

Comparative Study of Growth Regulatory Activities of Six Medicinal Plants Leaves Extracts on Two Summer Vegetable Seeds

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Abstract

This research was studied in petridish and pot condition using aqueous extracts of leaves of six medicinal plants viz., Arjun (*Terminalia arjuna*), Wood Apple (*Aegle marmelos*), Indian Lilac (*Azadiracta indica*), Green Chirayta (*Andrographis paniculata*), Malabar Nut (*Justica adhatoda*) and Ashoka tree (*Saraca asoca*) for investigating the presence of biologically active substances. The seeds of two summer vegetable crops such as, cucumber (*Cucumis sativus*) and yard long bean (*Vigna unguiculata*) were tested for germination and seedling growth treating with aqueous extract of six medicinal plant leaves. The chemical investigation on effective plant extract was also attempted. The aqueous extract of arjun leaves enhanced the germination of seeds. The growth of shoot and root length of cucumber and yard long bean showed more than those of other while the aqueous extract of Green Chirayta reduced and delayed germination, growth of shoot and root length of cucumber and yard long bean seeds enhanced compared with control. The thin layer chromatography (TLC) examination of ethanol extract of arjun leaves showed five distinct compounds at Hexane : Ethyl acetate (3:1 v/v). The separation and purification of these five distinct compounds are under in process which will be reported in due course.

Key Word: Arjun, Green Chirayta, Germination, Ethanol Extract.

1. Introduction

Bangladesh is a developing country and more than 80% people are directly and indirectly related to agriculture. Diverse crops are cultivated in different areas. Quick growing crops are the best way of

achieving return. Regarding these plants extracts play a vital role for increasing crop yield like growth promoter. The available of medicinal plants demands the isolation, separation, purification and characterization of physiologically active principles which are actually useful in the field of agriculture.

In plant kingdom, most of the plants have effective medicinal value, growth regulatory, herbicidal and pesticide effects and also toxic values. According to WHO, around 80% of the world's (5.86 billion) inhabitants depend on traditional medicine for their primary health care, majority of which use plant on their active principles [1].

However, several investigation reported that the effect of leave extract were affected different seed germination, growth development of various vegetable [2,3]. Islam [4].found that plant material such as extract or powder of Bitter ground, Karanja, Mehedi and Urmoi did not adversely affect seed germination. Reports are that crop residues are known to have growth regulatory chemical as well as physical effect on the growth of several crops and weeds. Above information was 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 not

available about the effect of different extract of Arjun, Wood Apple, Indian Lilac, Green Chirayta, Malabar Nut and Ashoka tree on germination of some selected vegetable crops.

Therefore, the present study was undertaken to examine the influence of some important summer and winter vegetable available in Bangladesh on germination of Cucumber and Long Yard's bean and the primary growth rate with emphasis on their chemical investigation on effective extracts.

2. Materials and Method

2.1 Experimental Site

The experiment was conducted in the laboratory and research field, Department of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh, during March 2013 to June 2014 for the study of the effects of some medicinal plants on two summer vegetable crops. The individual stored medicinal plant extracts were investigated as following sequential treatments for both vegetable seeds.

- | | |
|--------------------------------------|----------------|
| a) Aqueous extract of Arjun tree | T ₁ |
| b) Aqueous extract of Wood Apple | T ₂ |
| c) Aqueous extract of Indian Lilac | T ₃ |
| d) Aqueous extract of Green Chirayta | T ₄ |
| e) Aqueous extract of Malabar Nut | T ₅ |
| f) Aqueous extract of Ashoka tree | T ₆ |
| g) Water or control | T _c |

2.2 Selection and Collection of Medicinal Plants

The leaves of Medicinal plants were collected from Hajee Mohammad Danesh Science and Technology University campus. Leaves were collection from sunlight getting portion of a plant for the study of their biological activity and their

chemical investigation. The selected medicinal plants were Arjun (*Terminalia arjuna*), Wood Apple (*Aegle marmelos*), Indian Lilac (*Azadiracta indica*), Green Chirayta (*Andrographis paniculata*), Malabar Nut (*Justica adhatoda*) and Ashoka tree (*Saraca asoca*).

