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Effects of aqueous extract of some herbal plant leaves on seed germination and seedling growth of bean (*Lablab niger*) and borboti (*Vigna unguiculata*)

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Abstract

An experiment was conducted on aqueous extracts of leaves of some herbal plants viz., bohera (*Terminalia belirica*), horitoki (*Terminalia chebula*), tulsi (*Ocimum gratissmum*) and arjun (*Terminalia arjuna*) for investigating the presence of biologically active substance. The seeds of two vegetable crop such as, country bean (*Lablab niger*) and borboti (*Vigna unguiculata*), were testing for germination and seedling growth treating with aqueous extract of four herbal plant leaves. The chemical investigation on effective plant extract was also attempted. The aqueous extract of horitoki significantly reduced and delayed germination, growth of shoot length and root length of country bean, and borboti seeds compared with control whereas, bohera increased and enhanced germination, growth of shoot length and root length of these same vegetable crops. The maximum germination was in country bean, and borboti seeds within 4 and 3.77days respectively, treated with the leaves extract of bohera compared with control. Shoot lengths were 15.63 and 15.83 cm and root length were 8.44 and 8.65 cm for country bean, and borboti seedlings, respectively, due to the presence of growth regulator. The aqueous extract of horitoki showed the lowest and late germination at 5 and 4.77days and minimum shoot length were 12.01 and 12.76 cm, and root length were 5.56 and 5.82 cm for country bean, and borboti seedlings, respectively, due to the presence of growth inhibiting substances or toxic compounds. The growth promoting effect of bohera leaves extract on the tested vegetable crops seemed due might be the presence of biologically active substances. The thin layer chromatography (TLC) examination of chloroform extract of bohera leaves showed four distinct compounds at Hexane: Ethyl acetate (3:1, $^{v}/_{v}$) while horitoki leaves showed three distinct compounds at Hexane: Ethyl acetate (5:1, $^{v}/_{v}$).

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Introduction

The Plant releasing chemical into the environment may have either deleterious or beneficial effects on the other plants growing in their vicinity. On the other hand, the synthetic or chemical which are toxic and require careful handling where as botanical or organic herbicide and pesticides which can be safely handled and have no high toxicity. Allelopathy, a phenomenon known as, 'chemical interactions' is also phenomenon induced by secondary metabolites. International Allelopathy Society (IAS), defines allelopathy as 'it studies any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influence the growth and development of agricultural and biological systems (IAS,1996).Thus herbal plants may play key role in phytotoxic inhibition of other plants. It is easier to screen allelopathic plants from medicinal plants possibly because, there exist certain secondary metabolic compounds potent for curing many diseases of mankind (Nahrstedt *et al.,*1982). Earlier works (Anjum *et al.,* 2010) led out the confirmation to these possibilities.

