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COMPARATIVE GROWTH REGULATORY ACTIVITIES OF FRUIT LEAVES EXTRACTS AND ALLELOCHEMICALS ON PULSE CROPS

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ABSTRACT

The experiment was conducted on naturally occurring growth substances in aqueous extracts of some common fruit plant leaves *viz*., mango (T_2), black berry (T_3), jack fruit (T_4), litchi (T_5), wood apple (T_6), indian dillenia (T_7), papaya (T_8), banana (T_9), guava (T_{10}), olive (T_{11}) and water or control (T_1) on different pulse crops such as BARIMash3, BINAMug5, Lentil2, and bean with the attempt for chemical investigation on effective fruit leaves extract. The aqueous extracts of indian dillenia significantly increased the germination of BARIMash3, BINAMug5, Lentil2 and bean seeds also compared to control. The highest germination consequently 72.67%, 60.0%, 100% and 98.67% where maximum growth of BARIMash3, BINAMug5, Lentil2, and bean seeds showed compared with control. The effect of aqueous extract of indian dillenia showed the highest germination rate, root and shoot lengths in BARIMash3, BINAMug5, Lentil2, and bean seedlings. The thin layer chromatographic (TLC) examination of chloroform extracts of indian dillenia showed four distinct compounds at hexane: ethyl acetate (7:1, v/v). Crude compounds were purified and isolated by column chromatography.

Key words*: Aqueous extract, allelochemical, growth regulatory activity, pulse crops*

INTRODUCTION

Plants have an important role for human and other living organism and also for our environment. The plant kingdom supplies with food, fodder, shelter, wind breaker and provides raw materials for clothing and medicines. It is well known to all of us that most of the organic, bioorganic and also different types of toxic compounds are present in natural sources, especially in plant kingdom. The compound present in fruits leaves may undergo different types of interaction to produce allelochemical or other toxic compounds/chemicals, which may interrupt for germination of seeds. Some fruit leaves and crops secret allelochemicals and inhibitory substances which suppressed seed germination and seedling growth of neighbour crops ultimately reducing the yield of crops. Angiras (1987**)** showed the allelopathic effects of important weed species on germination and growth of maize and soybean seedlings. The allelopathic compound may be released from plants into the soil as either root exudates or decomposition products of their dead and worn out tissues. Sajjan *et al*.(1997) revealed the allelopathic effect of aqueous extracts of parthenium hystrerophorus plant parts on germination and seedling growth of some crops. Some fruit leaves have also been found to release volatile allelopathic compounds from their foliage which prove unhealthy to the nearby crop plants. Plant produces a wide variety of secondary metabolites that play important role in complex interactions among living organism in the natural environment, known as allelochemicals (Tripathi *et al* 1981). Now allelochemicals are used more generally for chemicals that mediate inter specific interaction. Plant eco-chemicals are the chemicals produced by plants that may be important role in complex interaction between plant-plant, plant-microorganism and plant-insect and plant-animal. A key deciphering the mechanism of allelopathic could be through and understanding of such soil processes as retention and transformation that affect the fate and transport of allelochemicals. The concept of chemical investigation on the extract of some fruit leaf is a new term and very much

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essential to protect the chemical pollution of the global environment (Roy *et al*., 2006a)**.** Plant also synthesis different hormones and acid like auxin, soprene, abscisic acid, ethylene, terpene, used as defense mechanism and growth parameter. Different types of naturally occurring organic and bioorganic compounds have been isolated from different types of plants, weeds and animal sources. Most of them have effective medicinal, insecticidal/pesticidal or toxic and growth regulatory values (Roy *et al*., 2006b). Therefore the study was undertaken to observe the biological activity *viz*. germination, radicle and plumule growth performance of BARIMash3, BINAMug5, Lentil2 and bean seeds using aqueous extracts of different fruit leaves.

MATERIALS AND METHODS

The experiment was conducted at the laboratory of Department of Agricultural Chemistry in Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. The tested leaves were wood apple (*Aegle marmelos*), indian dillenia (*Dillenia indica*), papaya (*Carica papaya*), olive (*Elaeocarpus floribundus*), blackberry (*Syzygium cumini*), guava (*Psidium guajava*), banana (*Musa sp*), litchi (*Litchi chinensis*), mango (*Mangifera indica*) and jackfruit (*Artocarpus heterophyllus*). The treatments under investigation for the study of pulse seeds germination were water (T_1), aqueous extract of mango leaves (T_2), aqueous extract of blackberry leaves (T_3), aqueous extract of jackfruit leaves (T_4), aqueous extract of litchi leaves (T_5), aqueous extract of wood apple leaves (T_6), aqueous extract of indian dillenia leaves (T_7), aqueous extract of papaya leaves (T_8), aqueous extract of banana leaves (T_9), aqueous extract of guava leaves (T_{10}) and aqueous extract of olive leaves (T_{11}). The freshly prepared aqueous extracts were applied on the seeds of selected pulse crops like BARIMash3, BINAMug5, Lentil2, and bean to observe their germination and growth performances.

