observed in root-tip mitosis are presented with photo-micrographs.

Statistical analysis

Analysis of Variance (STATISTICA 6.0, StatSoft, Inc. Tulsa, and U.S.A) was carried out for different traits at germination and seedling stages. A probability of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Documentation and classification of alien taxa

Present inventorization of the alien legume flora revealed occurrence of 40 species belonging to 21 genera (Table 1). Among the plant growth form, herbs constituted 48%, and it was followed by shrub (22%), tree (20%) and climbers (10%). Although possession of tendril is a common feature in papilionoid genera, the ability to climb has been taken as criteria to mention a climber. Several genera like *Acacia*, *Lathyrus*, *Phaseolus*, *Cassia*, *Crotalaria* etc. were found to possess three or more species (Table 1). About 87% of alien legumes are invasive in nature of which 8 species were primarily categorized as 'very high' invasive and 6 species are primarily designated as 'highly' invasive'. Twelve species were categorized as 'moderate' and 9 species showed 'low' invasiveness. Five species are not invasive in nature although they were alien in study areas (Table 1). Among the most invasive species, *Leucaena* and *Cassia* exhibited tremendous capacity to grow along roadside as well as deep inside the study areas. Cultivated fields and banks of water bodies were preferred by 20% and 10% species, respectively. Quadrat studies revealed high frequency of some of the legumes like *Leucaena leucocephala, Cassia sophera*, *C. tora*, *C. occidentalis*, *Crotalaria pallida*, *Melilotus alba and Phaseolus* in study sites (Table 1). The ratio of number of plants (cumulative of 400 quadrats/year) between cultivated field and roadside varied between 0.53-0.88, but it was close to 1.0 for *Cassia sophera* (0.98), and >1.0 for *Leucaena leucocephala*.
Documentation of spread of alien flora cumulative of 2800 total quadrats laid over the last seven years (2005-2011) revealed steep rise in number of certain legume species such as species of *Cassia, Crotalaria, Leucaena, Melilotus, Desmodium, and Phaseolus* (Fig. 2). Low to moderate rise was documented for other species. Some of these species particularly *Leucaena* reportedly possesses allelopathic potential, which has been postulated as one of the potent weapons for rapid introduction and seedling establishments of invasive legumes even in rough, polluted and nutrient-deficient terrain throughout the world (Lee, 2002).

Resource utilization of alien legumes by local people

The 40 legume species documented as alien flora in the present inventory have been used by local population as food, fodder, medicinal, ornamental, commercial (fishing, thatching, basket making, etc,), religious and other purposes (Table 1), revealing resource utilization by people in diverse ways. As local people reveled, wild beans (*Phaseolus* spp), *mungs* (*Vigna* spp), *khesari* (*Lathyrus sativus* L.) and *jangli matar (Lathyrus aphaca* L.) have considerable benefits in their daily life; seed flour as food supplement, making *besans*, *pokaras*, whole plant as fodder, soil fertilizer (mulching), and tender pod as vegetables. About 40% of total plants were used as food and fodder, while 20% plants were directly or indirectly utilized as medicinal purposes and rest of the plants had use in other economic and house-hold activities. Among the small-scale cottage industries, commercial 'shola' using *Aeschynomene americana* were found highly beneficial for local economics. Different types of wood works, another financially viable activity within the study area, are carried out with *Prosopis julifera* (Table 1). Talukdar and Talukdar (2012) recently documented diversity of different floras and identified legume resources in a sub-Himalayan river basin of West Bengal, and found extensive use of leguminous plants by people. Uses of leguminous plants as both food and forage by village folks have considerable significance as legumes are cheap source of plant protein with many essential amino acids, antioxidant flavonoids, and minerals (Dixon and Sumner,

Table 1. Alien and alien invasive legume species in selected study sites at Gangetic basin of West Bengal, India

