# Allelopathic effect of *Amaranthus spinosus* Linn. on growth of rice and mustard

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# Abstract

A study was conducted to evaluate the inhibitory effect of aqueous leaf extract of *Amaranthus spinosus* Linn. (family Amaranthaceae), an important medicinal plant, on growth activities of rice (*Oryza sativa* L.) and mustard (*Brassica campestris* L.) seedling. The growth parameters were significantly inhibited due to the activity of allelochemicals present in the weed. The germination of crop seeds as well as their root and shoot length were suppressed more than 50% with the treatment. Fresh weight (FW) of rice seedlings were reduced to 16.7 g, 16 g and 15 g with 1:20, 1:10 and 1:05 concentration as compared to 18.50 g of control. In case of mustard, there was 50% reduction in FW and 40% reduction in dry weight (DW) with the same concentration. Similar trend was also recorded for relative water content (RWC) where mustard plants showed a reduction up to 58%. Among the three enzymes studied, mustard was more sensitive to catalase and á- amylase and rice to peroxidase activity in terms of mUg<sup>-1</sup>FW. Similarly chlorophyll content was reduced to 50% for both test crops. Presence of phenols, ketones, flavonoids in the leaf leachate of *A. spinosus* suggest that the plant has allelopathic potentiality which might be responsible for the restricted growth activity of the crops. Comparative study revealed the inhibition of metabolic activity to be more in case of mustard than in rice and that the suppression was concentration depended. However, further research is necessary to confirm the presence of single compound responsible for this interaction.

KeyWords: Allelochemicals, Amaranthus spinosus, Germination, Growth, Rice, Mustard, Enzymes.

# Introduction

Allelopathy is the inhibitory or stimulatory interaction between two plant species. The term 'allelopathy' was first coined by Molisch (1937). In 1996, International Allelopathy Society defined the term as 'any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influence the growth and development of agricultural and biological systems (Roger et al., 2006). Allelochemicals are liberated by abscission and litter fall, volatilization, leaching of foliage by rain, and root exudates. These chemicals are generally water soluble in nature and species specific (Inderjit and Mallik, 2002). Phytotoxic effect of dominant plants are mainly due to the presence of phenolics that inhibit seed germination, plant growth and other physiological processes of the receiver plant (Khanh et al., 2007).

In accordance with the Plant Protection Act (7 U.S.C, 7701 et seq, 2000), the US government has designated certain plants as noxious weeds. These plants are weedy or invasive, some are harmful pests in parts of a country and valuable natives to others. *Amaranthus spinosus* Linn., also known as spiny amaranth or pig weed of the family Amaranthaceae is one such weed which is native

to tropical America and has spread to warmer areas of the world. Throughout India, the species is of common occurrence near railway tracks, village waste land, agricultural land and densely around the Bay of Bengal (Holm et al., 1991). This invasive nature of the weed is due to its allelopathic property and it spreads vigorously thereby reducing the grazing area. In Indian traditional system of medicine (Ayurveda), various parts of A. spinosus has been used to treat diabetes, internal bleeding, diarrhea, piles, bronchitis, excessive menstruation, skin disease, snakebite, as expectorant (Manik et al., 2011) and for many other treatments in different countries (Igoli et al., 2005). For this reason the plants are not destroyed and instead intentionally allowed by the local people to grow in and around the fields and also abundantly in northern India where it is used as food crop. However, chemical analyses of this species indicated the presence of a large number of compounds that are allelopathic in nature. The leaf extract contains betacyanins, phenolic compounds, glycocides, flavonone, streptozotocin-nicotinamide (Choudhury, 2012). Though not poisonous, A. spinosus when grown in nitrogen rich soil are known to concentrate nitrates in the leaves. It is inadvisable to eat this plant as nitrates are implicated in stomach cancer, blue babies and health problems like depression.

