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ALLELOPATHIC EFFECT OF *CASSIA OCCIDENTALIS* LEAVES ON MUSTARD SEEDS

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ABSTRACT
Cassia occidentalis was analyzed to evaluate the existence of allelopathic effect using fully viable seeds of mustard seeds (*Brassica campestris* L.) as bioassay material. The present study shows that mustard seeds pretreatment with various concentrations [1:5 and 1:10(w/v)] of *Cassia occidentalis* fresh leaf extracts and dry leaf leachates strongly reduced the percentage and speed of seed germination and TTC-stainability. Soluble carbohydrate and amino acid levels were rapidly increased in the leachates of seeds pretreated with Cassia leaf extracts and leaf leachates. Levels of insoluble carbohydrate and protein as well as activities of dehydrogenase and catalase enzymes were significantly reduced and soluble carbohydrate level as well as activity of IAA-oxidase enzyme was significantly increased in seed samples pretreated with leaf extracts and leaf leachates of *Cassia*. Existence of allelopathic effect of the plant species *Cassia* was confirmed from the reliable physiological and biochemical data of the present investigation.

KEY WORDS: allelopathy, *Cassia occidentalis*, mustard seeds.

INTRODUCTION

The interference in the growth of one plant by another can result either from competition which involves the removal of some factors (nutrient, water and light) from the environment, habitat or through chemical(s) released from one plant (donor) that effect to other (receiver) sharing the habitat. The phenomenon known as "allelopathy" is now considered as important as competition for influencing plant growth both in natural and agricultural ecosystem. In natural or man managed agro-ecosystems, neighboring plants may interact with the growth and development of other species. The term allelopathy signifies that interacting or inhibition of growth (Molish, 1937) both crop and weed species, by the release of chemicals from plant parts by leaching, root exudation, volatilization residue decomposition and other processes. These interactions are widely known in different groups of plants such as algae, lichens, crops, as well as annual and perennialweeds (Rice, 1984; Putnam, 1985; Horseley, 1991; Lawrey, 1993; Inderjit and Dakshini, 1994 a and b; Bhakat *et al*., 2005; Inderjit, 2005).

There is much evidence that allelochemicals liberated from certain weeds into the soil reduce crop growth(Rice, 1964, 1974, 1979; Putnam and Weston, 1986; Bhakat *et al*., 2002, 2003, 2005a, b, 2006, 2007; Bhattacharjee *et al*., 2003; Kanp *et al*., 2004).Approximately 6700 species, out of about 3,000,000 species of the flowering plants are recorded as weeds in agro-ecosystems of the world (Holm *et al*., 1979) of these, 76 weed species are categorized as "the World's Worst Weeds" (Holm *et al*., 1977).Only 15 species of the crops which supply 90% of the world's food occupy 75% of the world's tilled land (Harlam, 1975). Most of the food species belong to five families viz., Poaceae, Solanaceae, Convolvulaceae, Euphorbiaceae and Fabaceae and these families also include most of the common weeds. In crop subsystems of agro-ecosystem, crop species (often exotic) are selectively cultivated but weeds grow themselves in crop fields and interact with the crop species in various ways, including reduction in crop yields. Allelopathy is also an expression of the ecological phenomena which are normal constituents of the environment of the terrestrial plants (Datta and Sinha Roy, 1974; Rice, 1984).There are some common indices for assessing allelopathic action of plants or plant parts. These include, among others, germination behaviour and other physio-biochemical responses of test species (Bhattacharjee *et. al*., 2001, 2003; Bhakat *et. al*., 2002, 2005, 2006, 2007).