Preparation of aqueous leaf extracts: Aqueous extracts were prepared from fresh leaves as well as stems or whole plant of the selected fruit leaves species. For this, immature fruit leaves species *viz*. mango, banana, blackberry, jackfruit, litchi, wood apple, indian dillenia, papaya, guava and olive were collected randomly, selected and chopped and then it was made into paste by blender machine. Exactly 100 g of each fresh leaf was taken and cut into smaller pieces, then crushed using blender machine with the required amount of water and was then transferred to a 500 ml reagent bottle. Then 400 ml of water was added to it and kept for 72 h at room temperature (25±2°C) with regular interval of stirring. Therefore, after 72 h the aqueous slurry was filtered through Whatman filter paper No.1 and was taken in another 500 ml bottle. The filtrates of individual leaf extracts were stored and used for seed treatment and comprehensive study.

Seed setup for the investigation of pulse crops by petridish method: Petridish experiments were performed for pulse crops such as BARIMush3, BINAMug5, Lentil2 and bean seeds for the observation of germination rate, shoot and root growths. For this experiment, clean petridish with two sheets of filter paper was placed. For the investigation of pulse seeds growth, 15 ml of each aqueous extract were taken in each petridish then 25 seeds of each pulse crop were placed on filter paper that was placed on petridish and each treatment was replicated into three (3) times. The Petridishes were kept in natural diffused light under laboratory conditions at room temperature (26±2°C). To moisten the filter paper, 5 ml water was applied if the filter paper was dried in control only distilled water was used. Germination and growth parameters were recorded everyday and it was continued until germination completed. Lengths of root and shoot were recorded after 7 days. The collected data were analyzed statistically and differences among means were compared by Duncan's Multiple Range Test (DMRT).

Isolation of crude compounds from effective fruit leaves using chloroform: For isolation of crude compounds of the individual fruit plant, 100 g powder of the leaves was taken in a 2.5 l reagent bottle. Exactly 250 ml chloroform was added to it and then kept for 72 h with regular interval of shaking. After 72 h, it was filtered through Whatman filter paper No.1. After the collection of extract in 500 ml reagent bottle, 200 ml of chloroform was added to the residue again, the reagent bottle was again kept for next 72 h with also regular interval of shaking. After 72 h, it

was filtered. The chloroform extracts were combined together. The solvent was evaporated by using rotary film evaporator under reduced pressure and preserved in refrigerator at 4˚C for further investigation.

Examination of crude extracts or crude compounds by TLC

Thin Layer Chromatography (TLC) was applied to detect or identify the number of compounds or number of components present in a crude extracts. The R_f value of each component was calculated by using this formulae:

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

Preparation of TLC plates and compound detection

Thin Layer Chromatography was carried on glass plates (slides) coated with silica gel G type 60 (BDH, England). The glass plates were then washed with distilled water and dried well in an oven. The coating material with appropriate amount, 10 g of silica gel intimately mixed with 20 ml of distilled water was vigorously shaken in a 100 ml conical flask to yield a homogeneous suspension. Slurry was prepared by the slow addition with shaking 30 g of absorbent (silica gel) to 100 ml of dry chloroform in a wide racked capped bottle. A pair of glass slide were held together and dipped into the slurry, slowly withdrawn and allowed to drain momentarily while held over the bottle. The slides were parted carefully and placed horizontally in a rack; it was then dried in sunlight /in oven at 30-40 ^{0}C for 10-15 minutes (Furniss, *et. al.*, 1989).

The compounds were dissolved in the appropriate solvents and the solutions of the compounds were then spotted with thin glass capillary tube at one end of the plates. The plates were then placed vertically with the spotted end downwards in a solvent tank. Compounds are separated and detected different compounds as located in different places on the TLC.

Chemical investigation on effective fruit plant extract

For isolation the crude compound from indian dillenia plant, 100 g of leaves powder was taken in a 2.5 L reagent bottle with 500 ml chloroform and kept for 72 h with regular interval of shaking. It was then filtered through filter paper no.1. The filtrate was collected in a 500 ml bottle and 200 ml ethanol was also added again to the residues. This process was done for three times. These collected filtrates were combined together and was evaporated using thin film rotary evaporator under reduced pressure. The crude compound was checked by TLC (Furniss *et. al*., 1989).