Sl.no.	Species	Invasion Category	Life form	Nativity	Use
1	*Acacia auriculiformis* A. Cunn.ex. Benth	D	Tree	Australia	Bf
2	*Acacia farnesiana* (L.) Willd.	D	Tree	Trop. America	Fd, Cf, M
3	*Acacia holosericea*	B	Shrub	Australia	Or
4	*Aeschynomene americana* L.	C	Herb	Trop. America	Co, 'shola'
5	*Caesalpinia pulcherrima* (L.)Sw.	E	Shrub	Trop. America	Or
6	*Caesalpinia spinosa* (Molina) Kuntze	E	Shrub	Trop. S. America	Or
7	*Cajanus lineatus* (Wight & Arn.) Maesen	D	Herb	S. E. Asia	Fd, Cf
8	*Calliandra houstoniana*(Mill.) Standl.	C	Shrub	Trop. Africa	Cf
9	*Cassia alata* L.	D	Shrub	West Indies	M, Thatching
10	*Cassia fistula* L.	E	Tree	S. E. Asia	M, Or
11	*Cassia javanica* L.	C	Tree	S.E. Asia	Or, M
12	*Cassia occidentalis* L.	B	Herb	Trop. S. America	M, Bf
13	*Cassia sophera* L.	A	Herb	Trop. S. America	M, Bf
14	*Cassia tora* L.	A	Herb	Trop. America	M
15	*Clitoria ternatea* L.	B	Climber	S. E. Asia	M, sacred
16	*Clitoria annua* J. Graham.	D	Climber	S. E. Asia	Or
17	*Clitoria arborea* Benth.	D	Tree	Australia	M
18	*Crotalaria pallida* Dryand	A	Herb	Trop. America	Bf
19	*Crotalaria retusa* L.	A	Herb	Trop. America	Bf
20	*Cytisus scoparius* (L.) Link	C	Herb	Europe	M
21	*Delonix regia* (Boj. ex Hook.) Raf.	E	Tree	Trop. Africa	Or
22	*Desmodium elegans* DC.	A	Shrub	China	Or
23	*Desmodium triflorum* (L.) DC.	C	Herb	Trop. America	M
24	*Indigofera astragalina* DC.	D	Herb	Trop. America	Cloth washing
25	*Indigofera linifolia* (L.f.) Retz.	D	Herb	Trop. America	NU
26	*Lathyrus aphaca* L.	C	Herb	Mediterranean	M, Cf, mulching
27	*Lathyrus ochyrous* L.	C	Herb	Mediterranean	Cf
28	*Lathyrus sativus* L.	C	Herb	Mediterranean	Pulse, Fd, besan, Cf, veg
29	**Leucaena leucocephala* (Lam.) de Wit	A	Tree	Trop. America	Bf, basket making,
30	*Melilotus alba* Desv.	A	Herb	Europe	Insecticide
31	*Melilotus officinalis* L.	C	Herb	Europe	M
32	*Mimosa pudica* L.	B	Herb	Trop. S. America	M
33	*Mimosa pigra* L.	C	Shrub	Trop. America	M
34	*Phaseolus aureus* L.	B	Climber	Trop. America	Fd, Cf, veg
35	*Phaseolus coccineus* L.	A	Climber	Mediterranean	Fd, Cf
36	*Prosopis juliflora* (Sw.) DC.	C	Tree	Trop. S. America	Wood works
37	*Sesbania grandiflora*	D	Shrub	Trop. America	Bf, veg, M, Or
38	*Ulex europaeus* L.	E	Shrub	Europe	Or
39	Vigna *speciosa* (Kunth) Verdc	C	Herb	Trop. America	Cf, mulching
40	*Vigna sublobata* (L.) *Wilczek*	B	Herb	S. E. Asia	Pulse, 'bori', besan, Cf

* enlisted in database of world's 100 worst invasive; M-medicinal, Co-compost, Or -ornamental, Bf-biomass fuel, Cf-cattle feed, Fd-Food, Veg-Vegetables, NU-not in use, ** Invasion category based on F (%)-A-very high (91-100), B-High (71-90), C-moderate (51-70), D-low (21-50), E-Not invasive (0-20), F (%)= Frequency %= Number of quadrats in which a particular species occur /2800 × 100; cumulative of 400 quadrats /year during years of 2005-2011.

Table 2. Effect of leaf aqueous extracts of *Leucaena leucocephala* on germination and seedling growth parameters of target crop, grass pea (*Lathyrus sativus* L.)

Parameters	Leaf extracts concentrations (%)						
	0 (control)	10	20	30	40	F (treatments)	CD at 5%
Germination (%)	99.5 ± 0.8	99.0 ± 0.7	49.7 ± 0.6	40.8 ± 0.9	0.0 ± 0.0	24.72*	2.1
Shoot length (cm plant^{-1})	27.22 ± 0.3 (100)	26.19 ± 0.7 (96)	22.68± 0.5 (83)	18.14± 0.3 (66)	9.72± 0.09 (35)	39.99*	3.2
Root length (cm plant^{-1})	10.33 ± 0.1 (100)	10.41 ± 0.5 (100)	6.88 ± 0.1 (66)	4.13± 0.2 (39)	2.06 ± 0.06 (19)	31.09*	2.5
Dry mass of shoot (g) plant^{-1}	0.11 ± 0.04 (100)	0.11 ± 0.02 (100)	0.07 ± 0.02 (63)	0.05 ± 0.01 (45)	0.04 ± 0.00 (36)	37.07*	0.03
Dry mass of root (g) plant^{-1}	0.13 ± 0.04 (100)	0.11 ± 0.02 (84)	0.05 ± 0.02 (38)	0.03 ± 0.01 (23)	0.02 ± 0.00 (15)	45.23*	0.05