Secondary metabolites or allelochemicals are released by a plant into the environment by exudation from roots, leaching from stems and leaves or decomposition of plant material. Most of them have effective medicinal values, growth regulatory, herbicidal and pesticidal effects and also toxic values. Bohera (*Terminalia bellirica*), Horitoki (*Terminalia chebula*), Arjun *(Terminalia arjuna*), Tulsi (*Ocimum tenuiflorum*), extracts are commonly used as plant growth regulator or plant growth inhibitor. The plant product includes oil, extracts, dried leaves, fruits, seeds, rhizomes etc. Only Bohera based growth regulator have been evaluated as growth regulator of cereals, vegetables, cotton etc. The strongest inhibitory effect of aqueous extracts from *Terminalia chebula* on wheat seed germination, radical and plumule growth were reported by Tripathi *et al.*, (1981). Tripathi *et al.,* (1981) reported the strongest inhibitory effect of aqueous extracts of *Eupatorium adenophorum* on wheat seed germination, radical and plumule growth. Guenzi and Mc Calla (1962) showed that the water extract and the number of crop residues inhibited the germination growth of sorgam, corn and wheat in the laboratory experiment. They further reported that all residues contained water soluble substances that depressed the growth. An Experiment was conducted by B. Roy *et al.* (2006) on naturally occurring growth substances in aqueous extracts of some common weeds viz. Bothua (*Chenopodium album*), Bijli 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. Roy *et al.*, (2006) reported that banana plant extracts exerted a significant inhibition on seed germination of lettuce and the degree of inhibition increase with the increase in concentration of extract. Hong Gao *et al,* (2005) reported that Mammalian α-glucosidase inhibitory activity by *Terminalia chebula* Retz fruits were investigated. Regnault-Roger *et al.,* (2005) reported the bioactivity of 22 essential oils from aromatic and medicinal plants was tested upon *Acanthoscelides obtectus*, Coleoptera; Bruchidae, a pest of kidney bean, *Phaseolus vulgaris*. Trematerra *et al.*, (2002) tested fruits, extracts and metabolites of chilli, (*C. annum)* var. acuminatum, typical of the geographic areas of the molise region (central Italy) in an area for their attractive/repellent activity against adults of saw-toothed grain beetle (*Tribolium castaneum).*

However, 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). Islam (2002) found that plant material such as extract or powder of Bitter gourd, Karanja, Mehedi and Urmoi did not adversely affect seed germination. Reports are that some crop residues are known to have chemical (growth regulatory) as well as physical effect on the growth of several crops and weeds. Above information reported on germination, growth inhibitory or promoting influence, insecticidal activities as well as nutrient assimilation and other important biological activities of aqueous extracts of

plants leaves residues during germination of different crops and also in different insect pests. But data are not available about the effect of different extracts of Bohera (*Terminalia bellirica*), Horitoki (*Terminalia chebula*), Arjun *(Terminalia arjuna*), Tulsi (*Ocimum tenuiflorum*) on germination, plumule and radicle growth of some selected vegetables crops. Study of aqueous extract of leaves of fruit plants is of practical significance in crop farming system for the germination of seed and also crop growth rate. In view of this, the present investigation was framed to investigate the effects of aqueous extract of Bohera (Terminalia bellirica), Horitoki (Terminalia chebula), Arjun *(Terminalia arjuna*) and Tulsi (Ocimum tenuiflorum) on germination and seedling growth of cabbage and lady's finger seeds and isolate the different bioactive compounds from the effective aqueous extract. In view of this, the present investigation is framed with the following objectives:

a) Investigation of growth regulatory effect of aqueous extract of different herbal plants leaves.
b) Measurement of the active effect of aqueous extracts of the most effective herbal plants.
c) Isolation of biologically active compounds from the effective aqueous extracts.

Materials and methods

The experiment was conducted at research laboratory, Department of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh, for the study of the effects of naturally produced growth substances in four herbal plant leaves viz. beleric myrobalan, chebulic myrobalan, sweet basil, arjuna myrobalan on country bean and yard long bean along with the attempt for chemical investigation on the effective leaf extract.

Preparation of aqueous leaf extracts

100 gm of fresh and clean leaves were taken and cut into smaller pieces, it was then blended by using blender and was taken in a 500 ml reagent bottle and 400 ml of water was added to it. It was then kept for 72 hours at room temperature of 29±2°c and relative humidity of 85±5% with regular interval of stirring. After 72 hours the aqueous slurry was filtered through Whatmann filter paper No.1 and was taken in another 500 ml bottle. The filtrates of individual plant extract were stored and used for treating the seeds of vegetable crops along with water as a control and other comprehensive study.

