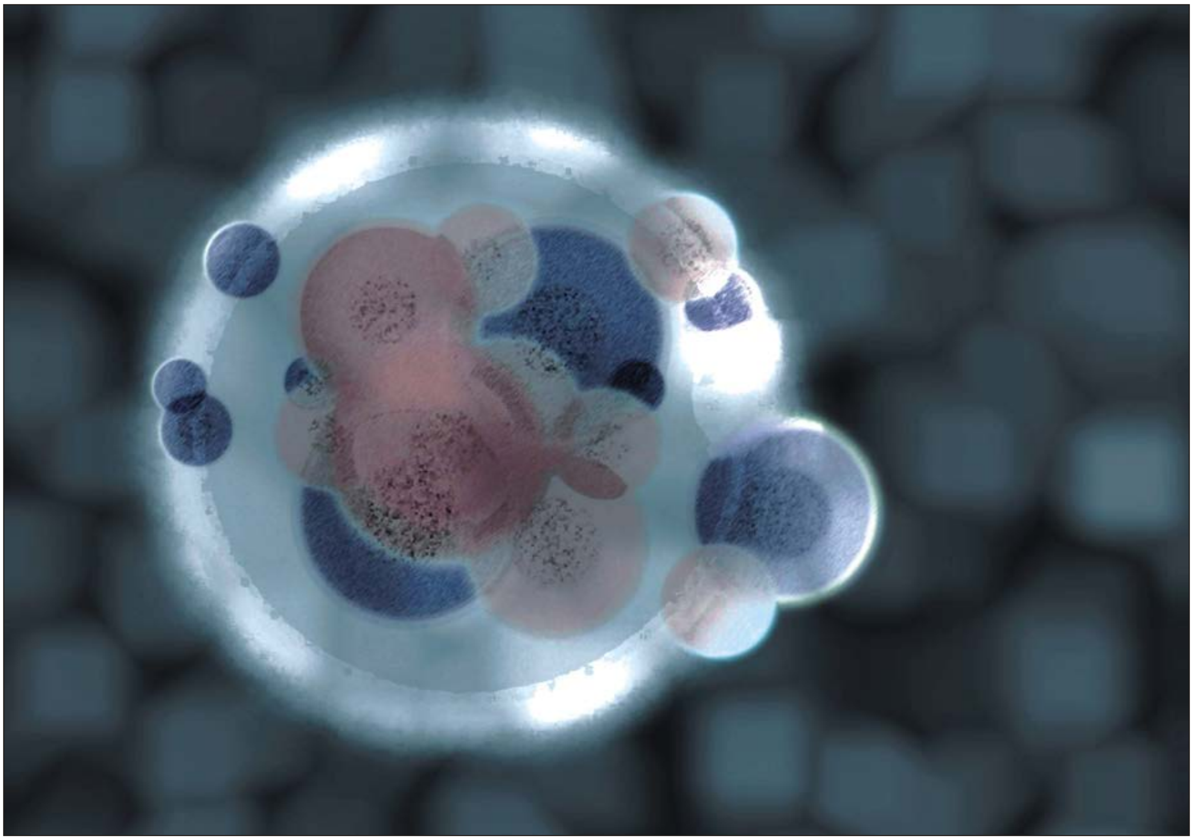

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Effect of Aqueous Extracts of Some Weeds on Germination and Growth of Wheat and Jute Seeds with Emphasis on Chemical Investigation

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Abstract: An experiment was conducted on naturally occurring growth substances in aqueous extracts of some common weeds viz., Bathua (*Chenopodium album*), Bhijli ghas (*Striga densiflora)*, Shetdrone (*Leucus aspera)*, Mutha (*Cyperus rotundus*), Chapra (*Eleusine indica)* and Khude Anguli (*Digitaria ischaemum*) with the attempt for chemical investigation on effective extracts. Boiled and unboiled extracts of all the weed species under test significantly reduced and delayed germination of wheat and jute seeds compared with control. The highest germination (96 and 85.3%) and maximum growth of wheat and jute were observed in seeds treated with control. The effect of boiled and unboiled extracts of Bathua (*Chenopodium album*) showed the lowest germination in seeds of wheat. The root and shoot length of wheat and jute were also decreased in presence of the weed extracts. The thin layer chromatography (TLC) examination of crude extract of Khude Anguli (*Digitaria ischaemum*) showed four separate compound at hex: ethyl acetate (20:1, v/v) and the crude extract of Bathua (*Chenopodium album*) showed three separate component at hex: ethyl acetate (10:1, v/v).