Leachable compounds from various parts of A. spinosus plant supplemented germination and growth of seedlings in various cultures (Ambika and Suma, 1999). Extracts of leaves and inflorescence of this weed drastically reduced the vegetative phases of Sinapis alba and Triticum aestivum (Datta and Bandyopadhyay, 1981). Aqueous leachates from leaf, stem, root of the plant has inhibitory effect on the growth of P. hysterophorus (Thapar and Singh, 2005). It is very likely that the same toxin (s) would affect many crop plants. Taking this viewpoint into account, a study was conducted with the leaf lechates of this weed to ascertain its inhibitory effect on growth performance of rice (Oryza sativa L.) and mustard (Brassica campestris L.), the two most important crop production of this region. *A. spinosus* is a major weed in rice and mustard fields in the region, where the study area is located.

### **Materials and Methods**

# Plant material

*Amaranthus spinosus* shoots were collected in bulk from the adjoining agricultural field areas of Habra, North 24 Parganas District, West Bengal, situated near India–Bangladesh border, about 50 kms from the proper town. Seeds of rice and mustard were collected from the local farmers of the study area during the period September to October 2013. These were authenticated by the Botanical Survey of India, Sibpur, Howrah, WB.

# Preparation of aqueous extract

Fresh leaves of *A. spinosus* were washed thoroughly with distilled water, shade dried to uniform moisture at room temperature  $(30 \pm 2^{\circ}C)$ for 10 days and crushed in a blender. 10 g of crushed material were soaked in 50 ml of double distilled water in corked conical flasks and kept on mechanical shaker for 20 h and filtered through Whatman No.1 filter paper. This filtrate served as stock aqueous solution of 1:5 concentration from which 1: 10 and 1:20 concentrations were prepared by dilution. The pH of the extract was measured with digital pH meter (Systronics, India).

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

Mustard (*Brassica campestris* L., cv B<sub>9</sub>) and rice (*Oryza sativa* L., cv Ratna) seeds were surface sterilized with 2% sodium hypochlorite solution for two minutes and washed thoroughly with distilled water. Single layer of Whatman No. 1 filter paper were kept in 9 cm sterile Petri dishes and divided into three sets. The first, second and third set received treatment of 10 ml of 1:5, 1:10 and 1:20 concentration of test extract and the fourth

set was treated with distilled water which served as control. In each prepared Petri dish, 10 surface sterilized mustard and rice seeds were placed separately. A total of six replication of each set were kept undisturbed in the dark chamber of the laboratory for 5 days. The numbers of germinating seeds were recorded on the sixth day and the length of radicle and plumule also measured. The emergence of visible radicles approximately 1 mm in diameter was taken as the index of germination. All results were expressed as percentage of their corresponding control. The percentage of germination was calculated by using the following equation:

Germination % =

<u>Average number of germinated seeds with treatment</u> x 100 Average no. of germinating seeds in control

The overall experimental procedure was followed according to Jacob et al. (2006). The treatments were arranged in completely randomized design (CRD).

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

To determine the direct effect of allelochemicals on crops in the field, 30 cm pots were filled with 600 g of soil collected from well ploughed mustard and rice growing fields such that the soil property could be considered constant. Then 30 surface sterilized seeds of mustard and rice were sown approximately 5 mm deep in their respective pots. The pots were divided into 4 sets for each crop with 4 replications. Set-1 pots received a daily dose of 100 ml aqueous leaf extracts of 1:5 concentration, set-2 of 1:10 concentration, set-3 for 1:20 concentration and set-4 or control pots received 100 ml of tap water. All the pots were kept in bright sunlight. After 12 days of germination, 10 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. FW and DW (determined by oven drying at 70°C for 24 h) of the seedlings were noted. Using the equation of Deef and Fatah (2008), the relative water content (RWC) was calculated as:

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

#### Determination of enzyme activity

Seedlings were harvested from their respective pots after 8 days of germination and enzyme assay was conducted. Crushed leaf sample were homogenized with phosphate buffer, centrifuged and the supernatant was taken for the determination of catalase, peroxidase and  $\alpha$ -amylase activity.