With considerable evidences adduced during the past few decades demonstrating the presence of inhibitory compounds in a wide variety of plant types and plant parts, the recent upsurge of interest in allelopathy, with major volumes of collected papers and books regularly published(Rice, 1984; Thomson, 1985; Waller, 1987; Rizvi and Rizvi, 1992; Inderjit *et. al*., 1999; Narwal, 1999)has established the topic as one of biological significance.Recently it is focused on establishing research procedures which may improve the credibility of evaluations of the allelopathic potential of the weed *Cassia occidentalis* which forms monospecific stands in different ecosystems in West Bengal. *Cassia* owing to its wide adaptability to different environmental conditions and habitats. Limited research has been done on the allelopathic effect or phytotoxicity of *Cassia* to other plants. There is a general mood of consensus now a days that invasive plants displace the local biodiversity through their harmful effects including allelopathy (Cronk and Fuller, 1995; Shiva, 1999; Bhattacharjee *et al*., 2001; Kanp *et al*., 2004). In fact, allelopathic action of any plants and plant parts affects

germination behaviour, seed metabolism and growth performance of target species which in turn may discourage a species from thriving, thus influencing the whole structure in course of time(Ghosh and Dutta, 1989).
Therefore, it was presumed that perhaps an allelopathic effect of *Cassia* on mustard seeds may be responsible for the inhibitory effect on seed germination behaviour and seed metabolism of target crop. Allelopathic effects may due to the presence of allelochemicals in *Cassia*, like different types of phenolic compounds, alkaloids, triterpenoides, essential oils and flavonoids, biocides, Juvenile hormones, growth hormones. They may be interacting with various physiological processes. Therefore, studies were conducted to test this hypothesis and laboratory experiments have confirmed this.
The objective of this investigation is to screen out the phytotoxicity of the leaf extracts and leaf leachates of *Cassia* on mustard seeds.

BOTANICAL CLASSIFICATION

Kingdom	Plantae
Divison	Rosopsida
Order	Fabales
Family	Fabaceae
Subfamily	Caesalpinioideae
Genus	*Cassia*
Species	*occidentalis*

MATERIALS AND METHODS

Fresh, mature and healthy leaves (100g) of *Cassia* (Family: Fabaceae) collected from Vidyasagar University campus, Paschim Medinipur were thoroughly homogenized using 300 ml double distilled water. The homogenate was strained using a fine cloth and then centrifuged at 5000 g for 15 minutes. The supernatant was then made up to 500 ml using double distilled water and this was considered 1:5 (w/v) proportion stock solution of leaf extract. From this stock solution another concentration grade in the proportion of 1:10 (w/v) was prepared using double distilled water. And this was taken as the two gradation fresh leaf extracts solution. Another lot of dry leaves (100g) sample of the *Cassia* was kept immersed in 300 ml double distilled water in 1000 ml beaker for 48 hours and the leachate was decanted in a separate beaker. The total volume of the leachate was then made up to 500 ml using double distilled water and this was taken as the 1:5 (w/v) proportion of leaf leachate. From this stock solution another concentration grade in the proportion of 1:10 (w/v) was prepared using double distilled water. And this was taken as the two gradations dry leaf leachate solutions of the plant species for allelopathic analysis. Fully viable mustard (*Brassica campestris* L.) seeds in five lots of 10 g each were surface sterilized with 0.1% $HgCl_2$ solution for 90 seconds.

The seed lots were then separately presoaked in the two concentration grade leaf extracts or leaf leachates or in distilled water for 6 hours and thus allowed the seeds for various physiological and biochemical tests. Data on seed germination behaviour (percent and speed of germination, T_{50}),TTC-stainability, leaching of sugars and amino acids and protein, soluble and insoluble carbohydrates level, activities of catalase, dehydrogenase and IAA-oxidase enzymes in seeds were recorded. To analyse percentage germination, three groups of 100 seeds i.e. 300 seeds of each treatment were transferred to separate Petri dishes containing filter paper moistened with 10 ml distilled water. Germination data were recorded after 120 h of seed soaking following the International Rules of Seed Testing (ISTA, 1976).The time for 50% germination of seeds (T50) was determined following the method described by Coolbear *et. al*. (1984).