Key words: Aqueous extracts, germination, chemical investigation, TLC

INTRODUCTION

Different types of naturally occurring organic, bioorganic compounds have been isolated from different types of plants, weeds and animal sources. Most of them have effective medicinal, insecticidal/pesticidal or toxic value. The different types of weed extracts in the crop field due to rainfall make aqueous extracts of toxic compounds, which may damage the germination and growth rate of crops. Because the direct or indirect effect of one plant to an adjoining plant through the release of chemical substances or toxic compounds have come to be known as allelopathy and is postulated to be one mechanism by which weeds interfere in crop growth and also in the germination of seeds (Ming, 1999; Jha and Dhakal, 1990; Duke; 1986). Weeds on plants releasing compounds into the environment may have either deleterious or beneficial effects on other plants growing in their vicinity. The plant-plant chemical compound interactions are of vital importance in the field of agriculture (Chou, 1990). Several investigators have reported that the effect of extracted primary and secondary metabolites from different weeds on germination, growth and development of various crops and some have insecticidal effects (Kohata *et al*., 2004; Liang *et al*., 1994; Saxena *et al*., 1994). Jones *et al*. (1999) also reported that some crop residues are known to have chemical (allelopathic) as well as physical effect on the growth of several crops and weeds. Above literature reviews basically reported on the germination, inhibitory influence, insecticidal activity as well as nutrient assimilation and other important biological activity of aqueous extract of different weeds/crop residues during germination of different crops/weeds and also in different insects. Study of aqueous extracts of weeds is of practical significance in crop farming system for the germination of seeds and also crop growth rate. Therefore, the present study was undertaken to examine the influence of some important summer and winter weeds available in Bangladesh on germination of wheat and jute seeds and their primary growth rate with emphasis on their chemical investigation on effective extracts.

MATERIALS AND METHODS

The experiments were conducted at Agricultural Chemistry and Biochemistry Laboratory of Hajee Mohammad Danesh Science and Technology University,

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Dinajpur, Bangladesh during July-December 2004 and January-June 2005 to study the effect of weed extracts on the germination and initial growth of wheat and jute. The entire plants including roots and flowers of 6 common summer weeds viz., Bathua (*Chenopodium album*), Bhijli ghas (*Striga densiflora),* Shetdrone (*Leucus aspera),* Mutha (*Cyperus rotundus*) Chapra (*Eleusine indica*) and Khude Anguli (*Digitaria ischaemum*) were freshly collected, chopped and macerated. In a set, fresh weed mass (250 g) of each weed species was boiled in one liter water and kept for 3 days with stirring a regular interval. The extracts were filtered through Whatman filter paper No. 1. The filtrates were used as boiled extracts of weeds. In another set, 250 g fresh weed mass were immersed in one liter water for 7 days at room temperature. The mixtures were stirred several times a day. The extracts were filtered and used as unboiled extract. Thus boiled and unboiled extracts of each weed species were prepared and used in the experiment (Prasad and Srivastava, 1991).

