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Growth Regulatory Activities of Different Extracts of *Tinospora Cordifolia* on Some Vegetable Seeds with their Chemical Investigation

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Abstract
An experiment was conducted to investigate the growth regulatory activities of different extracts of *Tinospora cordifolia* on radish (*Raphanus sativus***)**, swamp cabbage (*Impoea aquatica*) and lady's finger (*Hibiscus esculentus*) with the attempt for chemical investigation of effective plant extract. The chloroform extract of *Tinospora cordifolia* significantly increased and enhanced germination, growth of shoot length and root length of radish and lady's finger whereas and delayed germination, growth of shoot length and root length of swamp cabbage seeds compared with control. In the same way, ethanol extract of *Tinospora cordifolia* significantly increased germination, growth of shoot length and root length of swamp cabbage followed by control and chloroform extract. Our study reveals that different extracts of *Tinospora cordifolia* contain growth regulatory active principle. Among the extracts, chloroform extract was shown better performance in terms of percent germination, growth of shoot and root length of radish and lady's finger. To find out effective compound from chloroform extract, thin layer chromatography was done and showed five distinct compounds. Further study is needed to determine structure for finding growth regulatory compound for agricultural usage.

Key words: Chemical investigation, Extract, Growth regulatory activity, Vegetables,

Introduction

There are different types of plant such as herbal or medicinal, fruit trees, woody, necrotic, herbaceous, shrubs, weeds etc. in plant kingdom. Most of them have effective medicinal values, growth regulatory, herbicidal and pesticidal 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 or their active principles (Gias Uddin, 1998). The most important of these bioactive compounds of plant are alkaloids, flavonoids, tannins and phenolic compounds. There are different types of medicinal plants in Bangladesh. We have found that there is worth of 11 million US dollars medicinal plant market in Bangladesh, which have been imported but not in the name of medicinal plants rather in the name of spices and other products (Bregum, 2004). The cultivation of medicinal plants in Bangladesh will lead to the conservation and also protect the biodiversity. One of the medicinal plants *Tinospora cordifolia* belongs to the *Menispermaceae* family and is commonly known as Gulancha or Tinospora in English and Giloya in Hindi. The plant is a glabrous climbing shrub found throughout Bangladesh, India, Myanmar and Sri Lanka typically growing in deciduous and dry forests. It is a widely used shrub in folk and ayurvedic systems of medicine. It is reported to possess anti-spasmodic, anti-inflammatory, anti-allergic, anti-oxidant properties (Singh *et al*., 2003). The root and stem of *T. cordifolia* are prescribed in combination with other drugs as an anti-dote to snake bite and scorpion sting (Zhao *et al*., 1991). *T. cordifolia* is widely used in Indian ayurvedic medicine for treating diabetes mellitus (Stanely *et al*., 2001). It is reported that the daily administration of either alcoholic or aqueous extract of *T. cordifolia* decreases the blood glucose level and increases glucose tolerance in rodents (Grover *et al*., 2001).

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 plant extracts play a vital role for increasing crop yield like growth promoter. The availability of medicinal plants demands the isolation, separation, purification and characterization of physiologically active principles which are actually useful in the field of agriculture. To our knowledge, there is scarcity of information on the phytotoxic effects of *T. cordifolia* on succeeding agricultural crops. The attention is being needed to the importance of rotation in medicinal plant or between medicinal herbs and other crops, due to strong allelopathic effects of medicinal plants (Basotra *et al.*, 2005; Guo *et al.*, 2006; Nazir *et al*., 2007). Various types of extracts of *T. cordifolia and shial mutra* having bioactive compound increase or decrease germination and growth rate of crops (Roy *et al.* 2004; Roy, S.R. 2006). The observation of the growth regulatory activities of different extracts of *T. cordifolia* have progressed research works in the field of natural product chemistry. Different experiments were conducted to understand the effect of different extracts of *T. cordifolia* on various diseases. But data are not available about the effect of different extracts of *T. cordifolia* on germination, plumule and radicle growth of some selected vegetables crops. Keeping this view in mind the research has been undertaken to investigate the growth regulatory effects of different extract of *T.*

cordifolia and isolate the different bioactive compounds from the effective extract of *T. cordifolia.*

Materials and Methods

Experimental Site

The experiment was conducted at research laboratory, Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh, during January 2011-December 2011.

