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Allelopathic effect of *Cassia tora* on seed germination and growth of mustard

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Abstract: *Cassia tora* L. (CT) is a widely growing weed in India with medicinal value and has been reported to show allelopathic potential causing biological suppression on crops. The present study was conducted to observe its suppression on *Brassica campestris* L. (mustard). The rate of seed germination and the root length, shoot length, chlorophyll content, fresh weight (FW), dry weight (DW), and relative water content (RWC) of 10-day-old mustard seedlings were observed after treatment with aqueous extracts of the roots, leaves, and seeds of CT and compared with control sets treated with distilled water. A confirmatory chemical test was conducted to determine the presence of phenols, ketones, acids, and alcohols in the CT plant extracts. Different concentrations of the extracts showed inhibition (up to 100%) or deterioration in all of the parameters. Roots were more affected than shoots and the extract taken from leaves was more suppressive than that taken from other plant parts. The FW, DW, and RWC were considerably reduced upon treatment with the tested extracts. The extracts (>25%) also caused a reduction in the chlorophyll content. The overall results indicate the possible suppressive effect of allelochemicals (phenols, ketones, acids, and alcohols) present in CT on mustard. The overall results with regard to mustard plants followed the same trend of concentration-dependent inhibition in the order of leaf > seed > root.

Key words: Allelochemicals, allelopathy, *Cassia tora*, chlorophyll content

Introduction

Interactions between plants play a crucial role in natural ecosystems. Upon invasion, weeds compete with other plant species, causing adverse effects on natural vegetation, including crops (Rice, 1984). They are a major threat to agricultural systems and cause qualitative and quantitative declines in productivity. The invasive nature of weeds may be due to their allelopathic property. This property is regulated by the presence of various suppressive allelochemicals, which are secondary metabolites like terpenoids and phenolics (Khanh et al., 2007) that have specific actions. Generally, allelochemicals are produced at a later stage of plant development and influence the growth and reproduction of associated plants. This is due to the water-soluble nature of these chemicals (Inderjit, 1996). Such influence can be positive (stimulatory) or negative (inhibitory). All of the chemicals present may not always be harmful, as beneficial interactions have also been reported (Foy & Inderjit, 2001). Recently, a study showed that the allelopathic effect of *Cassia occidentalis* L. causes

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the suppression of seed germination and growth in *Parthenium hysterophorus* L., a detrimental weed in India (Knox et al., 2011). Allelochemicals are present throughout the plant body, though their concentration differs from part to part. The production of allelochemicals is widely influenced by genetics as well as environmental factors at different growth stages (Yu et al., 2003).

Cassia tora L. is an obnoxious, aggressive, annual, herbaceous weed of the family Leguminosae that grows throughout the tropical and subtropical regions of the world. The plant is native to south-eastern Asia, Fiji, northern Australia, Africa, and Latin America (Parson & Cuthbertson, 1992). It also grows well in Japan, Malaysia, Burma, Bangladesh, and Vietnam. Throughout India, the species is of common occurrence in warm, moist climates, especially those in the middle and southern parts of the country. It grows very aggressively, competing with crops for environmental resources and releasing toxic chemicals into the surrounding soil. This plant has been referred to as the most economically destructive weed throughout the country (Oudhia, 1999). The plant causes no harmful effects on animals or humans, however. The leaves and seeds of *Cassia tora* are used in Ayurvedic medicine for the treatment of cough, leprosy, ringworm, colic, flatulence, dyspepsia, constipation, bronchitis, and cardiac disorders (Maity et al., 1998). The seeds are also used as an alternative to coffee. Inorganic forms of the weed extract were reported to have natural pesticidal and fungicidal activity by Kim et al. (2004). For this reason, the plants are not destroyed and are instead allowed by the local people to grow near crop fields. Chemical analysis of this species, however, has indicated the presence of a large number of compounds that are allelopathic in nature. The aqueous extract of the whole plant and leaves produces an inhibitory allelopathic effect on many weeds. The powdered leaves of *C. tora* also affect the growth and metabolism of associated weeds. Leaves of the plant have been found to be rich in phenols, tannins, and glycosides (Prasad et al., 2006). The presence of phenols, glycosides, ketones, and cassitoroside in seeds and roots was reported by Choi et al. (1995) and Kitanaka and Takido (1986).