a. Assay of peroxidase activity

Ten seedlings each from the treated and control set were homogenized separately in pre-chilled conical glass homogenizer with cold phosphate buffer (0.05 M, pH 6.1). A tissue : buffer ratio of 1:5 was used. The homogenate was centrifuged at 10,000 x g for 15 min and the supernatant was then used directly for peroxidase assay which was done using the method of Chance and Machly (1955). The assav mixture consisted of 0.5 ml tissue extract. 5 ml of buffer, 1 ml H<sub>2</sub>O<sub>2</sub> and 1 ml catechol (0.5%). The change in the colour intensity of oxidized catechol at 420 nm was measured in Klett-Summerson Colorimeter and the results were expressed as milliunit per gram fresh weight of tissue (mUg<sup>-1</sup>FW).

b. Assay of catalase activity

Plant material was prepared in the same way as in the assay of peroxidase activity. Catalase activity was assayed using the method of Gasper and Lacoppe (1968). The assay mixture consisted of 1 ml tissue extract, 5 ml buffer pH 7 and 1 ml  $H_2O_2$  (0.2 vol). The reaction mixture was kept undisturbed for 15 min, transferred to 5 ml of 10%  $H_2SO_4$  and then titrated against 0.005 N KMnO<sub>4</sub>solution. Catalase activity was expressed as given earlier for peroxidase activity.

c. Assay of  $\alpha$ -amylase activity

The assay procedure was the same as adapted

by Bernfeld (1955) and modified by Dure (1960). Ten seedling from the treated and control set were homogenized separately. The homogenate was centrifuged at 10,000 x g for 15 min in cold. The supernatant fraction was heated to 70°C for 5 min to kill β-amylase and then assayed for  $\alpha$  - amylase activity. The assay mixture consisted of 0.8 ml tissue extract, 0.5 ml of 0.1M citrate buffer (pH 5.0) and 0.5 ml starch solution (1%), was held at 30°C for 5 min and the reaction was stopped by the addition of 2 ml of 1% dinitrosalysilic acid (colour reagent). The tubes containing the mixture were heated for 5 min. in a boiling water bath for the development of colour and diluted to 50 ml. The optical density of the solution containing the brown reduction product was measured colorimetrically by means of a green filter (No. 54) and a blank was prepared in the same manner by excluding the tissue extract.  $\alpha$ - amylase activity was expressed as milliunit per gram fresh weight of tissue (mUg<sup>-1</sup>FW)/ Preparation of the colour reagent: To start with, 1g 3, 5-dinitrosalicylic acid was dissolved in 20 ml of 2N NaOH and 50 ml water. Then 30 g of Rochelle salt was added to it and the volume was made up to 100 ml with distilled water

# Determination of Chlorophyll content

Chlorophyll content was determined from 100 mg (fresh weight) leaf portion of 12 days old rice and mustard seedlings grown on pots and irrigated with various concentration of *A. spinosus* leaf extract. Leaves were washed thoroughly with 0.1 % HgCl<sub>2</sub> for 1 min and repeatedly with distilled water for surface decontamination and air dried. They were then suspended in 10 ml of di-methyl sulphoxide (DMSO), incubated at 65°C for 1 h with slight stirring for complete extraction of chlorophyll and filtered. Fresh DMSO was added to make the final volume up to 10 ml. The estimation of chlorophyll thus recovered in DMSO was measured at 663 nm and 645 nm in a Beckman spectrophotometer. The

total chlorophyll content was calculated according to the equation of Arnon (1949), with Hiscox and Israelstan (1979) modification as follows:

Total chlorophyll ( $\mu g/ml$ ) =

6.45 x <sup>A</sup>663 + 17.72 x <sup>A</sup>645.