For analysing TTC-stainability, for 100 seeds samples of dehusked mustard seeds were allowed to imbibe in 0.5% TTC (2, 3, 5-triphenyl tetrazolium chloride) solution (w/v) in Petri dishes for 24 hours in dark condition. Percentage TTC-stainability was recorded taking samples from the embryonal axes of the mustard seeds. Sugar and amino acid levels were analysed from the common seed leachates obtained after immersing 1 g seeds in 10 ml deionized distilled water for 24 hours followed the method of McCready *et. al*. (1950) and Moor and Stein (1948) respectively. Soluble and insoluble carbohydrate levels were analysed from seed kernels by the method of McCready *et al*. (1950). Protein level was analyzed from the seed kernels following the method of Lowry *et al*. (1951). Extraction and estimation of the enzyme catalase and IAA-oxidase were done by the method described by Snell and Snell (1971) modified by Biswas and Choudhuri (1978) and Gordon and Weber (1951) respectively. For the assay of this enzyme the blank was taken as zero time control. The activities of these enzymes were expressed as $[(\Delta A \times Tv)/(t \times v)]$, where ΔA is the absorbance of the sample after incubation minus the absorbance of the zero time control, Tv is the total volume of the filtrate is the time (minutes) of incubation with the substrate and v is the volume of the filtrate taken for incubation (Fick and Qualset, 1975). For analyzing dehydrogenase activity the TTC-stained embryonal axes of the 100 seeds of each treatment were extracted with 5 ml of 2-methoxyethanol and OD values of the solution were recorded at 520 nm. This

method was adopted after Rudrapal and Basu (1979) with slight modification. All the data were statistically analysed at the treatment and replication levels and least significant difference (LSD) values were calculated at 95% confidence limits (Panse and Sukhatme,1967).

Figure1. Inflorescense of *Cassia occidentalis*

Figure 2.Whole plant of *Cassia occidentalis*

Figure 3. Plant material collection from the field.

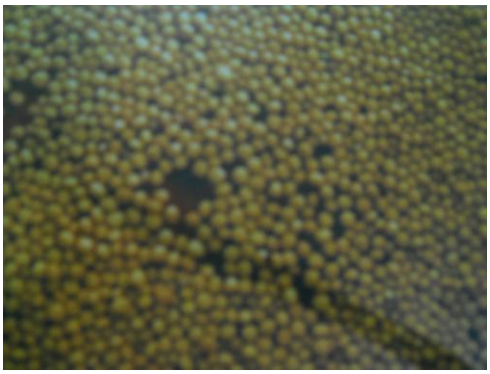
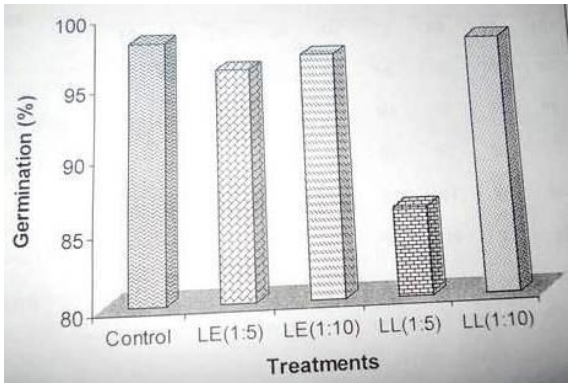


Figure 4. Mustard seeds



LE= Leaf extract, LL= Leaf leachate

Figure 5. Effect of seed pretreatment with leaf extracts and leaf leachates of *Cassia* on percentage germination of mustard seeds.

RESULTS

Effect on germinability (Figure-5) clearly revealed that percentage germination of mustard seeds were strongly inhibited by two concentration grades of fresh leaf extracts and dry leaf leachates of *Cassia*. The allelopathic effect of leaf leachate (1:5) was found inhibitorier than that of leaf extracts and the data shows that the more concentrated leachate and extract were more injurious. Effect on the speed of seed germination (Table-1) shows that in control sample percentage germination of mustard seeds increase with the advancement of the germination period as recorded from 12 to 120 hours. However, both leaf extracts and leaf leachates of *Cassia* rendered inhibition on seed germination during the observation periods recorded at 12-hours intervals. The inhibitory effect was found to be much more

significant at primary observation periods and the effect of dry leaf leachate (1:5)was found to be more significant than fresh leaf extracts.