The objective of the present study was to determine for the first time the allelopathic effect of aqueous extracts of the root, leaf, and seed of *C. tora* on the germination and growth performance of seedlings of mustard, an important oil-yielding crop of the study area.

Materials and methods

Study area

The experimental area, Habra, is situated near the Indo-Bangladesh border, about 50 km from the town proper. Locals in the area depend solely on agriculture.

Preparation of the aqueous solution

Cassia tora (sickle senna) plants were collected at the preflowering stage from the areas surrounding mustard-growing fields. They were washed thoroughly with distilled water and air-dried at room temperature for 96 h. The root and leaf portions were separated, chopped into 1-cm long pieces, and crushed. Ripe and dried seeds were also collected and crushed in a blender, and 10 g of these crushed materials were soaked separately in a corked, conical flask containing 100 mL of distilled water. They were kept on a mechanical shaker for 24 h and filtered. This served as the stock solution from which other concentrations (25%, 50%, 75%, and 100%) were prepared by way of dilution.

Determination of percentage of germination, root length, and shoot length

Mustard seeds were first surface sterilised with 2% sodium hypochlorite solution for 2 min and washed thoroughly with distilled water. Next, 4 sets of autoclaved petri dishes were prepared, each containing a single layer of Whatman No. 1 filter paper and 5 mL of test extract for each concentration (25%, 50%, 75%, and 100%) of roots, shoots, and seeds. The petri dishes treated with distilled water were taken as a control and considered to be set 0. In each prepared petri dish, 10 surface sterilised mustard seeds were placed. A total of 4 replications of the sets with the previously described concentrations were kept undisturbed at room temperature (24 ± 2 °C) in the laboratory for 5 days. The number of germinating seeds was recorded on the sixth day, as were the

root and shoot lengths of the mustard seedlings. The emergence of a radical approximately 1 mm in diameter was taken as the index of germination.

Determination of fresh weight, dry weight, and relative water content

To observe the direct effect of allelochemicals on crops in the field, 20-cm pots were filled with 300 g of soil collected from mustard-growing fields. Throughout the study, the soil collected from well-ploughed mustard fields was used, such that the soil property could be considered constant. Ten surface-sterilised mustard seeds were sown approximately 5 mm deep in each pot. The pots were divided into 3 sets. Set 1 received a daily dose of 50 mL of root extract of various concentrations (25%, 50%, 75%, and 100%). Similarly, sets 2 and 3 were treated with leaf and seed extracts, respectively. The control set was treated with water collected from the water source available to the mustard field. All of the pots were kept in bright sunlight and 3 replications were conducted for each treatment. After 10 days, the seedlings were uprooted from each pot, keeping the root system intact. They were washed under slow-flowing tap water until the adhering soil particles were removed and then soaked between paper towels. Afterwards, the fresh weight (FW) and dry weight (DW; determined by oven-drying at 70 °C for 24 h) of the seedlings were noted. Using the equation of Deef and Abd El-Fattah (2008), the relative water content (RWC) was evaluated as:

$$RWC\% = (FW - DW) / FW \times 100.$$

Determination of chlorophyll content

Chlorophyll content was determined from the leaf portion of 10-day-old potted mustard seedlings irrigated with various concentrations of *Cassia tora* root, leaf, and seed extracts, as described above. For the determination, 100 mg of leaf tissue was suspended in 10 mL of 80% acetone, mixed well, and kept in the dark for 24 h at 4 °C. Supernatant was withdrawn after centrifugation (5000 rpm) for 10 min. The optical density was measured at 663 and 645 nm in a Beckman spectrophotometer. The chlorophyll content was calculated according to the method adopted by Arnon (1949).

All of the above experiments were repeated twice and the mean of the readings was subjected to statistical analysis.