Column chromatography was performed using silica-gel as a standing phase and crude extract as a mobile phase. After column chromatography, it was attempted for determination of structure of purified compounds by ^{1}H-NMR (^{1}H-Nuclear Magnetic Resonance) and IR (Infrared spectroscopic) studies.

RESULTS AND DISCUSSION

Effect of aqueous extract of fruit leaves on black gram var. BARIMash3 germination

The aqueous extracts of indian dillenia significantly increased the germination of BARIMash3 seeds as compared to control (Table 1). The highest germination rate (72.6%) was found in BARIMash3 treated with the extracts of indian dillenia leaves. The aqueous extract of litchi showed the germination percentage of BARIMash3 seeds (70.2%) compared with control. Indian dillenia and guava inhibited the germination percentage which was 72.6% and 71.7% compared with control. Other extracts had similar effect on seed germination of BARIMash3. The superior increasing percentage of seed germination was found in seeds treated with indian dillenia possibly due to the allelopathic effects as well as the inhibiting substance present in the aqueous extract.

Root length

The highest root length of BARIMash3 seedlings (2.85 cm) was recorded in seeds treated with indian dillenia which was expressive different from the rest sources. The second highest root length (2.43 cm) was obtained in seeds treated with papaya which was statistically similar as

control. The third highest root length (2.39 cm) was found in seeds treated with litchi followed by seeds treated with indian dillenia and papaya which were statistically identical. The root length (1.92 cm) was found in seeds treated with mango. The root length (1.66 cm) was found in seeds treated with black berry followed seeds treated with jackfruit, banana, guava and wood apple which were statistically analogous (Table 1). The root length of BARIMash3 seedling (1.53 cm) was obtained in seed treated with wood apple aqueous extract, which was statistically identical with olive (1.28 cm). The most remarkable root length of BARIMash3 seedling was found in seeds treated with indian dillenia which might be due to the presence of some allelopathic chemicals.

Shoot length

Table 1 shows the effect of fruit leaves aqueous extracts of the superior shoot length of BARIMash3 seedling (2.92 cm) was recorded in seeds treated with indian dillenia, which was increased the development of shoot compared with control. The second highest shoot length (2.32 cm) was obtained in seeds treated with black berry which was statistically same as control (T_1). The third highest shoot length (2.29 cm) was found in seeds treated with banana followed by seeds treated with olive (2.07 cm) followed by seeds treated with jackfruit (2.03 cm) and treated with guava (1.51 cm). The shoot length of BARIMash3 seedling (0.89 cm) was recorded in seed treated with wood apple which was statistically similar with papaya (1.45 cm) aqueous extract. The result indicated that indian dillenia, aqueous extract increased the shoot length of BARIMash3 due to the presence of some allelochemicals.

Table1. Effect of fruit leaves extracts on germination and primary growth of black gram var. BARIMash3

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)
Water (control)	66.60ab	2.53 a	2.58ab
Mango	61.30ab	1.92 b	1.59cd
Black berry	6.00ab	1.67bc	2.32abc
Jack fruit	48.00 b	1.63bc	2.03abc
Litchi	70.20cd	2.39 a	2.12b
Wood apple	5.33c	1.53bc	0.89d
Indian dillenia	72.60 a	2.85 a	2.92 a
Papaya	58.60ab	2.43a	1.45cd
Banana	46.70b	1.60bc	2.29abc
Guava	71.70 a	1.57bc	1.91bc
Olive	56.00ab	1.29c	2.07abc
SD	6.22	0.159	0.2840

Effect of aqueous extract of fruit leaves on mungbean var. BINAMug5 germination

The highest germination percentage (60.0%) was found in seeds treated with indian dillenia (Table 2). The second highest germination percentage (40%) was obtained from seeds treated with mango. Third highest germination of BINAMug5 was recorded in seeds treated with guava followed by olive, jackfruit, papaya and banana which were statistically identical due to the presence of some allelochemical substances.

Root length

Table 2 shows that the highest root length (3.43 cm) of BINAMug5 seedlings was recorded in seeds treated with indian dillenia and the second highest root length of BINAMug5 seedlings (2.43 cm) was found in seeds treated with papaya which was statistically identical. The root length of BINAMug5 seedling (2.39 cm) was recorded in the seeds treated with litchi over the control.

The root length of BINAMug5 seedling (2.24 cm) was found in seeds treated with mango followed by black berry, jackfruit and guava which were statistically over the control or others.