Where, <sup>A</sup>663 and <sup>A</sup>645 represents density values at 663 and 645 nm respectively.

All the above experiments were repeated thrice and the mean of the readings was subjected to statistical analyses.

# Phytochemical screening

Fresh leaves of *A. spinosus* were oven dried at 28° C for 72 h, and crushed in a mixer to make a powder. This powder sample as well as aqueous extract was analysed in the laboratory to identify the presence of phenols, flavonoids, tannins and ketones. Phytochemical screening was done using standard procedures as performed by Ayoola et al. (2008).

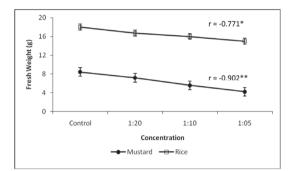
- a. Test for Phenols: 1 g of the sample was taken in a test tube containing 5 ml of ethanol and shaken for 2 min before the addition of 5-6 drops of neutral  $FeCl_3$ . The change in colouration was observed.
- b. Test for Flavonoids: 1 ml of extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution and the change in colouration was seen.
- c. Test of Ketones: 1 g of the sample was added to 5 ml of ethanol in a test tube and shaken for 2 min. 2, 4-Dinitro Phenylhydrazine (Brady's reagent) was added to it and allowed to react for 3-4 min until the appearance of precipitate.
- d. Test for Terpenoids (Salkowski test): 1 g of the powder sample was dissolved in 5 ml of ethanol. To it 2ml of chloroform was added and then 3 ml of concentrated  $H_2SO_4$  was poured slowly to form a layer. Formation of colour was observed.

#### Statistical analyses

The data were subjected to one-way analysis of variance and the mean values were separated at p < 0.05 applying 2-sample t-test and correlations were observed. The statistical analyses was performed using SPSS 20.0 software.

#### **Results and Discussion**

The present investigation showed that the aqueous leaf extract of A. spinosus (pH = 7.45) seriously affected crop species with both concentrations. Seed germination (Table 1) was decreased to 70% with 1:10 concentration for both the crops whereas it was 40% (for mustard) and 50% (for rice) with 1:5 concentration. The root and shoot lengths measured revealed a drastic reduction with extract treatment as compared with control treatment. They were reduced to almost 30% in case of mustard and about 50% in case of rice with 1:5 concentration. Though 1:10 concentration of extract showed gradual reduction, a marked suppression was noted with 1:5 concentration in both the test plants. Inhibition was more for mustard than rice seedlings (Table 1). The average FW of 12 days old rice seedlings were 15  $g \pm 0.55$ with 1.5, 16.00 g  $\pm$  0.55 with 1:10 and 16.5 g  $\pm$ 0.03 with 1:20 concentration as compared with 18.50 g  $\pm$  0.08 of control, whereas it was 4.23 g  $\pm$  0.05 with 1.5, 5.60 g  $\pm$  0.62 with 1:10 and 7.1 g  $\pm$ 0.30 with 1:20 concentration as compared to 8.45 g  $\pm$  0.31 of control in case of mustard (Fig. 1). Although treatment with 1:20 concentration had very little impact on both the seedlings but higher concentration reduced the weights by almost 50% as that of control. DW of 12 days old seedlings were significantly affected upon treatment (Fig. 2). It was 8.63 g  $\pm$  0.05, 9.00 g  $\pm$  0.35, 8.80 g  $\pm$ 0.30 and 10.31 g  $\pm$ 0.27 in case of rice and 0.34 g  $\pm$  0.33, 0.55 g  $\pm$  0.76, 1.25 g  $\pm$  0.42 and 1.95 g  $\pm$  0.56 for mustard with concentrations of 1:5, 1:10, 1:20 and



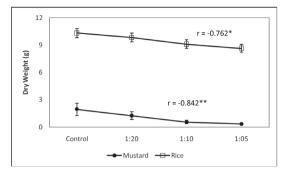
control respectively. The same trend was also