Chemical analysis of *Cassia tora* plant parts

Insect-free, disease-free plants of *C. tora* with luxuriant growth were collected from the mustard field and were kept in the oven at 28 °C for 72 h. The dried sample was then crushed in a mixer to make powder. Powdered samples of roots, leaves, and seeds of *C. tora* plants were analysed in the laboratory to identify the presence of phenols, using the ferric chloride test; ketones, using Brady's test; acids, using the sodium bicarbonate test; and alcohols, using the acetyl chloride test.

First, 1 g of the sample was taken in a test tube containing 5 mL of ethanol and shaken for 2 min before the addition of 5-6 drops of neutral $FeCl_3$; the change in colouration to bluish green or red was observed. This confirms the presence of phenols. In the same way, 1 g of the sample was added to 5 mL of ethanol in a test tube and shaken for 2 min. 2,4-Dinitrophenylhydrazine (Brady's reagent) was added to it and allowed to react for 3-4 min until the appearance of orange precipitate, which confirms the presence of ketones. To test for the presence of acids, 1 g of the sample was mixed with 10 mL of distilled water in a test tube and heated slightly. Solid $NaHCO_3$ was sprinkled into the aqueous extract slowly, resulting in the effervescence of CO_2 (confirmed by burning a match stick). To test for the presence of alcohol, 1 mL of aqueous extract, prepared as described above, was treated with 2 mL of acetyl chloride and heated gently. This allows the effervescence of the HCl gas to change the colour of litmus paper.

Statistical analysis

Using standard procedures of statistical data analysis (including the software BioStat 2009, version 5.7.8.1, and the inbuilt mathematical functions of Microsoft Excel 2007), the effects of different organ extracts of *C. tora* were correlated with the rate of germination, root length, shoot length, FW, DW, RWC, and chlorophyll content of mustard. In Figures 1-7, showing change in these parameters, the bars represent the standard deviation of measurements.

Results

Aqueous extracts of various organs of *Cassia tora* affected the germination of mustard seeds at all concentrations. Inhibition was greater with 100%

extract than with the other concentrations. Leaf extracts showed the maximum inhibition, with germination reduced to 80% with dilute extract and no germination at all observed with a 100% concentration. Seed extracts also reduced germination when the concentration was increased from 25% to 50% and higher. Treatment with root extract, however, had little effect on germination, with almost 60% of seeds germinated at the highest concentration. For comparison, this percentage of inhibition (60%) was attained using 75% concentrations of seed extract and 50% concentrations of leaf extract (Figure 1).

The root and shoot lengths of 5-day-old treated seedlings were much lower in comparison to those of the control. With all treatments, the lengths of roots showed more inhibition than the lengths of shoots. Both roots and shoots grew well with 25% extract, but gradually declined with concentrations of 50% and above for all of the organ extracts. Treatment with 100% and 75% root extracts followed a reverse trend, where shoot length was affected more than the length of roots. The control set showed the length of roots (Figure 2) and shoots (Figure 3) to be almost equal.

The FW (Figure 4) and DW (Figure 5) of 10-day-old mustard seedlings were significantly affected upon treatment with the 4 different concentrations of root, leaf, and seed extracts of *C. tora*. Although

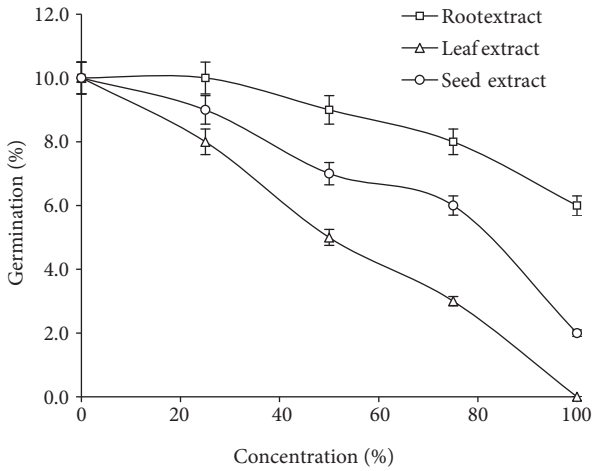

Figure 1. The effects of increasing concentrations of different organ extracts of *Cassia tora* on the germination of mustard (n = 10). The bars indicate standard deviation.