Shoot length

The highest shoot length (3.37 cm) of BINAMug5 seedlings were recorded in seeds treated with indian dillenia and the second highest shoot length (2.67 cm) was recorded in seeds treated with olive. The shoot length of BINAMug5 seedlings reduced in seed treated with litchi, banana, jackfruit and guava with statistically analogous. The increasing tendency of shoot length in aqueous extract indian dillenia might be due to the presence of some chemicals or other allelochemicals.

Table2. Effect of fruit-leaves extracts on germination and primary growth of BINAMug5

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)
Water (control)	37.3bc	2.40 b	2.62abc
Mango	40.0b	2.24 b	1.57de
Black berry	23.3 c	1.67 c	1.69cde
Jack fruit	28.3bc	1.42 c	1.91bcd
Litchi	21.6 c	2.39 b	2.30bcd
Wood apple	2.33d	1.10 c	0.77 e
Indian dillenia	60 a	3.43 a	3.37 a
Papaya	28.3bc	2.43 b	1.33de
Banana	25bc	1.6 c	1.99bcd
Guava	31.7bc	1.42 c	1.83bcd
Olive	30.7bc	1.34 c	2.67ab
SD	4.738	0.1862	0.2955

Effect of aqueous extracts of fruit leaves on lentil var. Lentil2 germination

The highest germination (100 %) was found in seeds treated with indian dillenia. The second superior seed germination (96%) was observed in seeds treated with olive. The least germination (48.67%) was recorded in seeds treated with wood apple and 71.67% was found in seeds treated with papaya which was statistically identical over the control and other treatments probably due to the presence of some allelochemicals or toxic chemicals present in aqueous extract of respective fruit leaves.

Root length

From Table 3, it is no doubt to say that the highest root length (5.10 cm) of Lentil2 seedlings was recorded in seeds treated with indian dillenia and the second highest (3.39 cm) was found in seeds treated with olive which was statistically same. The root length of Lentil2 (3.34 cm) was found in seeds treated with guava. Root lengths of Lentil2 treated with mango (3.32 cm) and papaya (2.96 cm) were statistically identical compared with the control and other treatments. The superior root length of Lentil2 seedlings was found in seeds with aqueous extracts of indian dillenia perhaps due to the presence of some allelochemicals.

Shoot length

The highest shoot length of Lentil2 seedlings (7.41 cm) was recorded in seeds treated with indian dillenia. The second highest shoot length of Lentil2 seedling (6.40 cm) was found in control. The third highest shoot length of Lentil2 seedlings (5.92 cm) was found in seeds treated with jackfruit.

Shoot lengths of Lentil2 seedlings treated with papaya (4.88 cm) and mango (4.36 cm) were observed. Rests of all treatments were statistically similar. The increasing tendency of shoot length in aqueous extract of indian dillenia might be due to the presence of some toxic chemicals.

Table 3. Effect of fruit leaves extracts on germination and primary growth of lentil var. Lentil2

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)
Water (control)	82.7bc	2.09bcd	6.40ab
Mango	81.3bc	3.32abc	4.36abc
Black berry	84.0b	2.55bcd	3.17bc
Jack fruit	76.0bc	3.70ab	5.92abc
Litchi	74.7bc	1.58cd	2.71c
Wood apple	48.7d	2.81bcd	4.52abc
Indian dillenia	100.0 a	5.10a	7.41a
Papaya	71.7 c	2.96bcd	4.88abc
Banana	80.0bc	1.23d	3.75bc
Guava	78.7bc	3.34abc	4.25abc
Olive	96.0a	3.39abc	4.17abc
SD	3.38	0.123	1.01

Effect of aqueous extract of fruit leaves on bean germination

The highest germination (98.67%) was found in seeds treated with indian dillenia. The second highest germination on bean seeds (96%) was recorded in control. The germination (80.7%) was recorded in seeds treated with papaya and 86% germination was observed in seeds treated with olive which was statistically identical over the control and other treatments. The increasing tendencies of germination of bean seeds were probably due to the presence of some allelochemicals in aqueous extract of respective fruit leaves.

Root length

The highest root length (5.53 cm) of bean seedlings was recorded in seeds treated with indian dillenia as compared to control. The root length of bean (3.04 cm) was found in seeds treated with olive and 2.94 cm root length was noted in seeds treated with mango which were statistically identical. The superior root length of bean seedling was found in seeds treated with aqueous extract of indian dillenia.