Collection of T. cordifolia and summer vegetable seeds

T. cordifolia stems were collected from Chalksudum of Chirirbandar under Dinajpur district. Radish (*Raphanus sativus)*, Swamp cabbage (*Impoea aquatic)* and Lady's finger (*Hibiscus esculentus*) seeds were selected due to their short growth period and available in sub-tropical countries. The seeds of these vegetables were collected from the Dinajpur seed market. The purity percentages and germination percentages of these seeds were 95 and 90, respectively.

Preparation of aqueous extracts of T. cordifolia

About 50 gm of fresh stems of *T. cordifolia* were cut into smaller pieces and blended by blender and taken into a 1000 ml reagent bottle with water up to the mark. It was kept for 72 hours at room temperature with regular interval of stirring. After 72 hours the resulting brownish and dark solution were filtered through three layers of Whatman No. 1 filter paper and was taken into another 1000 ml bottle. The filtrates of plant extract were stored and used for treating the seeds of vegetable crops.

Preparation of chloroform extract and ethanol extract of T. cordifolia

Ten kilogram of green and fresh stem were cleaned and cut into small pieces. The small pieces of stem were dried in sun light for 7 days followed by oven at 70^{o} C for 48 hours. The dried stem was grinded by grinding machine and obtained about 4 kg powder. Two kilogram powder was dissolved in five liter absolute chloroform (96%) and another two kilogram in absolute ethanol (98%) and incubated for 72 hours for a suspension. These suspensions were filtered with thin and clean cloth and finally filtered by filter paper. The suspension was dried by BUCHI Rota vapor R-114 connected with BUCHI water bath B-480 at 70^{o}C. The dried extract was weighed by digital balance. Five percent solution of each extract was prepared with water for treating the seeds of vegetable crops. The individual plant extracts were investigated as following sequential treatments:

a) Water or control (T_c)
b) Aqueous extract of *T. cordifolia* (T_1)
c) Chloroform extract of *T. cordifolia* (T_2)
d) Ethanol extract of *T. cordifolia* (T_3)

Set up for the investigation of vegetable crop seeds

Thirty six petridish were cleaned and divided into three groups for three vegetables seeds and two sheets filter papers were placed on it. For each vegetable seeds, petridish were divided into four treatments with three replications each petridish separately (Fig 1). 15 ml of each aqueous, chloroform, ethanol extract and distilled water put in each petridish according to experimental design. After that 25 seeds of each vegetable crop were kept in each petridish. The petridishes were placed in natural diffused light under laboratory conditions at 29±2°C temperature and relative humidity of 85±5%. Five ml of water was applied per day per petridish to keep constant moisture. The percent germination, shoot length, root length and completion of germination were recorded.

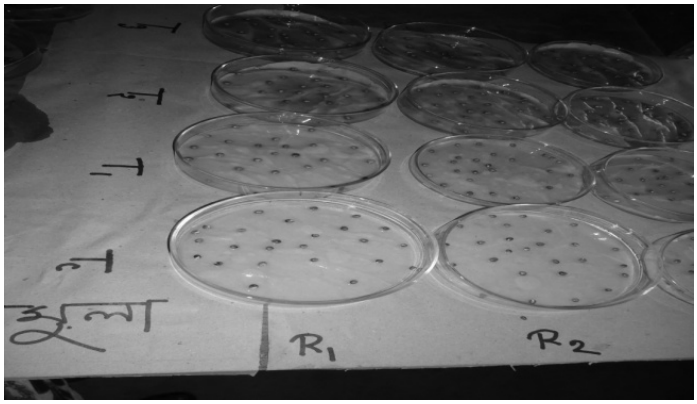

Fig. 1. Seeds set up for the investigation

Identification of effective extracts

analyzed statistically using Duncan's Multiple Range Test (DMRT). After analyses these data, it was observed that the chloroform extract of *T. cordifolia* enhance the germination, shoot length and root length of radish and Lady's finger and inhibit germination, shoot length and root length of swamp cabbage compared with control.