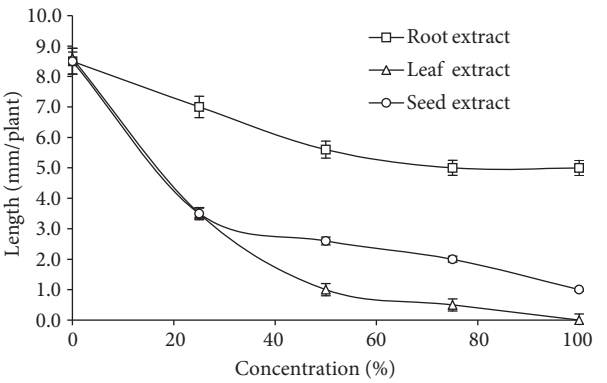

Figure 2. The effect of increasing concentrations of different organ extracts of *Cassia tora* on the root length of mustard (n = 10). The bars indicate standard deviation.

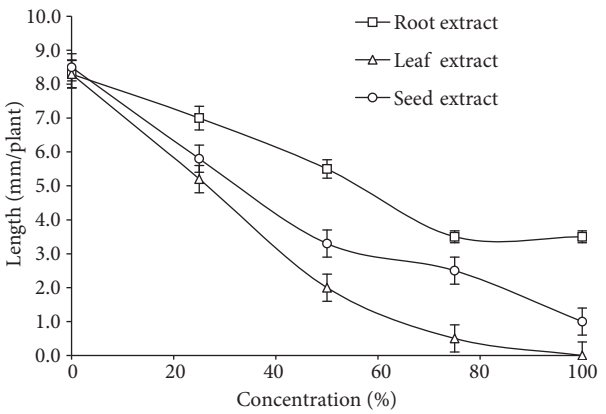

Figure 3. The effect of increasing concentrations of different organ extracts of *Cassia tora* on the shoot length of mustard (n = 10). The bars indicate standard deviation.

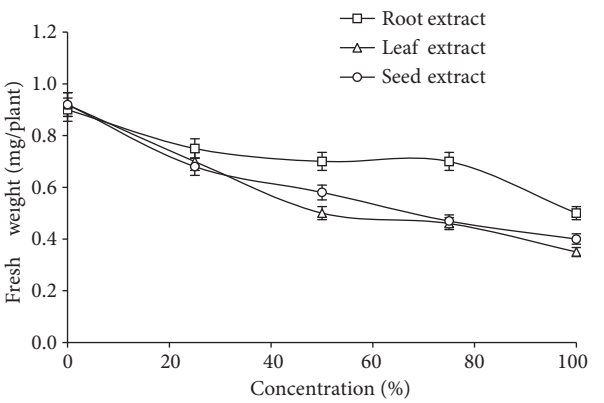

Figure 4. The effect of increasing concentrations of different organ extracts of *Cassia tora* on the fresh weight of mustard (n = 10). The bars indicate standard deviation.

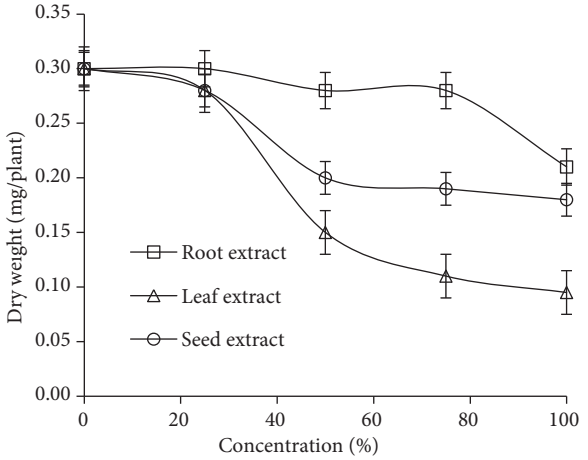

Figure 5. The effect of increasing concentrations of different organ extracts of *Cassia tora* on the dry weight of mustard (n = 10). The bars indicate standard deviation.