*Figure 1.* Effect of *Amaranthus spinosus* extract on fresh weight of rice and mustard in different concentrations. Bars indicate standard deviation (correlation co-efficient r significant at 0.05\* and 0.01\*\* level)

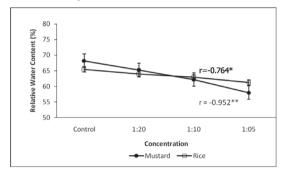
Concentration	Mustard			Rice		
	G	R	S	G	R	S
C	100.00	6.00	4.00	100.00	4.00	3.50*
	$\pm 0.00$	±1.24	±0.24	$\pm 0.00$	±0.91	±0.06
1:5	70.00	5.00***	2.15***	72.50	3.80***	2.95
	±0.25	±1.75	±2.36	±0.30	±2.10	±0.05
1:10	40.20**	$2.05^{*}$	0.80	50.50*	2.05	1.60
	±1.05	±1.52	±0.03	±0.72	±0.03	±0.63
1:20	80.00	5.80**	3.50	85.00	4.20	3.20*
	$\pm 1.05$	±1.25	±1.05	± 1.25	±0.25	±1.25

*Table 1.* Percentage germination (G in %), root length (R in cm) and shoot length (S in cm) of mustard and rice seedlings upon treatment with different concentrations of *A. spinosus* leaf extract.

't' value significant at 0.05 (\*), 0.01 (\*\*) and 0.001 (\*\*\*) level.



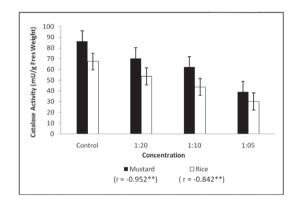
*Figure 2*. Effect of *Amaranthus spinosus* extract on dry weight of rice and mustard in different concentrations. Bars indicate standard deviation. (correlation co-efficient r significant at 0.05\* and 0.01\*\* level)



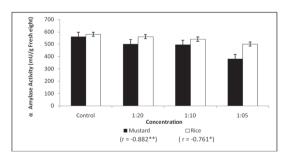
*Figure 3.* Effect of *Amaranthus spinosus* extract on relative water content of rice and mustard in different concentrations. Bars indicate standard deviation. (correlation co-efficient r significant at  $0.05^*$  and  $0.01^{**}$  level)r = -0.764\*

observed with regard to the percentage of RWC of seedlings (Fig. 3). Though RWC showed little difference in case of rice and mustard plants receiving 1:20 concentration (64%) but fell slightly upon treatment with 1:5 concentration (61.25 % and 58%) with respect to their control (65.5 % and 68.2%).

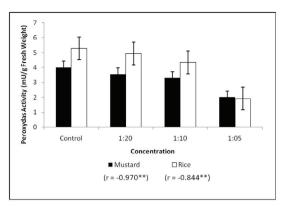
It is very difficult to separate secondary effects from primary causes of growth process in plants. Inhibition or stimulation of specific enzymes may be one of the causes of growth and development. Results showed inhibition of catalase, peroxidase and  $\alpha$ - amylase enzyme activities in rice as well as mustard seedlings. Catalase (Fig. 4) and  $\alpha$ - amylase (Fig. 5) showed more inhibition in both crop plants with all concentrations of *A. spinosus* leaf extract. Peroxidase activity (Fig. 6) in rice was lowered to less than 50% of control, (1.92 milli unit per g FW from 5.3 mUg<sup>-1</sup> FW for rice and 2.00 mUg<sup>-1</sup> FW from 4.00 mUg<sup>-1</sup> FW in control) in case of both the crops with 1:5 concentration. In case of 1.5 concentration, catalase activity was inhibited to 30.20 mUg<sup>-1</sup> in rice and 39.00 mUg<sup>-1</sup> in mustard compared to 67.35 mUg<sup>-1</sup> and 86.20 mUg<sup>-1</sup> of controls respectively. Similarly, with 1:5 concentration,  $\alpha$ -amylase activity was reduced to 380 mUg<sup>-1</sup> FW in case of mustard and 500 mUg<sup>-1</sup> FW in case of rice compared to 560 mUg<sup>-1</sup> FW and 580 mUg<sup>-1</sup> FW of their respective controls. Of the