Data are means ± standard error of three replicates, *Values varied significantly at (P<0.05) level across treatments in ANOVA. Values within bracket are in relation to control in percentage.

2003). Fruits and flowers of the ornamental legume *Sesbania grandiflora* have been used by village folks in different ailments, as the plant is one of the richest natural sources of vitamin A (Padmaja *et al*., 2011). The huge potential of under-utilized and 'poor man's' legume like *Lathyrus* in sustainable agro-biodiversity, relay-cropping and maintenance of soil nutrition has been recognized in recent decade and genetic improvement programs have been undertaken in these

Table 3. Mitotic index (MI%) values and chromosome abnormalities (%) of *Lathyrus sativus* L. root-tip meristem cells induced by *Leucaena leucocephala* leaf extracts at different concentrations (0, 10, 20, 30 and 40%) of treatments

Mitotic parameters	Concentrations (%)					Percentage of total aberrant cells	F	CD at 5%
	0 (control)	10	20	30	40			
MI	15.20 ± 4.2	14.90 ± 3.9	9.90 ± 2.8	7.35 ± 2.0	3.39 ± 0.9	-	25.09*	2.3
Interphase and prophase								
Micronuclei	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.89 ± 0.2	0.89 ± 0.2	325.10*	0.0
Metaphase								
Stickiness	0.00 0± 0.0	0.001 ± 0.0	0.48 ± 0.1	0.51 ± 0.3	0.49 ± 0.4	1.48 ± 0.9	10.50*	0.05
Breaks	0.00 ± 0.0	0.00 ± 0.0	0.21 ± 0.09	0.88 ± 0.3	0.96 ± 0.2	2.05 ± 0.11	10.04*	0.18
c-mitosis	0.00 ± 0.0	0.00 ± 0.0	0.53 ± 0.2	0.79 ± 0.6	0.81 ± 0.7	2.13 ± 1.2	19.27*	0.21
Anaphase								
Sticky bridge	0.00 ± 0.0	0.007 ± 0.0	0.37 ± 0.3	0.65 ± 1.1	0.09 ± 1.0	1.62 ± 1.8	22.19*	0.17
Laggard	0.00 ± 0.0	0.00 ± 0.0	0.21 ± 0.7	0.51 ± 1.1	0.10 ± 1.7	1.52 ± 2.1	20.39*	0.23
Diagonal orientation	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.33 ± 0.5	0.00 ± 0.0	0.33 ± 0.5	9.95*	0.001
Multipolar movement	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.28 ± 0.1	0.002 ± 0.0	0.28 ± 0.3	10.06*	0.00
Telophase								
Diagonal movement	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.23 ± 0.02	0.00 ± 0.0	0.23 ± 0.0	5.69*	0.004

Data are means (± SE) of three replicates with a total of 4500 divisional stage studied, *parameters differed significantly ($P<0.05$) across treatments by ANOVA.

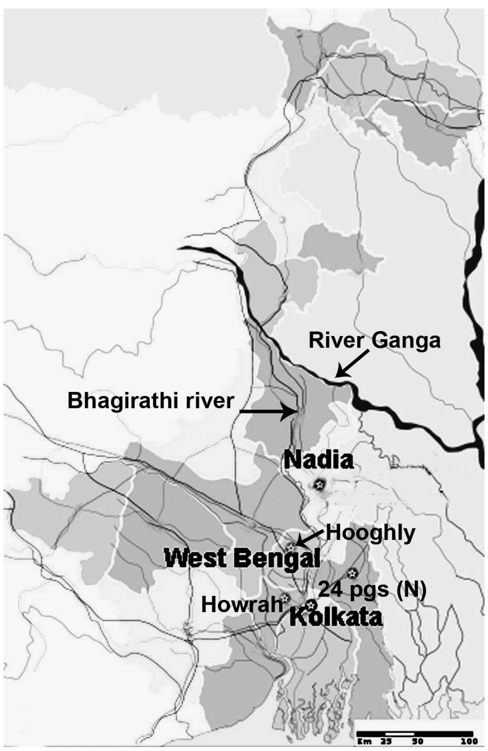

Fig. 1. Study areas of Gangetic West Bengal with districts of Nadia, Hooghly, Howrah, North 24 Pgs, and Kolkata