Shoot length

The superior shoot length of bean seedling (7.90 cm) was recorded in seeds treated with indian dillenia and the second highest shoot length was found in control. The third highest seed germination 4.49 cm was found in mango. The shoot length of bean seedling (3.44 cm) was recorded in seeds treated with jackfruit which was followed by guava 3.44 cm. The shoot length of bean (3.14 cm) was found in seeds treated with wood apple extract which was identical. The shoot growth of bean in aqueous extract of indian dillenia increased due to the presence of some unknown allelochemicals.

Chemical investigation of the effective fruit leaves

The results in this experiment indicates that the aqueous extracts of different plant species had increasing activity on germination, root and shoot lengths of pulse crops such as BARIMash3, BINAMug5, Lentil2 and bean seeds. It is very interesting that the increasing tendency of

Table 4. Effect of fruit leaves extracts on germination and primary growth of bean

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)
Water (control)	96.0a	2.48 b	5.36 b
Mango	69.3b	2.94 b	3.49bcd
Black berry	36.3d	1.85b	2.18cd
Jack fruit	66.7bc	1.87 b	3.44bcd
Litchi	64bc	2.87 b	2.05 d
Wood apple	42cd	2.24 b	3.14cd
Indian dillenia	98.7 a	5.53 a	7.90 a
Papaya	86.7ab	1.37 b	2.49cd
Banana	85.3ab	1.47 b	2.04 d
Guava	68b	1.89 b	3.41bcd
Olive	86ab	3.04 b	4.20bc
SD	6.048	0.159	0.462

germination, root and shoot lengths was observed in aqueous extracts of indian dillenia in case of BARI Mash3, BINAMug5, Lentil2 and bean. It is a great challenge for the farmer's of our country to identify which compound is responsible for this type of activity. For this reason, crude compounds were extracted from the powder of indian dillenia fruit leaves with different non-polar and polar solvents like chloroform.

TLC of chloroform crude of wood apple leaves extract

The TLC of chloroform extract of indian dillenia was showed distinctly four compounds at hexane: ethyl acetate (7:1, v/v). The present findings suggested that the crude had four different compounds designated as Bl_1, Bl_2, Bl_3, and Bl_4 respectively. It showed the intensity of non-polar compound like Bl_1 was too much high in comparison with others. These compounds were distinguished in iodine tank and the R_f value was calculated

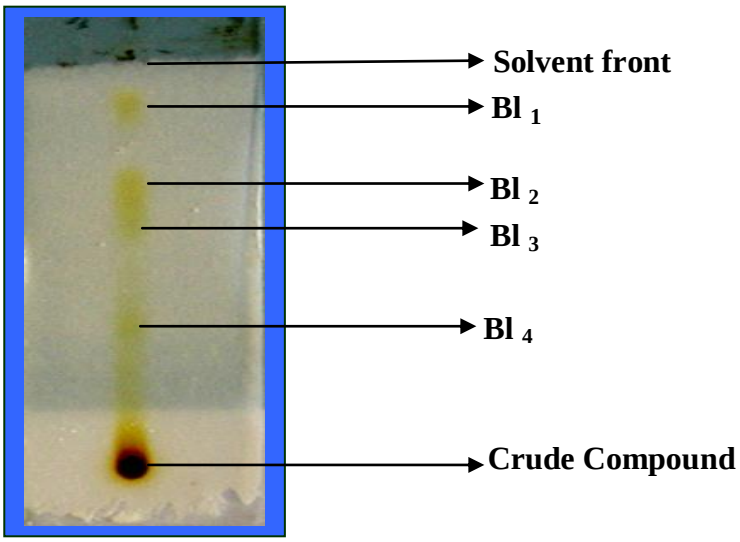

Figure1. TLC of chloroform crude extract of wood apple using the solvent hexane and ethyl acetate in the ratio of (7:1, v/v).

Table 5. R_f values of chloroform crude extract of wood apple

Name of plant species	Ratio of hexane and ethyl acetate	Detected component	R_f value
Wood apple *(Aegle marmelos)*	7:1	Bl_1	0.95
		Bl_2	0.75
		Bl_3	0.55
		Bl_4	0.33

The compounds with the higher R_f value indicated most nonpolar compound and compounds/components having the least R_f value indicated most polar compounds.

Considering the comparative activities of aqueous extract of tested plant species, indian dillenia showed strongly increasing activity on germination rate, root and shoot growths of BARIMash3, BINAMug5, Lentil2 and bean. The most effective extract was the indian dillenia among the tested extracts. The inhibitory activity might be due to the presence of some allelochemicals or toxic chemicals present in the mentioned extract comparison with others. This study indicated that fruit leaves extract have some beneficial effects on germination, root and shoot growths of BARIMash3, BINA Mug5, Lentil2 and bean seeds because indian dillenia may be contain allelochemicals which may responsible for this type of activity.

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