Chemical investigation of effective extract of T. cordifolia

Thin Layer Chromatography (TLC) technique was used to detect the presence of compounds in a crude extract in

The collected data were which R_f value of each component was calculated by using this formula (Furniss *et al.*, 1989):

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

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 values (Fig. 2 & 3

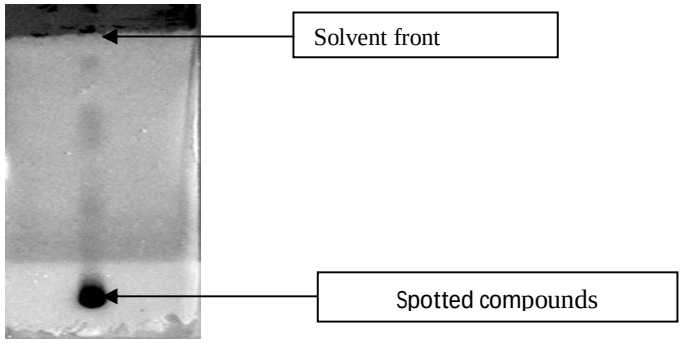

Fig. 2. Thin Layer Chromatographic Plate

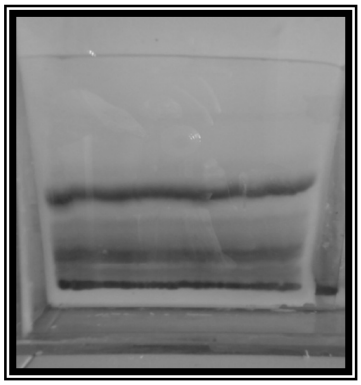

Fig. 3. Preparative TLC

Test for sterols of different purified compound

After purification of different crude compounds, the isolated purified compounds were subjected to test for sterol by Salkowaski reaction and Libermann-Burchard reactions.

Results and Discussions

The results showed that growth regulatory activity in different extracts of *T. cordifolia.* The result of the study has been presented in Tables (1, 2 & 3).

Table 1. Effects of *T. cordifolia* (gulancha) stem extract on percent germination of radish, swamp cabbage and lady's finger seeds

Treatments	% Germination								
	Radish			Swamp Cabbage			Lady's finger		
	1st day	3rd day	6th day	1st day	3rd day	6th day	1st day	3rd day	6th day
Control (water)	28.00 a	56.00 a	84.00 b	30.67 a	56.00 a	74.67a	29.30a	41.33b	61.33a
Aqueous Extrct	18.67 b	34.67 b	57.33 c	12.00 b	34.67 b	61.33b	12.00b	29.33c	44.00b
Chloroform extract	29.33 a	54.67 a	96.00 a	16.00 b	44.00ab	74.67a	36.00a	50.67a	64.00 a
Ethanol Extract	25.33 a	53.33 a	82.67 b	36.00 a	52.00 a	78.67a	16.00b	49.33ab	58.67a

Data expressed in mean value; different letter in a column differ significantly at 5% level of significance (as per DMRT)

Table 2. Effects of *T. cordifolia* (gulancha) stem extract on shoot length of radish, swamp cabbage and lady's finger seeds

Treatments	Shoot length (cm)								
	Radish			Swamp cabbage			Lady's finger		
	5th day	8th day	11th day	5th day	8th day	11th day	5th day	8th day	11th day
Control (water)	0.9933 b	3.163 a	4.567ab	0.9267 b	4.067 b	5.233 b	1.637 a	3.667 a	5.380 b
Aqueous Extract	1.380 b	2.250 a	2.433 b	1.580 a	1.093 c	1.303 d	1.467 b	1.750 b	0.0000 d
Chloroform extract	2.373 a	2.427 a	5.873 a	1.573 a	4.033 b	4.900 c	1.617 a	3.563 a	5.737 a
Ethanol Extract	1.220 b	2.013 a	3.787 ab	1.893 a	5.233 a	5.867 a	1.527 b	3.280 a	5.163 c

Data expressed in mean value; different letter in a column differ significantly at 5% level of significance (as per DMRT)

Table 3. Effects of *T. cordifolia* (gulancha) stem extract on root length of radish, swamp cabbage and lady's finger seeds

Treatments	Root length (cm)								
	Radish			Swamp Cabbage			Lady's finger		
	5th day	8th day	11th day	5th day	8th day	11th day	5th day	8th day	11th day
Control (water)	1.627 ab	2.827 a	4.767 a	1.273 a	1.4793 a	4.380 b	1.353 a	3.017 a	5.033 b
Aqueous Extract	1.060 b	1.620 a	1.120 b	0.7667 a	0.8633 b	0.9633 c	1.257 a	1.330 b	0.45 d
Chloroform extract	1.727 a	3.153 a	6.047 a	1.247 a	2.837 a	4.417 b	1.340 a	3.177 a	5.300 a
Ethanol Extract	1.180 ab	1.873 a	3.500 ab	1.347 a	3.057 a	5.500 a	1.303 a	2.617a	4.733 c