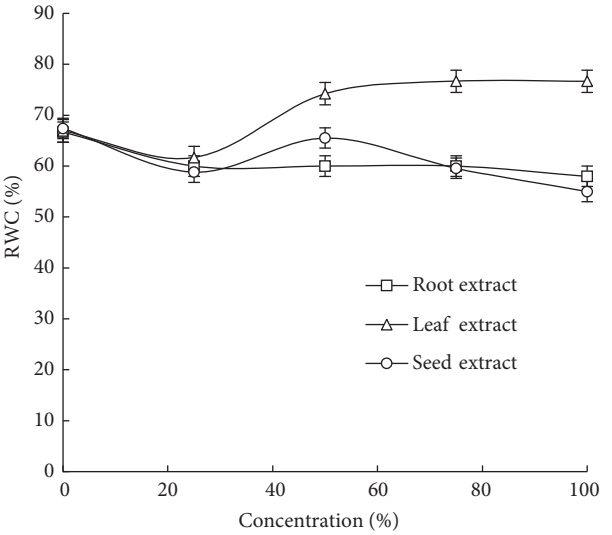

Figure 6. The relative water content in the extracts of different organs of *Cassia tora* (n = 10). The bars indicate standard deviation.

treatment with 25% extract of all organs had very little impact on the FW and DW of mustard seedlings, higher concentrations reduced the weights to almost half with 50% leaf extract and to 20% when the concentration was increased compared to the control. With 25% root extract, the DW was found to be almost equal to that of the control. Both the FW and DW of treated seedlings were reduced more by exposure to the leaf extract than by the extracts of seeds and roots. The same trend was also observed with regard to the percentage of RWC of seedlings (Figure 6). RWC rose in plants receiving the leaf extract application, whereas it fell off rapidly in those receiving seed extract and more slowly for those receiving root extract.

The total chlorophyll content of mustard seedlings collected from pots irrigated with different concentrations of test extracts of *C. tora* plant organs showed a gradual decrease with increasing concentrations of the extracts (Figure 7). With the 25% extract, the reduction was similar in all cases. Seedlings turned yellowish when the concentration was increased from 25% to 75% and 100%, despite being kept in bright sunlight. This brought down the chlorophyll content to almost one-third of that seen in the control. Results indicated the reduction to be concentration-dependent and that the maximum reduction of chlorophyll was observed with the treatment of leaf extracts.

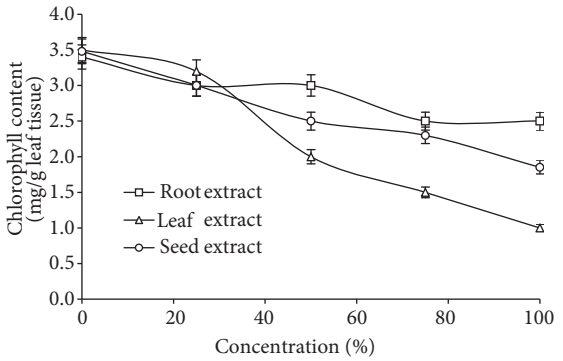

Figure 7. The effect of increasing concentrations of different organ extracts of *Cassia tora* on the chlorophyll content in the green shoot portion of mustard (n = 10). The bars indicate standard deviation.

In comparative analysis, when the relative effect of all of the studied parameters was considered, the inhibitory effect of leaf extract of *C. tora* was the highest and that of root extract was found to be the lowest. Regarding the correlation analyses, the correlation coefficient of each observation was determined to be significant ($P < 0.05$).

Laboratory analysis of the roots, leaves, and seeds of the *C. tora* plant showed the presence of different groups of chemicals in different parts. The development of a bluish green colour during the ferric chloride test confirmed the presence of phenol

in the leaf and seed samples. A bright orange red precipitate with Brady's reagent showed that there was a sufficient number of ketones present in the roots and seeds. While the effervescence of CO_2 in the sodium bicarbonate test indicated the presence of acids in the leaf, the effervescence of HCl gas (which was responsible for changing the colour of the litmus in the acetyl chloride test) showed alcohol to be present in the roots and leaves.