2.3 Selection and Collection of Summer Vegetables

The vegetables were selected on the basis their short life and growth period and also availability in sub – tropical countries. The seeds of these vegetables were collected from the Dinajpur seed market. The germination percentages of these seeds were 90% to 95% respectively. The following vegetable crops were selected Cucumber (*Cucumis sativus*), Yard long bean (*Vigna unguiculata*).

2.4 Preparation of Aqueous Extracts of Leaves of Medicinal Plants

Two hundred gram of fresh and clean dry leaves was taken and cut into smaller pieces, it was then blended by using blender and was taken in a 1000 ml reagent bottle and 800 ml of water was added to it. It was then kept for 72 hours at room temperature of $18 \pm 2^{\circ}\text{C}$ and relative humidity of $75 \pm 5\%$ with regular interval of stirring. After 72 hours the aqueous slurry was filtered through Whatman filter paper No.1 and was taken in another 500 ml bottle. The filtrates of individual plant extract was stored and used for treating the seeds of vegetable crops along with water as a control and other comprehensive study.

2.5 Set up for the Investigation of Vegetable Crop Seeds on Petridish

Petridish experiment was done for Yard long bean and Cucumber seeds for the observation of germination percentage; shoot growth and root growth etc. For this experiment, clean petridish with two sheets filter papers was used. For the

investigation of germination percentage, growth and development of vegetable seeds, fifteen 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 seeds of each vegetable crop were kept in each petridish and each treatment was replicated into five times. The Petridises were kept in natural diffused light under laboratory conditions at $29\pm 2^{\circ}\text{C}$ temperature and relative humidity of $85\pm 5\%$ after placing. 5 ml of water was

2.6 Set up for the Investigation of Vegetable Crop Seeds on Pot

Pot experiment was done for Cucumber and Yard long bean seeds for the observation of germination percentage; shoot growth and root growth etc. For this experiment, clean medium size pot was used. For the investigation of germination percentage, growth and development of vegetable seeds, 15 ml of each aqueous extract was put in each pot. In control, only distilled water was used and amount of distilled water was also same. Then twenty five seeds of each vegetable crop were kept in each pot and each treatment was replicated into five times. The pot were kept in natural diffused light under field conditions at $29\pm 2^{\circ}\text{C}$ temperature and relative humidity of $85\pm 5\%$. 15 ml of water was used per day per pot to keep constant moisture [5]. In control, only water was added if necessary per day per pot. In this experiment, all subsequent observations were recorded and it was started from March 2013 – June 2014. 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 data were subjected to analyze and compared by the DMRT method.

used per day per pot to keep constant moisture [5]. In control, only water was added if necessary per day each petridish. In this experiment, all subsequent observations were recorded and it was started from March 2013. After setting the experiment, the germination percentages, shoot length, root length and completion of germination were recorded. Effect of different treatments on morphology of seedlings was also recorded. The data were subjected to analyze and compared by the DMRT method.

2.7 Isolation of Crude Compounds from Effective Medicinal Plant Using Ethyl Alcohol

For the isolation of crude compounds of the individual medicinal plants, 100 gm of the leaves of effective medicinal plants leaves was taken in a 2.5 litter reagent bottle and 300 ml ethyl alcohol was added to it. It was then kept 72 hours with regular interval of shaking. After 72 hours it was filtered by using Whatman filter paper No. 1. The extract was collected in 500 ml reagent bottle and 300 ml of ethyl – alcohol was added to the residue again, the reagent bottle was again kept for next 72 hours with also regular interval shaking. After 72 hours it was then filtered. The extracting process was repeated for at least three times. The ethyl alcohol extracts of individual plant were combined together. The solvent was evaporated by using thin film rotary evaporator under reduced pressures at a temperature 55°C . Crude extracts were stored in refrigerator at 0°C for further investigation.