Data expressed in mean value; different letter in a column differ significantly at 5% level of significance (as per DMRT)

Effect of *T. cordifolia* (gulancha) extracts on radish

Percent germination

The percent germination was counted in 1st, 3rd and 6th days presented in Table 1. In the 1st day, the highest germination was found in T_2 (29.33%) followed by T_c and T_3. The lowest germination was recorded in T_1 (18.67%). In 3rd day, the highest germination was found in T_c (56.00%) followed by T_2 and T_3. The lowest germination percentage was recorded in T_1 (34.67%). In 6th day, the highest germination was found in T_2 (96.00%) and the lowest germination was recorded in T_1 (57.33%).

Shoot length

Shoot length of radish was significantly influenced by different types of stem extracts (Table 2). After 5 days after sowing (DAS), chloroform extract of *T. cordifolia* shows the highest shoot length (2.373 cm) whereas the lowest shoot length (0.9933 cm) was recorded in control treatment. The highest shoot length of radish seedling was found in T_2 i.e. chloroform extract of *T. cordifolia* (2.427 cm) at 8 DAS that was statistically similar to others. At 11 DAS the highest shoot length was recorded in T_2 (5.873 cm) and the lowest was found in T_1 (2.433 cm). At 14 DAS, The highest shoot length of radish seedling was found in T_2 i.e. chloroform extract of *T. cordifolia* (6.633 cm) followed by T_c and T_3. On the other hand the lowest shoot length was recorded in T_2 (2.667 cm). Possibly chloroform extract of *T. cordifolia* contain some growth regulatory or other bioactive substance which may responsible for enhancing the shoot length of radish.

Root length

Root length of radish was significantly influenced by different types of stem extract (Table 3). After 5 DAS, chloroform extract of *T. cordifolia* showed the highest root length (1.727 cm) which was similar to T_c and T_3 treatments, whereas the lowest root length (1.06 cm) was recoded in T_1 treatment. The highest root length of radish seedling was found in T_2 i.e. chloroform extract of *T. cordifolia* (3.153 cm) at 8 DAS that was statistically similar to others. At 11 DAS the highest root length was recorded in T_2 (6.047 cm) and the lowest was found in T_1 (1.12 cm). At 14 DAS, The highest root length of radish seedling was found in T_2 i.e. chloroform extract of *T. cordifolia* (6.333 cm) followed by T_c and T_3. On the other hand the lowest shoot length was recorded in T_2 (1.00 cm). Possibly chloroform extract of *T. cordifolia* contain some growth regulatory or other bioactive substance which may responsible for enhancing the root length of radish.

Effect of *T. cordifolia* (gulancha) extract on swamp cabbage

Percent germination

The percent germination was counted in 1st, 3rd and 6th days presented in Table 1. In the 1st day, the highest germination percentage was found in T_3 (36.00%) followed by T_c treatment. The lowest percent germination was recorded in T_1 (12.00%). In 3rd day, the highest germination percentage was found in T_c (56.00%) followed by T_2, T_3 and T_1 (34.67%), respectively. In 6th day, the highest germination percentage was found in T_3 (78.67%) similar to that of T_c and T_2 treatments, respectively. On the other hand the lowest germination percentage was recorded in T_1 (61.33%).

Shoot length

Shoot length of swamp cabbage was significantly influenced by different types of stem extracts (Table 2). After 5 DAS, Ethanol extract of *T. cordifolia* showed the highest shoot length (1.893 cm) followed by T_1, T_2 and Tc (0.9267 cm) treatment, respectively. The highest shoot length of Swamp cabbage seedling was found in T_3 i.e. Ethanol extract of *T. cordifolia* (5.233 cm) and the lowest shoot length was recorded in T_1 (1.093 cm) in the 8 DAS. At 11 DAS the highest shoot length was recorded in T_3 (5.867 cm) and the lowest was found in T_1 (1.303 cm). Possibly ethanol extract of *T. cordifolia* contain some growth regulatory or other bioactive substances which may responsible for enhancing the shoot length of swamp cabbage.

Root length

Root length of swamp cabbage was significantly influenced by different types of stem extract (Table 3). After 5 DAS, Ethanol extract of *Tinospora cordifolia* was the highest root length (1.347 cm) which was statistically similar to all other treatments. The highest root length of Swamp cabbage seedling was found in T_3 i.e. ethanol extract of *Tinospora cordifolia* (3.057 cm) followed by T_c , T_2 and T_1 (0.8633 cm), respectively at 8 DAS. At 11 DAS the highest root length was recorded in T_3 (5.50 cm) and the lowest was found in T_1 (0.9633 cm). Possibly ethanol extract of *T. cordifolia* contain some growth regulatory or other bioactive substance which may responsible for enhancing the root length of swamp cabbage.