Discussion

Aqueous extracts of various concentrations of roots, leaves, and seeds of *Cassia tora* had varying degrees of inhibition on the germination and growth of mustard seeds, reflecting the allelopathic potential of the plant. Higher concentrations of the extracts had a higher degree of germination inhibition with all of the plant organs. Concentrated aqueous extracts contain a greater amount of inhibitory substances than those actually found in the fields, where a portion is leached or degraded into the soil. For this reason, lower concentrations (50% and 25%) were also tested. In the experiments using lower concentrations, there was almost no inhibition observed with the root extracts, although the leaf and seed extracts showed some germination inhibition. Allelochemicals suppress the mitotic activity of young cells, resulting in the inhibition of seed germination (Rice, 1984). Leaf extract was seen to be more inhibitory, which may be due to the presence of certain chemicals such as emodin, stigmasterol, β-sitosterol, β-D-glucoside, freindlen, succinic and d-tartaric acids, and uridine. The inhibition of seed germination was found to be concentration-dependent (Oudhia, 1999).

When compared to the control, the root length inhibition was greater than that of the shoots. This may also be due to the contact of the roots with the filter paper, leading to constant absorption of the extract solution. According to Wu et al. (2001), *C. tora* extract affects benzo[a]pyrene-mediated DNA damage of cells, resulting in growth inhibition.

To compare the allelopathic effect tested in the laboratory with what actually happens in the field, the FW and DW of seedlings were recorded by growing them in pots irrigated with the inhibitor extracts. The concentrated extract of test organs delayed germination and suppressed FW. All concentrations of leaf extract affected the DW and RWC of mustard seedlings more than the extracts taken from seeds and roots. In a previous study of barley and wheat, the elongation and DW of seedlings were reported to be reduced by the walnut allelochemical juglone (5-hydroxy-1,4-naphthoquinone) in a similar pattern (Terzi & Kocaçalışkan, 2010). Macro- and micronutrient absorption and IAA oxidase in plant root cells is inhibited by various allelochemicals (Yang et al., 2004), which may lead to the observed reductions in DW, FW, and RWC of germinating mustard seedlings.

Being the most important component of the pigment system, chlorophyll molecules play a major role in photosynthesis. The significant reduction of chlorophyll content seen with all concentrations may be due to the inhibition of chlorophyll biosynthesis, the stimulation of chlorophyll-degrading substances, or both (Yang et al., 2007). Patterson (1981) found a marked reduction in the concentration of chlorophyll in leaves of soybean plants following treatment with a number of allelopathic compounds. Higher concentrations of all organ extracts were found to cause mosaic chlorosis, resulting in the yellowing of leaves of potted seedlings and thereby causing the reduction in the chlorophyll content.

A large number of chemicals were found to be present in different organs of the *C. tora* plant. The presence of ketones, alcohols, and sterols in the root, as well as phenols, alcohols, terpenes, and acids in the leaf and lactones, ketones, and phenols in the seeds of *C. tora*, throws a light on its inhibitory activity (Patil et al., 2004; Thapar & Singh, 2006). On the other hand, *Brassica campestris* contains 1% sinigrin, glucosinolate, isothiocyanate, fatty oil, and glycerides of erucic acid (Turk & Tawaha, 2003; Bernat et al., 2004). Though terpenes and phenols are well-known allelopathic compounds, the role of other chemicals and higher salts present in the extracts cannot be ruled out. Overall inhibition may be due to the activity of a single chemical having multiple phytotoxic effects (Einhellig & Rasmussen, 1979) or to an interaction of various chemicals of *C. tora* with those of mustard.

In this comparative study, though all 3 organ extracts showed significant allelopathic potential, the degree of inhibition seemed to be highest in the case of the leaf extract of *Cassia tora*.

Conclusion

From the present study, it is evident that, despite being overlooked by farmers due to its demand in Ayurvedic medicine, *Cassia tora* is considerably phytotoxic to the growth and development of mustard plants. In order to avoid the long-term accumulation of phytotoxins, necessary steps should be adopted to check its occurrence in and around crop fields.

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