Growth of Vegetable Crops

Petridish experiment was done for country bean, and Yardlong bean seeds for the observation of germination percentage; shoot growth and root growth, plant height etc. For this experiment, clean petridish with two sheets filter papers were used. For the investigation of germination percentage, growth and development of vegetable seeds, 15 ml of each aqueous extract was put in each petridish. In control, only distilled water was used and amount of distilled water was also same. Then twenty five (25) seeds of each vegetable crop were kept in each petridish and each treatment was replicated into three times. The petridishes were kept in natural diffused light under laboratory conditions at 29±2°c temperature and relative humidity of 85±5% after placing. 5 ml of water was used per day per petridish to keep constant moisture (Dubey, 1973). In control, only water was added if necessary per day per petridish. In this experiment, all subsequent observations were recorded and it was started from 16th June, 2009. After setting the experiment, the germination percentages, shoot length, root length and completion of germination were recorded. Effects of different treatments on morphology of seedlings were also recorded. The collected data were analyzed statistically and the co-efficient of variance and means were compared by using Duncan's New Multiple Range Test (DMRT).

Technique for Shoot and Root Growth Measurement

Ten healthy seedlings were taken from each replication of all treatments for measurement of shoot and root length. Each replication of individual treatments was averaged the root and shoot lengths measured individual treatment finally.

Isolation of Crude Compounds from Effective Herbal Plant Using Chloroform

For isolation of crude compounds of the individual herbal plant, 100 g of the leaves of effective herbal plant's powder was taken in a 2.5 L reagent bottle and 250 ml chloroform was added to the powder and it was then kept 72 h with regular interval of shaking. Then it was filtered by using Whatman filter paper No.1. The extract was collected in 500 ml reagent bottle and 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 then filtered. The extracting processes were repeated for at least three times. The chloroform extracts of individual plant were combined together. The solvent was evaporated from the extract by using rotary film evaporator under reduced pressure. The different types of plant extracts made by chloroform were Horitoki (18.67 g) and Bohera (18.38 g). After evaporation the semisolid crude was stored in refrigerator at 0˚C for further investigation.

Isolation of Crude Compounds from individual Herbal Plant using Ethyl-alcohol

To the residue after chloroform extract, 200 ml of ethyl-alcohol was added to it. It was kept for 72 h with several interval of shaking. After 72 h it was filtered by using Whatman filter paper No.1. The extract was collected in 500 ml reagent bottle and 200 ml of ethyl-alcohol 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 then filtered. The process was also repeated for three times. The ethyl-alcohol extracts of individual plant were combined together. The solvent was evaporated by using rotary film evaporator under reduced pressure. The different types of plant extracts by using ethyl-alcohol were Horitoki (18.67 g) and Bohera (18.38 g). Both the extracts were also stored in refrigerator at 0˚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 formula:

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

TLC was prepared for the bohera leaves extract as the most potential and compounds were detected by using the solvent hexane and ethylacetate in the ratio of 5:1 (v/v).

Results and discussion

Effect of aqueous extract of herbal leaves on Country bean

Germination

The highest germination percentage was in 6.3 days found in seeds treated with water (T_o) and statistically identical. Where the lowest germination percentage was in T_2 (4.8 days) was found in seeds treated with Bohera. The second highest germination percentage was obtained from seeds treated with T_1 (5.8 days), T_3 (5.3 days) and T_4 (5.3 days) which were statistically similar. Similarly the highest seed germination percentage was obtained from the seeds treated with water but germination gradually increases in case of Bohera extract treated seed (Table 1).

Shoot length

Shoot length of swamp cabbage at different days after sowing influenced significantly by the effects of different fruit leaf extract (Table 2). At 10 Days after sowing (days) with the leaf extract of Bohera showed the highest shoot length (16.43 cm) whereas the lowest shoot length (12.81 cm) was recorded with Horitoki. Other treatments showed more or less similar statistical results at the same time. The highest shoot length of swamp cabbage seedling was found with Bohera extract (22.05 cm) at 15 days that was statistically different from others. And other treatments showed moderate results and leaf extract of Horitoki showed the lowest value (18.05 cm). At 20 days, 25 days and 30 days the highest shoot length was recorded in case of seed treated with Bohera leaf extract (45.63 cm, 62.06 cm and 81.82 cm, respectively). The other treatments perform

moderately at different days after sowing (days). The increasing tendency of shoot length in aqueous extract of Arjun treated seedlings might be due to the presence of some growth regulatory materials. The highest shoot length of country bean seedling was found in *Terminalia belirica* aqueous extract possibly due to some growth regulator or other bioactive substances present in these species.