The germination of wheat (var. Satabdi) and jute seeds (var. Deshi) was studied by petridish method. Two sets of trials, each with boiled and unboiled extracts for each crop, was performed. Twenty five seeds of each species were placed in each Petridish lined with double layer of filter paper and treated with weed extracts, 15 mL per petridish for wheat and 15 mL per petridish for jute and control set with distilled water was run simultaneously at every time. The filter papers were kept constantly moist with distilled water. The experiment was laid out in randomized complete block design with three replications at a room temperature 28±1°C. Germination (%) and days required for complete germination was recorded in each case. The linear growth of root and shoot were separately recorded, for wheat and jute at 10 and 7 days after seed placing respectively. The collected data were analyzed statistically and the differences between means were compared by using Duncan's New Multiple Range Test (DMRT).

Three hundred and fifty milliliter effective unboiled extracts (UBE) and 200 mL of $CHCl_3$ were taken in a separating funnel. It was shaken for 2-5 minutes. It was allowed to stand with the help of stand and clamp. Two layers were detected as organic layer and aqueous layer. The $CHCl_3$ layer (organic layer) was separated and collected in a conical flask. The same procedure was followed for three times for the purpose. The all-organic layers were combined together. Excess amount of anhydrous Na_2SO_4 was added to it to remove the excess water. It was then filtered using ordinary filter paper (Whatman No. 1). All the filtrate were combined and evaporated under reduced pressure using rotary film evaporator. After evaporation 0.8 g crude extract (Khude anguli) and 0.9 g crude extract (Bathua) were isolated and stored in refrigerator. Thin layer chromatography and column chromatography for crude extract were performed with silica gel 60F 254 (Merck) and silica gel for column chromatography, respectively.

Preparation of TLC plate: Slurry was prepared by the slow addition with shaking 30 g of absorbent (silica gel) to 100 mL of dry chloroform in a wide-recked capped bottle. A pair of microscopic slides were held together and dipped into the slurry, slowly with drawn and allowed to drain momentarily while held over the bottle. The slides were parted carefully and placed horizontally in arack; it was then dried in sunlight/ in oven at 30-40°C for 10-15 min (Furniss *et al.*, 1989).

RESULTS AND DISCUSSION

Effect of weed extracts on wheat: The effect of weed extracts on germination and growth of wheat (Table 1) revealed that boiled extract of Shetdrone, Bhijli ghas and Bathua significantly reduced the germination of wheat by 18.1, 23.6 and 100%, respectively, compared with control. The unboiled extracts of the same weed also decreased germination of wheat seed by 27.8, 11.1 and 100%, respectively, over control. The lowest germination was observed in wheat seeds (0%) treated with boiled and unboiled extract of Bathua. The highest germination (96%) was in wheat seeds treated with control. All the boiled and unboiled extracts significantly delayed germination of wheat seed compared with the control. Root lengths of wheat seedlings were significantly reduced by boiled and unboiled extracts of weed species compared with the control. The root length was the lowest in wheat because of wheat were treated separately with both boiled and unboiled extract of Bathua. The effect of the control treatment showed maximum root length (9.42 cm). Shoot length of wheat was also significantly decreased by boiled and unboiled weed extracts except those of unboiled extracts of Bhijli ghas and Shetdrone. Among different weed extract under study, the lowest shoot length (2.92 cm) was recorded with unboiled extract of Bathua and maximum shoot length (6.08 cm) was found over control. The reduction of germination also decreased the shoot and root length of wheat possibly due to aqueous extracts of Bathua as well as allelopathic effects of this species. Similar result was also observed in other cases (Prasad and Srivastava, 1991).

Effect of weed extracts on jute: Table 2 shows that boiled and unboiled extracts of different weed species significantly inhibited the germination of jute seeds over control. The highest germination (85.3%) was found in seeds treated with control. The germination was

Table 1: Effect of weed extracts on germination and primary growth of wheat

Treatment	Germination (%)	Days to complete germination	Root length (cm)	Shoot length (cm)
Water (control)	96.0a	4.0c	9.42a	6.08a
Bathua (UBE)	0.00d	7.0a	0.99d	2.92b
Bhijli ghas (UBE)	85.3ab	6.0b	7.78b	5.19a
Shetdrone (UBE)	69.3c	6.0b	6.29b	4.96a
Bathua (BE)	0.00d	7.0a	1.34d	3.22b
Bhijli ghas (BE)	78.6bc	6.0b	6.25b	3.08b
Shetdrone (BE)	73.3bc	6.0b	4.35c	3.66b
$S_{\bar{x}}$	3.85	0.26	0.52	0.41

BE= Boiled extract, UBE = Unboiled extract

In a column, means followed by same letter(s) did not differ significantly at 5% level of probability.