Effect of *T. cordifolia* (gulancha) extract on lady's finger

Percent germination

The highest germination percentage was counted in 1st, 3rd and 6th days presented in (Table 1). In the 1st day, highest germination percentage was found in T_2

(36.00%) followed by T_c treatment. The lowest germination was recorded in T_1 (12.00%). In 3rd day, the highest germination was found in T_2 (50.67%) which was followed by T_3 treatment. The lowest germination was recorded in T_1 (29.33%). In 6th day, the highest germination percentage was found in T_2 (64.00%) followed by T_c and T_3, respectively. On the other hand the lowest germination was recorded in T_1 (44.00%).

Shoot length

Shoot length of lady's finger was significantly influenced by different types of stem extracts (Table 2). After 5 DAS, chloroform extract of *Tinospora cordifolia* showed the highest shoot length (1.617 cm) which was similar to T_c treatment. The lowest shoot length (1.467 cm) was recoded in T_1 treatment. The highest shoot length of lady's finger seedling was found in T_2 i.e. chloroform extract of *T. cordifolia* (3.563 cm) followed by T_c, T_3 and T_1 (1.75 cm), respectively at 8 DAS. At 11 DAS the highest shoot length was recorded in T_2 (5.737 cm) and no shoot was found in T_1 (00 cm). Possibly chloroform extract of *T. cordifolia* may contain some growth regulatory or other bioactive substances which may responsible for enhancing the shoot length of lady's finger.

Root length

Root length of lady's finger was significantly influenced by different types of stem extracts (Table 3). After 5 DAS, chloroform extract of *T. cordifolia* showed the highest root length (1.34 cm) which was similar to all other treatments. At 8 DAS, the highest root length of ladys finger was found in T_2 i.e. chloroform extract of *T. cordifolia* (3.177cm) that was statistically similar to T_c and T_3 treatments. On the other hand the lowest root length was found in T_1 (1.33 cm). At 11 DAS the highest root length was recorded in T_2 (5.30 cm) and the lowest was found in T_1 (0.45 cm), respectively. Possibly chloroform extract of *T. cordifolia* may contain some growth regulatory or other bioactive substances which may responsible for enhancing the root length of lady's finger.

Chemical investigation of chloroform extract of T. cordifolia

The results in this experiment indicate that the chloroform extract of *T. cordifolia* have increasing or inhibitory activity on germination, shoot and root length of early growth vegetables. To find out this active compound Thin Layer Chromatography (TLC) of chloroform extract of *T. cordifolia* was done and showed five distinct compounds at Hexane: Ethylacetate (5:1 v/v). This result suggested that it contained five distinct compounds, designated as F_1, F_2, F_3, F_4 and F_5 respectively (Table 4). These compounds were detected in iodine tank and the R_f value were calculated by using the formula (Furniss *et al.,* 1989).

Table 4. R_f values of detected components of *Tinospora cordifolia*

Name of the Plant Species	Ratio(Hexane:Ethylacetate)	Detected component	R_f value
Tinospora cordifolia	5:1	F_1	0.94
		F_2	0.81
		F_3	0.72
		F_4	0.45
		F_5	0.18

Chemical test for sterol for isolated fraction

The results of the different chemical test were presented in Table 5. The fractions F_3, F_4 and F_5 were showed both the Salkowaski and Libermann Burchard reaction positive which indicated that F_3, F_4 and F_5 may be steroid type compounds.

Table 5. Chemical tests for sterol for isolated fractions

Name of fraction	Salkowaski reaction	Liebermann-Burchard reaction
Fraction 1	-ve	-ve
Fraction 2	-ve	-ve
Fraction 3	+ve	+ve
Fraction 4	+ve	+ve
Fraction 5	+ve	+ve

Conclusions

Finally, we can say chloroform extract of *T. cordifolia* enhance the germination percentage, shoot and root length of radish and lady's finger in all respects comparison with other treatments. Therefore, it can be said that chloroform extracts of *T. cordifolia* may contain growth regulatory or other bio-active substances. Further investigation including separation of individual fractions and structure determination of active compounds are essential to make conclusive remarks.

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