*Figure 4.* Effect of *Amaranthus spinosus* extract on catalase activity of rice and mustard in different concentrations. Bars indicate standard deviation. (correlation co-efficient r significant at 0.01\*\* level)



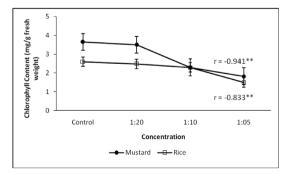
*Figure 5.* Effect of *Amaranthus spinosus* extract on  $\alpha$  amylase activity of rice and mustard in different concentrations. Bars indicate standard deviation. (correlation co-efficient r significant at 0.05\* and 0.01\*\* level)



*Figure 6.* Effect of *Amaranthus spinosus* extract on peroxidase activity of rice and mustard in different concentrations. Bars indicate standard deviation. (correlation co-efficient r significant at 0.01\*\* level)

three enzymes studied, mustard was more sensitive to catalase and  $\alpha$  -amylase where as it was rice in case of peroxidase activity.

The total chlorophyll content of rice and mustard seedlings collected from pots irrigated with different concentrations of test extracts of *A. spinosus* showed a gradual decrease with increasing concentration of extract (Fig. 7). Rice seedlings showed yellowish patches upon treatment, despite being kept in bright sunlight. Chlorophyll content was reduced to almost half as compared to control in both the cases.



*Figure 7*. Effect of *Amaranthus spinosus* extract on chlorophyll content of rice and mustard in different concentrations. Bars indicate standard deviation. (correlation co-efficient r significant at 0.01\*\* level)

The production of allelopathic compounds in A. spinosus is widely influenced by genetic as well as environmental factors at different growth stages. Various growth performances of seedling showed negative response to the increasing concentration of the aqueous extract reflecting allelopathic potential of the weed. Allelochemicals suppressed the mitotic activity of young cells and brings about decrease in cell number or cell elongation or both, resulting in the inhibition of metabolic activities (Rice, 1984). This may have led to the reduction in percentage of seed germination as well as root and shoot length. Both the crops showed reduced rate of germination as compared to control. Alteration of soil properties, nutritional status and activity of microorganisms also play a major role in growth activities of plants. To keep these factors constant, investigation of FW, DW, chlorophyll content and enzyme assays of rice and mustard seedlings were recorded by growing them in pots containing the same soil as that in the field and irrigated with the inhibitor extracts.

Leaf extract of A. spinosus suppressed FW and also affected the DW and RWC of both crop plants in different concentrations. FW and percentage of RWC reduced more in mustard than in rice. The reduction of dry matter production indicates interference by the toxic substances from the extract with cell division, nutrient uptake and transport. In case of barley and wheat seedlings, Terzi and Kocacaliskan (2010) reported about the inhibition of both elongation and DW by the walnut allelochemical juglone (5-hydroxyl-1-4naphthaquinone) in a similar pattern. Macro and micro nutrient absorption and IAA oxidase in plant root cells is inhibited by various allelochemicals (Yang et al., 2004), which may lead to the observed reduction in DW, FW and RWC of growth activities of mustard and rice seedling. Beside possible allelochemicals, higher salts and chemical concentration in the extract might possibly cause an osmotic stress during seed germination and seedling growth. As reported by Bosy and Reader (1995), soil surface residue may also prevent

seedling emergence physically in addition to releasing allelochemicals. Growth of seedling is more affected by the allelopathic interaction than the seed germination.