Table 2: Effect of weed extracts on germination and primary growth of Jute

Treatment	Germination (%)	Days to complete germination	Root length (cm)	Shoot length (cm)
Water (control)	85.3 a	2.3 e	2.54 a	2.31 a
Mutha (UBE)	73.3 b	4.6 bc	1.03 b	1.94 b
Chapra (UBE)	68.0 bc	4.6 bc	1.00 b	1.86 b
Khude Anguli (UBE)	72.0 b	4.3 c	0.76 c	1.59 c
Mutha (BE)	64.0 c	3.3 d	1.16 b	1.73 bc
Chapra (BE)	64.0 c	5.3 ab	1.21 b	2.25 a
Khude Anguli (BE)	72.0 b	5.6 a	0.47 d	0.98 d
$S_{\bar{x}}$	2.39	0.28	0.07	0.08

decreased by 15-25% by boiled extracts of weeds compared with control and the lowest germination (64%) was recorded with Chapra and Mutha extracts treated seeds. Unboiled extracts of weeds reduced germination by 14 -21% over control and germination was the lowest (68%) in seeds treated with unboiled extract of Chapra. The germination period significantly increased in seeds treated with weed extract over control. Root lengths of jute seedlings were significantly reduced by boiled and unboiled extracts of weed species compared with control. The least root length (0.47 and 0.76 cm) was observed in seedlings treated with boiled and unboiled extracts of Khude Anguli. The effect of control showed maximum root length (2.54 cm). Shoot length of jute was also significantly decreased by boiled and unboiled weed extracts except those of boiled extract of Chapra.

The least shoot length (0.98 cm) was observed in seeds treated with boiled extract of Khude Anguli and the highest shoot length (2.31 cm) was found over control. The decreasing tendency of germination, root and shoot length in aqueous extract treated seedlings might be due to the presence of some toxic compounds or other allelopathic materials. Similar results were observed in Mohant and Soni (1979) that aqueous extract of Chandlai (Amaranthus) significantly inhibited the growth of root and shoot of Jowar seedlings due to presence of growth regulating substances in it. Kulshrestha and Mohant (1973) reported that aqueous extract of Bapji (*Ocimum omericana*) had a phytotoxic effect on seed germination and seedling growth of Sarson. Angiras *et al.* (1988) observed harmful effect of boiled extracts of white sedge and goat weed on Chickpea (*Cicer arietinum* L.).

It is interesting that decreasing tendency of germination, root and shoot length in aqueous extracts of Bathua in case of Wheat and Khude Anguli in jute, is a great challenge for us that why and which compound is responsible for this activity. For that, we separated organic layer from both aqueous extract. Boiled and unboiled extract both showed decreasing tendency but we emphasized on unboiled extract, over boiled extract because there is some possibility for decomposition of some valuable compounds due to high temperature.

The TLC of crude extract of Khude Anguli (*Digitaria ischaemum*) showed interestingly clear four compounds at hexane: ethyl acetate (20:1, v/v) (Fig. 1a). This result suggested that it contained four distinct compounds, designated as Khu_1, Khu_2, Khu_3 and Khu_4, respectively. Here also the intensity of non-polar part like Khu_1 was too much high. Similarly, TLC of crude extract of Bathua (*Chenopodium album*) showed that it contained three compounds at hexane: ethyl acetate 15:1, 10:1, v/v, respectively (Fig. 1b). From Fig. 1b it indicated that the intensity of the compound Bat_1 is too much high, indicates a major compound comparison with Bat_2 and Bat_3. In both Fig. 1a and b, the compounds were detected in iodine tank by calculating the R_f value using the following formula by the method of Furniss *et al.* (1989).

$$R_f = \frac{\text{Distance traveled by the component}}{\text{Distance traveled by the solvent front}}$$

R_f value of each component for both species is shown in Table 3.