Table 1. Effect of seed pretreatment with leaf extracts and leaf leachates of *Cassia* on speed of germination of mustard seeds.

Treatments	Percentage germination after hours									
	12	24	36	48	60	72	84	96	108	120
Control	27	70	87	91	94	98	98	98	98	98
Leaf extract(1:5)	0	18	78	89	93	94	95	96	96	96
Leaf extract(1:10)	3	60	91	96	97	97	97	97	97	97
Leaf leachate(1:5)	3	11	52	78	80	83	84	85	86	86
Leaf leachate(1:10)	0	37	84	90	91	93	95	95	98	98
LSD(P=0.05)	2.65	1.77	1.54	1.43	1.21	1.01	NS	NS	NS	NS

NS: Not significant

Table 2. Effect of seed pretreatment with leaf extracts and leaf leachates of *Cassia* on time (hours) to 50% germination (T_{50}) and TTC-stainability pattern of mustard seeds.

Treatments	T_{50} values of germination	TTC-stainability pattern (%)		
		TTC-stained (%)	Full coloured	Partially coloured
Control	18.5	100	100	0
Leaf extract(1:5)	30.1	100	73	27
Leafextract(1:10)	22.4	100	79	21
Leaf leachate(1:5)	35.3	100	68	32
Leaf leachate(1:10)	30.1	100	85	15
LSD (P=0.05)	2.03	NC	4.35	1.65

NC: Not calculated

Table 3. Effect of seed pretreatment with leaf extracts and leaf leachates of *Cassia* on leaching of free soluble carbohydrates and amino acids from mustard seeds.

Treatments	Soluble carbohydrates (mg/g/10 ml)	Amino acids (mg/g/10 ml)
Control	0.23	0.10
Leaf extract (1:5)	0.60	0.26
Leaf extract (1:10)	0.38	0.20
Leaf leachate (1:5)	0.89	0.30
Leaf leachate (1:10)	0.31	0.15
LSD (P=0.05)	0.06	0.04

Time required for 50% seed germination was found to be significantly high in the fresh leaf extracts and dry leaf leachates-pretreated samples (Table-2). Treatment of the mustard seeds with leaf extracts and leaf leachates could not alter gross TTC-stainability, however a differential result was noted. Here more concentrated leaf leachate (1:5) of *Cassia* significantly decreased the percentage of full colour seed staining. It was found that the leaching of soluble carbohydrates and amino acids was higher when mustard seeds underwent pretreatment with the dry leaf leachate (1:5) of *Cassia* and the magnitude of leaching was comparatively less in dry leaf leachate (1:10) and fresh leaf extracts treatment (Table-3).

Internal soluble carbohydrate level was increased in seed samples irrespective of the treatments. On the other hand, insoluble carbohydrate level was found to be significantly reduced in seeds pretreated with leaf extracts and leaf leachates of *Cassia* (Table-4).

Table 4. Effect of seed pretreatment with leaf extracts and leaf leachates of *Cassia* on changes of soluble and insoluble carbohydrate contents in kernels of mustard seeds.

Treatments	Soluble carbohydrates (mg/g wet wt.)	Insoluble carbohydrates (mg/g wet wt.)
Control	13.38	17.15
Leaf extract (1:5)	17.88	10.50
Leaf extract (1:10)	16.01	11.35
Leaf leachate (1:5)	20.63	8.58
Leaf leachate (1:10)	14.71	12.25
LSD (P=0.05)	1.25	0.78

Table 5.Effect of seed pretreatment with leaf extracts and leaf leachates of *Cassia* on the changes of protein content and dehydrogenase activity in the kernels of mustard seeds.

Treatments	Protein (mg/g wet wt.)	Dehydrogenase (Δ OD/100 seeds/5 ml)
Control	48.0	0.41
Leaf extract (1:5)	38.4	0.27
Leaf extract (1:10)	41.7	0.32
Leaf leachate (1:5)	35.0	0.22
Leaf leachate (1:10)	45.2	0.37
LSD (P=0.05)	3.14	0.05

Table 6. Effect of seed pretreatment with leaf extracts and leaf leachates of *Cassia* on the changes of Catalase and IAA-Oxidase activities in the kernels of mustard seeds.