directions (Talukdar, 2009b, 2011c). Identification of wild legumes as alien species in the present study assumes significance for three reasons: first, their utilization in crop improvement, second, their role/effect in alteration of legume-pollinator relationship with existing native cultivars in the invaded region, and third, as many parts of the study area is arsenic-contaminated, their potential to accumulate toxic

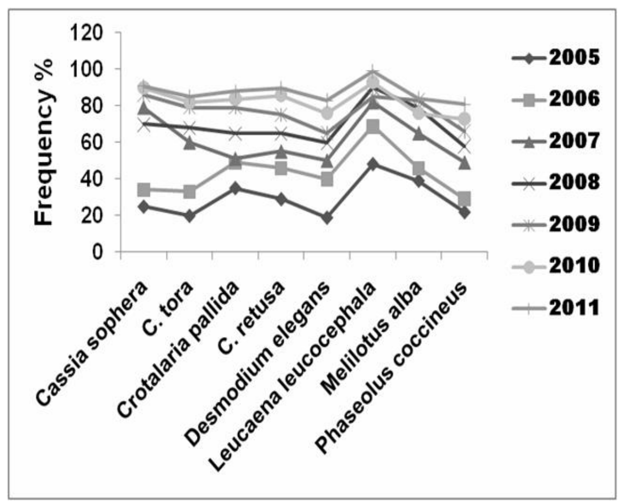

Fig. 2. Frequency of eight highly invasive legumes in the study areas during the study periods of 2005-2011, data represented are cumulative of 400 quadrats laid /year.

metals in edible part and concomitant risk to consumers. As biological invasions are related to climate change and pollution (Kriticos *et al*., 2003; Thuiller *et al*., 2007; Crooks *et al*., 2011), legumes used as food as well as forage purposes (*Phaseolus*, *Vigna*, *Lathyrus*) needs special attention.

Nativity of documented alien flora

Contribution of different geographical regions in terms of nativity of documented flora was shown in table 1. Tropical America alone accounted for nearly 35% plants, followed by share of South-East Asia (15%), tropical South America (12%), Europe and the Mediterranean (10% each), Australia (7%), tropical Africa (5%), and other regions (6%). Among the 40 taxa documented in the present inventory, one legume

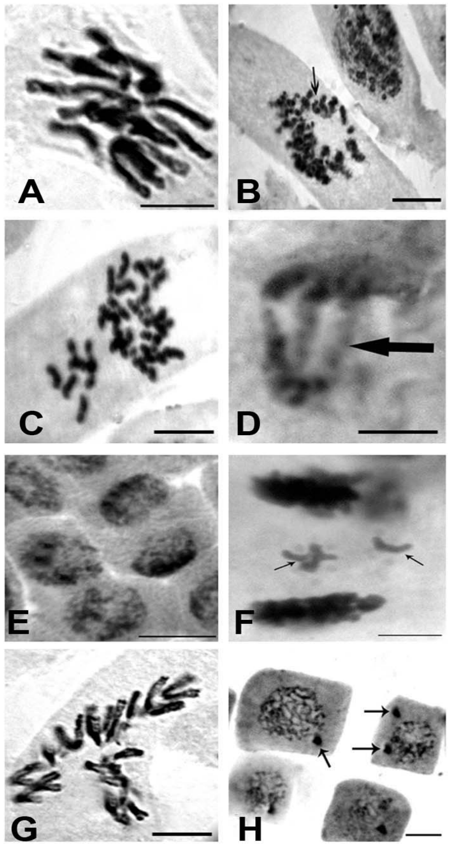

Fig. 3. Mitotic abnormalities observed in root-tip mitosis of *Lathyrus sativus* L. plant induced by different concentrations (0, 10, 20, 30 and 40%) of leaf aqueous extracts of *Leucaena leucocephala*; (A) sticky metaphase, (B) chromosome breaks (→), (C) c-mitosis, (D) bridge (→) formation at anaphase, (E) diagonal orientation, (F) lagging (→) chromosome/s, (G) multipolar tendency, and (H) micronuclei (→) formation at interphase and early prophase. Bar represents 1SD=10 µm.

species, *Leucaena leucocephala* are enlisted as world's 100 worst invasive species (Lowe *et al*., 2000). The dominance of this aggressive legume has been attributed to the presence of strong allelopathic effects on native species due to presence of mimosine, an allelo-chemical in its leaves (Yeung *et al*., 2002).