2.8 Examination of Crude Extracts or Crude Compounds by Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is one of the easiest techniques, by which we are able to detect or indentify the presence of the number of compounds or number of components present in a crude extract.

Crude compound in which R_f value of each component was calculated by using this formula:

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

3. Results and discussion

The results express the growth regulatory and enhancement activity of different aqueous extracts of medicinal plants leaves for seed germination, growth and development on both vegetables (Cucumber and Yard long bean). The result of the study has been presented in Tables 1, 2, 3, 4, 5, 6

3.1 Effects of Aqueous Extracts of Medicinal Plant Leaves on Cucumber and Yard Long Bean in Petridish

3.1.1 Germination Percentage

In Table 1, after two days germination of cucumber, treated with the aqueous extracts of Green Chirayta (T_4) and Arjun (T_1) were 20.20 % and 29.60 % respectively. The final date of seed germination of Cucumber i.e. after five days of the highest germination was observed (93.00 %) of Cucumber seeds treated with aqueous extract of Arjun tree (T_1) and which was statistically different from others but similar to control (T_c). Conversely, the lowest germination (65.60 %) of Cucumber was recorded in seeds treated with the extracts of Green Chirayta (T_4), which value was also not same from others treatment.

On the other hand, two days after in Table 1 the germination percentage of Yard long bean, the aqueous extracts of Green Chirayta (T_4) and Arjun (T_1) were 24.20 % and 28.70 % respectively. The final date of seed germination of Yard long bean i.e. after five days of seed showing the highest germination (93.20 %) of Yard long bean was found in seeds treated with aqueous extract of Arjun tree (T_1) and which value was statistically different from others but

About five different compounds were detected by thin layer chromatography (TLC) and separated by preparative TLC and calculated to find out five distinct R_f value in table 7.

similar to control (T_c). Whereas, the lowest germination (65.80 %) of Yard long bean was recorded in seeds treated with the extracts of Green Chirayta (T_4), which was also statistically different from others.

The highest germination of Cucumber and yard long bean seeds treated with aqueous extracts of Arjun tree possibly due to some growth regulatory or enhancing the lowest germination of Green Chirayta.

3.1.2 Shoot length

The effect of leaves extract of medicinal plants on the shoot length of Cucumber was significant throughout the growth period (Table 2). Shoot length of Cucumber was observed at different day's after showing (DAS). At 10 DAS shoot length of Cucumber was highest (5.70 cm) that is statistically similar to others and the shortest (3.88 cm) was recorded in T_4 (Green Chirayta) treatment. However, at 18 DAS, the highest shoot length (6.84 cm) was observed in the treatment applied with Arjun tree leaves extract and the shortest shoot length (3.15 cm) was recorded in the treatment with application of Green Chirayta leaves extract which was statistically different from other treatments.

In contrary, the effect of medicinal plants leaf extract on the shoot length of Yard long bean was markedly throughout the growth period (Table 2). At different day's shoot length of Yard long bean was calculated. At 10 DAS shoot length of yard long bean was more or less same. While the shortest (3.92 cm) was recorded in T_4 treatment. At 18 DAS, the maximum shoot length (6.14 cm) was observed in the

treatment T₁. As well as the lowest shoot length (3.96 cm) was recorded in the treatment with the application of Green Chirayta leaf extract which was statistically not same to other treatments.

3.1.3 Root length

From Table 3 root length of cucumber was observed at different day's and applied above same leaf extract. At 10 DAS shoot length of Cucumber was highest 3.54 cm which value similar to others. While, the shortest (2.50 cm) was recorded in T₄ (Green Chirayta) treatment. At 18 DAS, the highest root length (4.97 cm) was observed in the treatment applied with Arjun tree leaf extract and the shortest root length (2.63 cm) was recorded in the

treatment T₄ with application of Green Chirayta leaf extract which was statistically vary from others.