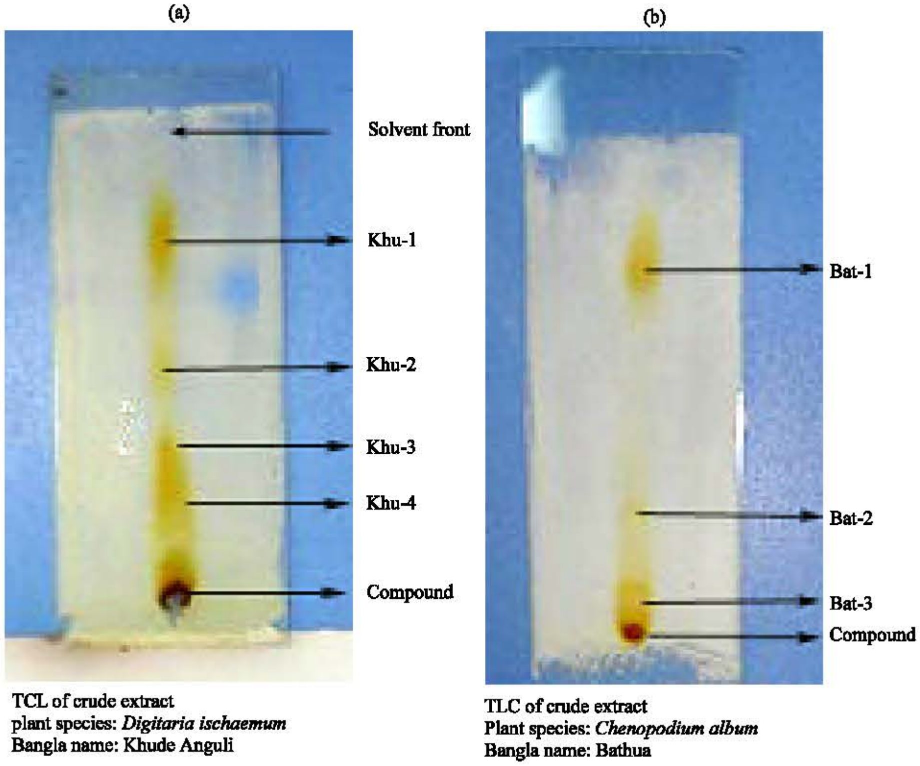

Fig. 1: Thin layer Chromatography (TLC) for different plant extracts. a and b represent TLC for Khude Anguli and Bathua extracts.

Table 3: R_f values of four components in two species.

Name of plant species	Detected component	R_f value (cm)
Khuda Anguli	Khu_1	0.769
	Khu_2	0.596
	Khu_3	0.327
	Khu_4	0.192
Bathua	Bat_1	0.740
	Bat_2	0.203
	Bat_3	0.074

Compounds showing the higher R_f value indicate most non-polar compound and compounds/components showing least R_f value indicate most polar compound. In case of Khude Anguli we designed four components as Khu_1, Khu_2, Khu_3 and Khu_4 according to their polarity. Similarly for Bathua, we designed as Bat_1, Bat_2 and Bat_3 as three components according to their order of polarity. Now we are in the way to purify the fractions of Khude Anguli and Bathua individually by column chromatography. After purification we will again set up an experiment for their germination test on respective seeds. The structure of the effective component will be determined by ^{1}H-NMR, 1R and Mass spectroscopy study, which will be reported on due course. It is difficult to draw a definite conclusion from this study. But from this result it is better to suggest the farmer to remove the weeds Khude Anguli (*Digitaria ischaemum*) and Bathua (*Chenopodium album*) rapidly during germination of jute and wheat seeds.

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