Table 1. Effect of some herbal plant leaf extract on percent germination of country bean and yard long.

Treatment	% Germination							
	Country bean				Yard long bean			
	Days to first germination	Time to 50% germination	Vigor index	Germination index	Days to first germination	Time to 50% germination	Vigor index	Germination index
T_0	6.3 a	6.8 a	44.53 d	11.89 c	5.27 a	5.77 a	44.3 d	10.86 c
T_1	5.8b c	5.9 b	46.89 c	12.59 bc	4.77 bc	4.87 b	45.86 e	11.56 bc
T_2	4.8 d	5.05 c	57.13 a	14.86 a	3.77 d	4.02 c	56.1 a	13.83 a
T_3	5.3 cd	5.55 b	48.82 b	12.93 b	4.27 cd	4.52 b	47.79 b	11.9 b
T_4	5.3 cd	5.5 b	49.7 b	13.17 b	4.27 cd	4.47 b	48.67 b	12.14 b
LSD(.05)	1.53	1.22	1.66	1.37	0.5	0.19	0.63	0.34
CV (%)	6.48	6.85	2.21	4.48	5.45	5.82	1.18	3.45

* Means followed by same latter (s) did not differ significantly at 5% level by DMRT.

T_0 = Control

T_1 = Horitoki leaf extract

T_2 = Bohera leaf extract

T_3 = Arjun leaf extract

Table 2. Effect of some herbal plant leaf extract on shoot length of country bean and yard long bean seeds.

Treatment	Shoot length (cm)									
	Country bean					Yard long bean				
	Days to first germination (DAS)					Days to first germination (DAS)				
	10 DAS	15 DAS	20 DAS	25 DAS	30 DAS	10 DAS	15 DAS	20 DAS	25 DAS	30 DAS
T_0	12.81 d	19.02 d	27.39 e	42.82 e	61.25 d	12.21 d	18.42 d	26.79 e	42.22 e	60.65 d
T_1	13.36 cd	20.59 c	29.58 d	50.58 d	66.34 c	12.76cd	19.99 c	28.98 d	49.98 d	65.74 c
T_2	16.43 a	22.85 a	45.63 a	62.06 a	81.82 a	15.83 a	22.25 a	45.03 a	61.46 a	81.22 a
T_3	14.48 b	21.56 b	37.66 b	56.37 b	71.10 b	13.88 b	20.96 b	37.06 b	55.77 b	70.5 b
T_4	14.04bc	21.34 bc	32.27 c	51.65 c	66.49 c	13.44bc	20.74 bc	31.67 c	51.05 c	65.89 c
LSD (.05%)	1.49	1.57	1.28	1.64	3.71	0.89	0.97	0.68	1.04	3.11
CV (%)	4.85	3.77	1.93	2.1	4.11	4.25	3.17	1.33	1.5	3.51

*Means followed by same latter(s) did not differ significantly at 5% level by DMRT.

Root length

Root length of country bean seedling at different days after sowing was lowest at 10 days and showed an increasing trend up to 30 days (Table 3), At 10 days after sowing (DAS days) seed treated with Bohera showed the best result (8.91 cm) whereas Horitoki showed the lowest root length (6.08 cm). The highest root length of country bean was found from (11.93cm) at 15 days that was statistically different from others whereas the lowest root length (9.06cm) was recorded with Horitoki leaf extract. And other treatments showed moderately similar results for the

same days after sowing. At 20 days, 25 days and 30 days the highest root length was recorded in case of seed treated with Bohera (17.25cm, 21.61 cm and 8.91 cm, respectively). The other treatments perform moderately at different days after sowing (days). The increasing tendency of root length in aqueous extract of Arjun treated seedlings might be due to the presence of some growth regulator and the lowest root length of country bean seedling was obtained in seed treated with horitoki aqueous extract. The highest root length of country bean seedling was found in seeds treated with bohera might be due to the presence of some growth regulator or other compounds present in the respective aqueous extract.