In contrast, the effect of leaf extract of medicinal plants on the root length of Yard long bean was significant throughout the growth period (Table 3). Root length of Yard long bean was observed at different day's after showing (DAS). At 10 DAS shoot length of yard long bean was statistically similar. While the shortest (1.77 cm) was recorded in T₄ (Green Chirayta) treatment. Lastly, at 18 DAS, the highest root length (4.49 cm) was observed in the treatment applied with Arjun tree leaf extract and the shortest root length (2.03 cm) was recorded in the treatment T₄. Which value showing from others.

Table 1: Effect of some medicinal leaves extract on germination percentage of cucumber and yard long bean seeds in petridis

Treatments	Germination Percentage (%)							
	Cucumber				Yard long bean			
	Two DAS	Three DAS	Four DAS	Five DAS	Two DAS	Three DAS	Four DAS	Five DAS
T ₁	29.60a	83.50a	89.40a	93.00a	28.70a	83.20a	89.60 a	93.20a
T ₂	21.60b	73.20b	68.00cd	73.70cd	25.60b	74.20b	67.40cd	73.20cd
T ₃	22.20b	68.40c	85.80ab	79.60b	24.20b	68.60c	85.80ab	79.60b
T ₄	20.20b	66.00c	66.40d	65.60d	24.20b	66.40c	66.40d	65.80d
T ₅	20.60b	73.60b	72.80cd	78.00bc	25.60b	74.60b	72.80cd	78.00bc
T ₆	21.20b	72.40b	78.00bc	81.60ab	25.20b	72.40b	78.00bc	84.60ab
T _c	20.40b	74.80b	86.40ab	92.80a	25.40b	74.80b	86.40ab	92.80a

Means followed by the same letter did not differ significantly at 5% level by DMRT.

Table 2: Effect of some medicinal leaves extract on shoot length of cucumber and yard long bean seeds in petridis

Treatments	Shoot length (cm)					
	Cucumber			Yard long bean		
	10 DAS	14 DAS	18 DAS	10 DAS	14 DAS	18 DAS
T ₁	5.70 a	6.58 a	6.84 a	4.70 a	5.58 a	6.14 a
T ₂	5.27 a	4.56 b	5.36 bc	4.27 a	4.56 b	4.36 bc
T ₃	4.80 a	5.33 ab	4.25 c	4.60 a	5.33 ab	4.05 c
T ₄	3.88 b	3.87 c	3.15 d	3.92 b	3.87 c	3.96 d
T ₅	4.46 a	4.10 c	3.66 d	4.46 a	3.91 c	3.98 d
T ₆	5.57 a	6.20 a	6.20 ab	4.57 a	5.20 a	5.20 ab
T _c	5.30 a	5.31 ab	5.31 bc	4.30 a	4.83 ab	4.31 bc

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

Table 3: Effect of some medicinal leaves extract on root length of cucumber and yard long bean seeds

Treatments	Root length (cm)					
	Cucumber			Yard long bean		
	10 DAS	14 DAS	18 DAS	10 DAS	14 DAS	18 DAS
T ₁	3.54 a	4.87 a	4.97 a	2.77 a	3.87 a	4.49 a
T ₂	3.45 a	3.19 b	3.66 b	2.45 a	2.59 b	2.96 b
T ₃	3.20 a	3.38 ab	3.49 b	2.20 a	3.38 ab	2.92 b
T ₄	2.77 b	2.50 c	2.63 c	1.77 b	1.85 c	2.03 c
T ₅	3.07 a	2.77 c	2.69 c	2.54 a	2.97 b	2.16 c
T ₆	3.12 a	3.35 ab	4.04 ab	2.42 a	3.35 ab	3.54 ab
T _c	3.42 a	3.90 ab	3.46 b	2.42 a	3.39 ab	2.46 b

Means followed by the same letter did not differ significantly at 5% level by DMRT.