Effect of *Leucaena leucocephala* leaf extracts on germination and seedling growth parameters of *Lathyrus sativus* L.

The effect of *Leucaena leucocephala* L. leaf aqueous extracts (10-40%) on seed germination and seedling growth of *Lathyrus sativus* L. was studied in Petri dish and pot experiments, respectively. Germination was 99.5% in control, but reduced with increasing concentrations of the leaf extracts (Table 2). In comparison to control, germination decreased by about 50% at 20% extract concentration, and there was no germination at 40%. For seedling growth parameters, no significant change ($P<0.05$) was observed at control and 10% extract concentrations, whereas leaf extracts from 20% to 40% markedly reduced the shoot and root length as well as their dry weights of *Lathyrus* seedlings (Table 2). Compared to control, root lengths reduced by about 1.5-fold, 2.5-fold, and nearly 5-fold, whereas length of shoot decreased by about 1.2-fold, 1.5-fold and 2.8-fold at 20%, 30% and 40% extract concentrations, respectively (Table 2). Dry weight of plant parts in *Lathyrus* seedlings also decreased with increasing concentrations, exhibiting nearly 1.5-2.5-fold reduction for shoot and 2.5-4.7-fold for root across the treatments ranged between 20% and 40% (Table 2). Decreasing root length was accompanied with reduction in lateral branches and conspicuous absence of nodulation from 20% dose onwards in treated plants. No leaf injury was visible at control plants, but it became marked (level 1) at 20% and elevated to level 4 at 40% concentration. All the parameters of seedling growth showed highest inhibition at 40% aqueous extract, but the root length and its dry weight reduced by greater magnitude than those of shoot (Table 2). The results strongly indicated that aqueous extracts of *Leucaena* leaves at concentrations >10% substantially reduced the percentage of seed germination and seedling growth parameters of target plant *Lathyrus sativus* L as dose-dependent way and no germinated seeds were observed following 40% extract treatments. This indicated toxic level of germinability of grass pea seeds at this concentration. A number of previous studies have also suggested that the degree of growth inhibition increases with increasing extract concentrations (Laosinwattana *et al*., 2007, 2009). For seedling growth, plant extracts at 20% reduced the length and dry weight of root as well as length of shoot about 50% compared to control, and the inhibition increased at elevated concentrations. Interestingly, degree of reduction of both shoot and root length was identical at 20% treatment dose, but the root length was relatively more sensitive to aqueous extract than shoot length at elevated doses. Furthermore, nearly 2.5-fold reduction in dry weight of root was found at 20% treatment dose, where the same for shoot dry weight was achieved at 40% extract concentration. These results agree with earlier studies reporting that water extracts of allelopathic plants had more pronounced effects on root growth than on shoot growth (Batish *et al*., 2006; Siddiqui, 2007). Such an outcome might be expected because it is likely that roots are the first organ to absorb the allelochemicals from soil environment (Turk and Tawaha, 2002). Apart from inhibition of shoot growth, effect of *Leucaena* leaf extract on aerial part of target plant was also evidenced by conspicuous appearance of leaf injury and increase of its level as dose-dependent manner in pot bioassay. It, therefore, becomes clear that *Leucaena* leaf extracts effectively inhibited the seed germination and growth of *Lathyrus* seedlings, and the result is in agreement with reports regarding inhibition of germination and growth of different crop legumes like *Pisum*, *Vigna*, *Cajanas* and *Cicer*, treated with *Leucaena* leaf extract (Ahmad *et al*., 2008; Siddiqui *et al*., 2009). Oudhia (1999a, 2001) reported allelopathic effect of aqueous leaf extracts of non-legume plant species on germination and seedling vigour of grass pea plants. However, both growth promoting and inhibitory effects of allelopathy were observed in mung bean, soybean and chickpea (Oudhia *et al*., 1997; Oudhia, 1999b).

Chromotoxic effect of *Leucaena* leaf extracts on mitotic index and division abnormalities in root-tip cells of *Lathyrus sativus* L.