Table 3. Effect of some herbal plant leaf extract on root length of country bean and yard long bean seeds.

Treatment	root length (cm)									
	Country bean					Yard long bean				
	Days to first germination (DAS)					Days tofirst germination (DAS)				
	10 DAS	15 DAS	20 DAS	25 DAS	30 DAS	10 DAS	15 DAS	20 DAS	25 DAS	30 DAS
T_0	6.03 c	9.13 c	10.69 d	12.95 e	6.03 e	5.77 c	8.87 c	10.43 d	12.69 e	15.42 e
T_1	6.08 c	9.06 c	11.98 c	14.2 d	6.08 d	5.82 c	8.8 c	11.72 c	13.94 d	16.73 d
T_2	8.91 a	11.93 a	17.25 a	21.61 a	8.91 a	8.65 a	11.67 a	16.99 a	21.35 a	28.03 a
T_3	7.76 b	10.75 b	13.76 b	17.32 b	7.76 b	7.5 b	10.49 b	13.5 b	17.06 b	20.77 b
T_4	6.59 c	9.7 c	12.99 b	16.13 c	6.59 c	6.33 c	9.44 c	12.73 b	15.87 c	18.99 c
LSD(.05)	1.06	1.23	1.38	1.32	1.06	0.8	0.97	1.12	1.06	1.07
CV (%)	6.74	4.72	4.76	4	6.74	6.48	4.46	4.5	3.74	5.84

*Means followed by same latter(s) did not differ significantly at 5% level by DMRT.

Table 4. Rf values detected compounds of Horitoki (*Terminalia chebula*) with Chloroform solvent and Bohera (*Terminalia belirica*) with ethanol solvent.

Name of the plant species	Ratio of hexane and Ethylacetate	Detected component	R_f value
Horitoki (*Terminalia chebula*)	5:1	C_1	0.94
		C_2	0.72
		C_3	0.45
	Ratio of hexane and Ethylacetate	Detected component	R_f value
Bohera (*Terminalia belirica*)	3:1	P1	0.90
		P2	0.85
		P3	0.55
		P4	0.10

Effect of aqueous extract of herbal leaves on yard long bean

Germination

The highest germination percentage was in 5.27 days found in seeds treated with water (T_0) and statistically identical. Where the lowest germination percentage was in T_2 (3.77 days) was found in seeds treated with Bohera. The second highest germination percentage was obtained from seeds treated with T_1 (4.77days), T_3 (4.27days) and T_4 (4.27days) which were statistically similar. Similarly the highest seed germination percentage was obtained from the seeds treated with water but germination gradually increases in case of Bohera extract treated seed (Table 1).

Shoot length

Shoot length of yard long bean at different days after sowing influenced significantly by the effects of

different leaf extract (Table 2). The highest shoot length (15.83, 22.25, 45.03, 61.46 and 81.22) was obtained when seed treated with Bohera leaf extract (T_2) at 10 DAS, 15 DAS, 20 DAS, 25 DAS and 30 DAS followed by seed treated with Arjun leaf extract (T_3) which is identical with the result where seed treated with Tulsi leaf extract (T_4). On the other hand, lowest shoot length (12.21, 18.42, 26.79, 42.22 and 60.65) was obtained in seeds treated with water at 10 DAS, 15 DAS, 20 DAS, 25 DAS and 30 DAS.