3.2 Effects of Aqueous Extracts of Medicinal Plants on Cucumber and Yard Long Bean in Pot

3.2.1 Germination percentage

From the data in Table 4, the effect of leaves aqueous extracts medicinal plant on germination percentage of Cucumber seeds. At 2 DAS the maximum germination (52.80 %) was found in Cucumber seeds treated with aqueous extract of Arjun tree (T₁). Conversely, the minimum germination (39.60 %) was recorded in Cucumber seeds treated with

the application of Green Chirayta (T₄). Germination of Cucumber seeds at 5 day’s after showing the highest germination (96.10 %) was found in treatment T₁. . Conversely, the lowest germination (79.20 %) of was recorded in T₄, which was also statistically different from others.

On the other hand, same table 4 shows that, the effect of Medicinal plant leaves aqueous extracts on germination and growth of Yard long bean seeds. At 2 days after showing the highest germination (67.60 %) was found in seeds Yard long bean treated with aqueous extract of Arjun

tree (T₁), which was statistically different from others. Conversely, the lowest germination (47.60 %) was recorded in Yard long bean seeds treated with Green Chirayta (T₄), which was also statistically different from others. At the final date of seed germination of Yard long bean i.e. after 5 days of seed showing the upper germination (95.00 %) of Yard long bean was found in treatment T₁. As well as, the lowest germination (74.40 %) of Yard long bean was recorded in seeds treated with the extracts of Green Chirayta (T₄), which was also statistically different from others.

3.2.2 Shoot length

Table 5 shows that shoot length of Cucumber varied at different times experiment. At 10 DAS, shoot length (5.82 cm) was recorded the highest using the treatment T₁ and the lowest (4.38 cm) was recorded in Green Chirayta using (T₄) treatment. At 30 DAS the shoot length was statistically similar with T₁ (Arjun tree) treatments.

From Table 5, at different days shoot length of yard long bean was observed. At 10 DAS highest shoot length (7.96 cm) was observed in the treatment T₁ and the shortest (4.73 cm) was recorded in T₄

treatment. However, at 30 DAS, the highest shoot length (11.12 cm) was observed in T₁ (Arjun tree) treatment and others was statistically approximately same to T₁ (Arjun tree) treatment.

3.2.3 Root length

In Table 6, root length of Cucumber was observed at different day's after showing (DAS). At 10 DAS root length (4.02 cm) was observed in the treatment T₁ (Arjun tree) and another treatment near about same value Maximum root length (5.86 cm) was observed in the treatment T₁ (Arjun tree) at 30 days. Whereas minimum root length was observed (4.50 cm) in the treatment T₄ (Green Chirayta) and others value was near about same.

On the contrary, showing At 10 DAS, root length (4.03 cm) was observed in the treatment T₁, and another treatments value were not significantly different. . At 30 DAS, the root length (6.96cm) was observed in the treatment applied with Arjun tree leaf extract and the minimum root length (6.07 cm) was observed in the treatment T₄ (Green Chirayta).

Table 4: Effect of some medicinal leaves extract on germination percentage of cucumber and yard long bean seeds in pot

Treatments	Germination Percentage (%)							
	Cucumber				Yard long bean			
	Two DAS	Three DAS	Four DAS	Five DAS	Two DAS	Three DAS	Four DAS	Five DAS
T ₁	52.80 a	63.80 a	88.80 a	96.10 a	67.60 a	76.80 a	88.00 a	95.00 a
T ₂	47.20 ab	55.60 c	80.40 ab	82.40 b	66.60 a	74.60 ab	76.00 ab	84.80 ab
T ₃	42.00 b	61.20 a	70.40 bc	82.40 b	59.20 ab	65.60 b	74.00 b	84.00 ab
T ₄	39.60 bc	59.20 ab	63.20 c	79.20 b	47.60 c	58.00 c	76.00 ab	74.40 c
T ₅	46.40 ab	57.40 bc	70.40 bc	86.40 b	51.60 bc	63.00 bc	72.00 ab	81.60 bc
T ₆	45.20 ab	62.40 a	77.60 ab	88.80 ab	57.20 bc	63.40 bc	76.80 ab	89.60 ab
T _c	46.00 ab	62.80 a	80.80 ab	88.80 ab	61.20 ab	62.80 bc	76.00 ab	86.40 ab