Significant ($P<0.05$) differences in mitotic index (MI) values were observed between control and *Leucaena* leaf extract-

treated *Lathyrus sativus* L. root in respect of concentrations (Table 3), and differences between extract doses increased due to lower values of MI at elevated treatment doses (Table 3). The MI values in the control and 10% treatment changed non-significantly ($P<0.05$). However, extract concentrations ranging between 20% and 40% significantly ($P<0.05$) reduced MI values, and highest reduction (5-fold), compared with control, was observed at 40% dose (Table 3), indicating a dose-dependent decrease in MI value in the present material. The results also indicated that 10% concentration of *Leucaena* leaf extract was not inhibitory to *Lathyrus* root-tip mitosis, whereas concentration ranging between 20% and 40% was found highly inhibitory. The *Leucaena* leaf extracts also induced a variety of mitotic abnormalities in root-tip cells of *Lathyrus sativus* L (Table 3). At metaphase, percentage of cells with chromosome stickiness, breaks and frequency of C-mitosis increased significantly ($P<0.05$) at concentrations >10% (Fig. 3A-C). Chromosome stickiness has been considered as chromosome agglutinations displaying a sticky appearance, and usually being irreversible in nature, it is the most lethal type of cytotoxic effect in plants (El-Ghamery *et al*., 2003). Consequently, this led to formation of chromosome bridges through the rejoining of the sticky ends and may lead to chromosome breaks or fragmentation also (El-Ghamery *et al*., 2003). Conspicuous occurrence of chromosome stickiness, and breaks in the preset study is an indication that *Leucaena* leaf extract has the allelotoxic potential on the organization of chromatin, and suggests a possible cytogenetic mechanism by which this invasive legume exert its influence on target plant.
The anaphasic abnormalities occurred in the form of bridge formation (with or without fragments), lagging chromosomes, diagonal orientation, and multipolarity (Figs. 3D-G), whereas micronuclei (MN) formation occurred at interphase and prophase stages (Fig. 3H). While percentage of bridge formation increased across 20-40% doses, diagonal orientation and multipolarity occurred only at 30% extract concentration. On the other hand, MN was scored only at 40% extract treatment. Occurrence of c-mitosis, lagging chromosomes, multipolarity and diagonal orientations are considered strong indications of severe disturbances in mitotic spindle apparatus (Teerarak *et al*., 2010), induced by *Leucaena* leaf extracts on grass pea divisional cells. In general, it was observed that *Leucaena* leaf extract provoked a significant increase in the percentage of total mitotic abnormalities. Decrease in mitotic activities indicated mito-depressive effect of *Leucaena* leaf extract; while chromosomal abnormalities revealed its chromotoxic potential on *Lathyrus* chromosomes. Decrease in mitotic index and increasing chromosomal anomalies due to clastogenic potentials of different plant extract was also reported in roots of maize (Tūrker *et al*., 2008), *Vicia faba* (Sabita and Bhagirath, 2005; Haroun, 2010) and many other plants (Jose *et al*., 2008; Teerarak *et al*., 2010).

Formation of micronuclei (MN) in mitotic interphase and prophase is another important event encountered in *Leucaena* extract treated *Lathyrus* root-tip mitosis. Micronuclei (MN) formation was considered as an important biomarker for ascertaining chromotoxic/genotoxic damage of plants including legumes (Liu *et al*., 2003/4; Ünyayar *et al*., 2006). MN results from chromosome fragments or whole chromosome lagging during cell division, and high frequency of lagging chromosomes/fragments with concomitant rise in micronuclei formation at specific doses in the present material is in agreement with earlier observation on aneuploid lines of grass pea (Talukdar and Biswas, 2007, 2008; Talukdar, 2008). Remarkably, MN frequency in the present *Lathyrus sativus* plant was concentration-dependent, the specificity of which can certainly be used as a reliable cytogenetic parameter in assay of alleopathic effect of invasive plants on target crops.

CONCLUSION

In the present investigation, occurrence of alien legumes, their use and nature of invasiveness were documented, for the first time, in Gangetic West Bengal. Among the 40 species identified, *Leucaena* emerged as the worst invasive, showing highest frequency in quadrat study. The plant possessed strong allelopathic potential on crop legumes as evidenced by inhibition of germination and growth parameters of target crop, *Lathyrus sativus* L. Furthermore, *Leucaena* leaf extracts triggered cytotoxic damage as exhibited by significant decline in mitotic index and high chromosomal abnormalities in root-tip divisional cells of *Lathyrus*. Occurrence of leaf injury in target crop due to allelopathy was a unique phenotype, encountered in this study. Among the concentrations (0, 10, 20, 30 and 40%) tested, dose of 20% onwards was found inhibitory to toxic.

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