Dzyubenko and Petrenko (1971) reported the effect of root secretion of two crop species on catalase, and peroxidase activity of weed species. Aqueous leaf extract of A. spinosus showed inhibition of catalase, peroxidase and  $\alpha$ -amylase in mustard and rice seedling and in no case there was a stimulation of these enzymes even with the dilution of leaf extract. The inhibition may be attributed to the alteration of enzyme activity which affects the mobilization of storage compounds during germination. This is in contrary to the observation by Jankey and Muller (1976), who noted that umbelliferon caused a swelling response with increasing peroxidase level. Also Parish (1968) suggested that the activity of peroxidase increases with maturity and senescence. Our results showed at seedling stage, allelochemicals affected the peroxidase activity of both rice and mustard. Benoit and Starkey (1968) stated that tannins inactivated  $\alpha$ -amylase, dehydrogenase and other enzymes. In this study catalase and  $\alpha$ - amylase might be held responsible for the so called inhibitory activity of mustard and peroxidase for rice. However, all the values observed with different concentration of extracts were below the corresponding controls. This inhibition may be attributed to the alteration of enzyme activity, which affects the mobilization of storage compounds during germination.

Being the most important component of pigment system, chlorophyll molecules play a major role in photosynthesis. When the potted plants were treated with different concentrations of leaf extract, pigment content of both test species exhibited significant difference from control. Aqueous extract of *A. spinosus* showed different level of inhibition on different crops. This reduction may be due to the fact that allelochemicals either inhibit the biosynthesis of chlorophyll or stimulates the chlorophyll degrading substances or both. Petterson (1981) found a marked reduction in the concentration of chlorophyll in leaves of soybean plants following treatment with a number of allelopathic compounds. Higher concentration of extract caused mosaic chlorosis, resulting in the yellowing of leaves of potted rice and mustard seedlings thereby affecting chlorophyll content. Einhellig and Rasmussen (1993) reported that allelochemicals caused marked reduction in the chlorophyll pigment of test plants through their effect on the biosynthesis and denaturation of chlorophyll molecules.

Chemical tests of *A. spinosus* leaves in the laboratory indicated the presence of phenols when a bluish green colouration was observed with neutral FeCl<sub>3</sub> test. Formation of yellow colour indicated the presence of flavonoids and orange precipitate with Brady's reagent confirmed the presence of ketones. Similarly, a reddish brown colouration of the interface indicates the presence of terpenoids. In comparative analyses, when the relative effect of all the studied parameters were considered, inhibitory effect was found to be more for mustard seedlings with both concentrations.

As found by phytochemical screening, phenols, flavonoids, ketones and terpenoids present in the weed may be the cause of inhibition. Flavonoids are also phenolic compounds usually occurring in complex mixtures and responsible for inhibition of growth activities. Manik et al., (2011) found the presence of flavones, glucosides, ketones, quercetin, linoieic acid etc. in the leaves of same species. Presence of these chemicals is similar to our findings and throws a light on the inhibitory activity of aquous extract of A. spinosus. Oil crop Brassica campestris contains 1% sinigrin, glucosinulate, isothiocyanate, fatty oil and glycerides of eruric acid (Turk and Tawaha, 2003). According to Kato-Noguchi (2005), Oryza sativa contains phenolic acids, fatty acid, indoles and terpenes. Though phenols, ketones and terpenes 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 or to an interaction of various chemicals of *A. spinosus* with those present in rice and mustard.

From the present study it is evident that aqueous leaf extract of *A. spinosus* growing in and around agricultural fields contain a large number of phytochemicals, which may be responsible for the inhibitory growth activities of crop species. On the other hand, chemicals present in *Brassica campestris* and *Oryza sativa* or their degradation products may interact with those of the weed to bring about allelopathic suppressions. Further research is necessary to confirm the activity of a single chemical(s) present in this weed resulting in the inhibition of test crops. In order to avoid long term accumulation of phytotoxins, necessary steps should be adopted to check the occurrence of this invasive weed in and around crop fields.

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