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

Table 5: Effect of some medicinal leaves extract on shoot length of cucumber and yard long bean seeds in pot

Treatments	Shoot length (cm)					
	Cucumber			Yard long bean		
	10 DAS	20 DAS	30 DAS	10 DAS	20 DAS	30 DAS
T ₁	5.82 a	7.79 a	7.89 a	7.96 a	10.41a	11.12 a
T ₂	5.07 ab	7.03 bc	6.47 a	5.88 b	9.56 a	10.32 a
T ₃	4.82 ab	6.06 bc	7.05 a	6.05 b	10.05a	10.95 a
T ₄	4.38 b	5.41 c	7.51 a	4.73 c	9.45 a	10.13 a
T ₅	5.17 ab	6.18 bc	7.10 a	6.26 b	9.56 a	10.54 a
T ₆	4.43 b	7.69 ab	7.62 a	5.45 bc	9.64 a	10.24 a
T _c	4.90 ab	7.21 bc	7.65 a	6.20 b	9.41 a	10.33 a

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

Table 6: Effect of some medicinal leaves extract on root length of cucumber and yard long bean seeds in pot

Treatments	Root length (cm)					
	Cucumber			Yard long bean		
	10 DAS	20 DAS	30 DAS	10 DAS	20 DAS	30 DAS
T ₁	4.02 a	4.87 a	5.86 a	4.03 a	5.41 a	6.96 a
T ₂	3.27 ab	3.82 a	4.80 ab	3.13 b	4.16 a	6.68 ab
T ₃	3.57 ab	3.46 a	4.61 ab	3.59 b	4.93 a	6.49 ab
T ₄	3.01 b	4.29 a	4.50 b	3.24 b	5.20 a	6.07 b
T ₅	3.52 ab	4.11 a	4.77 ab	3.65 b	4.25 a	6.38 ab
T ₆	3.63 ab	3.64 a	4.81 ab	3.47 b	4.23 a	6.57 ab
T _c	3.68 ab	4.14 a	5.27 ab	3.07 b	4.78 a	6.11 b

Means followed by the same letter did not differ significantly at 5% level by DMRT.

3.3 Chemical Investigation on Aqueous Extracts of Leaves of Arjun

The excellent growth enhancing activity of aqueous extracts of leaves of Arjun on Cucumber and Yard long bean encourage us for their chemical investigation to know the reason what type of compound is responsible for such type of bioactivity. For this reason an attempt was taken to isolate the individual compound of five fractions of Arjun by thin layer chromatography (TLC), column chromatography and preparative TLC etc. Purifications under in progress, which will be reported in due course.

3.3.1 TLC Thin Layer Chromatography (TLC) of Ethanol Crude Extract of Arjun.

The TLC of ethanol extract of Arjun was done distinctly with five compounds at Hexane : Ethylacetate (3 : 1 v/v) (Table. 7), these results, designated as A₁, A₂, A₃, A₄ and A₅ respectively. Here also the intensity of non – polar compound like A₁ of Arjun was too much high compared with others. These compounds were detected in iodine tank and the following R_f values were calculated by using the formula [6].

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

Table 7: R_f values detected compounds of Arjun (*Terminalia arjuna*) with ethanol solvent

Name of plant species	Ratio of Hexane and Ethylacetate	Detected component	R_f value
Arjun	3:1	A ₁	0.94
		A ₂	0.82
		A ₃	0.73
		A ₄	0.61
		A ₅	0.41

4. Conclusions

Aqueous extracts of arjun enhances the germination, shoot and root growth of cucumber and yard long bean. Leaves of arjun may contain growth regulatory compounds and Green Chirayta reduced and delayed germination, growth of shoot and root length of cucumber and yard long bean seeds compared with control. The thin layer chromatography (TLC) examination of ethanol extract of arjun leaves showed five distinct compounds at Hexane : Ethyl acetate (3:1 v/v). Further investigation including separation of individual fractions and structure determination of active compounds are essential to make conclusive remarks.

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