Treatments	Catalase (unit /h/ g wet wt.)	IAA-Oxidase (unit /h /g wet wt.)
Control	117.3	1.25
Leaf extract (1:5)	78.2	2.50
Leaf extract (1:10)	90.0	2.23
Leaf leachate (1:5)	60.4	2.71
Leaf leachate (1:10)	105.1	2.11
LSD (P=0.05)	6.13	0.11

The seed samples pretreated with *Cassia* leaf extracts and leaf leachates treatment resulted in drastic decrease of the protein contents in seed kernels of mustard. Similarly dehydrogenase activity was found to be reduced by the leaf extracts and leaf leachates of *Cassia*, which clearly established the allelopathic potentiality of the species (Table-5).
It was also found that catalase activity was significantly reduced in seed kernels by seed pretreatment with fresh leaf extracts and dry leaf leachates of *Cassia*. On the other hand IAA-oxidase activity was increased in seed samples irrespective of treatments with two concentration grades leaf extracts and leaf leachates of *Cassia* (Table-6).

DISCUSSION

The present study shows that pretreatment of mustard seeds with fresh leaf extracts and dry leaf leachates of *Cassia* reduced percentage germination, speed of germination (Table-1) and TTC-stainability (Table-2) of seeds, enhanced T_{50} hours (Table-2),leaching of soluble carbohydrates and amino acids (Table-3),decreased insoluble carbohydrate (Table-4),protein levels as well as activities of dehydrogenase (Table-5) and catalase enzymes (Table-6).Soluble carbohydrate level (Table-4) and IAA-oxidase (Table-6) activity were increased by the pretreatments. Analysis of germination behaviour is considered to be a reliable index of evaluation of allelopathic action (Ghosh, 1979; Datta and Chakraborty, 1982; Bhattacharjee *et al.*, 2001; Bhattacharjee *et al.*, 2003; Nayek *et al.*,2004).Reduced seed germinability and TTC-stainability, slower rate of germination are important effects of allelopathic action of plants and such action is chiefly

exerted by a number of inhibitors of diverse chemical nature (Ghosh and Dutta, 1989).In this investigation the leaf extracts and leaf leachates induced inhibition of percentage and speed of seed germination is clear indicative of the allelopathic action of the test material. The relatively high allelopathic potential of *Cassia* was recorded from its stronger germination inhibitory capacity.On the other hand more concentrated leaf extracts and leaf leachates were more injurious than more diluted extract and leachate solutions. More concentrated plant extracts or leachates have more inhibitory compounds. Allelopathic action of *Cassia* can also be substantiated from the profuse leakage of soluble carbohydrates and amino acids which is indirect indicative of the damage of seed membrane.Membrane is the most important site of a seed which appears to be affected first by treatment with plant leaf extracts and leachates having strong allelopathic action (Nayek, 2000; Bhattacharjee *et al.,* 2001). Allelopathic potential of *Cassia* plants can also be corroborated from the present data on the leaf extracts and leaf leachates-induced reduction of insoluble carbohydrate and protein levels,as well as activities of dehydrogenasw and catalase enzymes and enhancement of soluble carbohydrate level and IAA-oxidase activity. Various inhibitors present in plants having allelopathic property reduce the overall metabolism of plants or plant parts, and particularly anabolic activities are reported to be strongly impaired (Datta and Chakraborty, 1982; Nayek, 2000; Bhakat, 2006, 2007).Results, therefore, point out that both the leaf extracts and leaf leachates of *Cassia* possess some chemicals which efficiently rendered allelopathic action on mustard seeds.

Thus, a conclusion can be made from this investigation using a number of physiological and biochemical indices that *Cassia* exert strong allelopathic effect on the test material. From the overall observations, high concentration of leaf leachate (1:5) to be more effective with respect to its allelopathic action on the present experimental plant than that of leaf extracts.

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