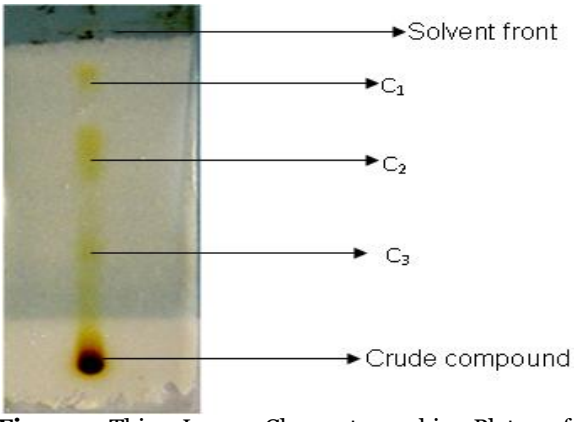

Fig. 1. Thin Layer Chromatographic Plate of *Terminalia chebula* (Horitoki).

Root length

Effect of leaf extract on root length of ladys finger (Table 3) varied significantly at different days after sowing (DAS). The maximum shoot (8.65, 11.67, 16.99, 21.35 and 28.03) length was obtained when seed treated with Bohera leaf extract (T_2) at 10 DAS, 15 DAS, 20 DAS, 25 DAS and 30 DAS. While the minimum root length (5.77, 8.87, 10.43, 12.69 and 15.42) was recorded seed treated water.

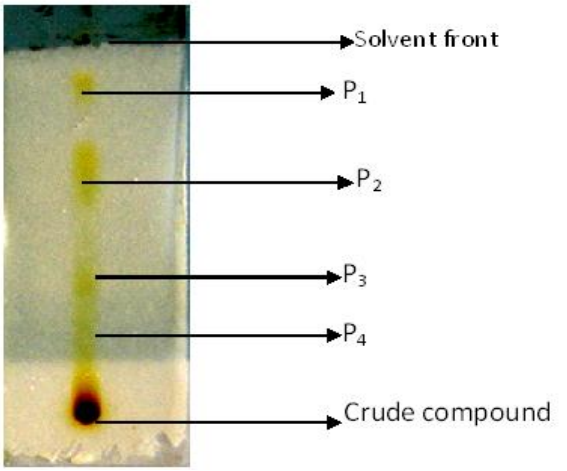

Fig. 2. Thin Layer Chromatographic Plate of *Terminalia belerica* (Bohera).

Chemical Investigation on Aqueous Extracts of Leaves of Herbal Plants

The results in this experiment indicates that the aqueous extracts of different plant species have increasing or inhibitory activity on germination parameters like time to get fast germination, time to get 50% germination, coefficient of germination, vigor index, germination index and final germination percentages and increasing on root and shoot length or early growth of vegetables. To find out this active compound Thin Layer Chromatography (TLC) of chloroform extract of Horitoki (*Terminalia chebula*) and ethanol extract of Bohera (*Terminalia belirica*) was done. The TLC (Thin Layer Chromatography) of chloroform extract of Horitoki (*Terminalia chebula*) was showed distinctly four compounds at Hexane: Ethylacetate (5:1 v/v), this result suggested that it contained three distinct compounds, designated as C_1, C_2 and C_3 respectively. The TLC (Thin Layer Chromatography) of ethanol extract of Bohera (*Terminalia belirica*) was showed distinctly four compounds at hexane: ethylacetate (3:1 v/v).This result suggested that it four distinct compounds, designated as P_1, P_2, P_3 and P_4 respectively. Here the intensity of non-polar compound like P_1 was too much high comparison with others.

Conclusion

This study interestingly indicated that herbal plant extracts have also strong biological activity in the field of agriculture. From this small scale study we may concluded that.

Aqueous extract of bohera significantly enhance the germination of vegetable crops.

It needs pot experiment as well as field experiment with bohera extract during cultivation of vegetable crops for final conclusion.

Leaves of bohera may contain some growth promoting and other bio-active substances.

It needs isolation of active compounds from leaves of bohera extract and their structure determination by

spectral study is also most essential.

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