J. Agrofor. Environ. 5 (2): 63-66, 2011 ISSN 1995-6983

Biological activity of four ornamental plant leaves on some vegetable seeds and chemical investigation of *Butea monosperma*

Sona R. Roy, B. Roy, Bikash C. Sarker, M. Nazim Uddin and Prodip K. Roy
Department of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh

Abstract: An experiment was conducted to assay biological activity such as germination inhibition or activation; root and shoot lengths growth inhibition or enhancement of aqueous extracts of some ornamental plants leaves and to identify the bioactive compounds. The aqueous extract of *Butea monosperma* significantly enhanced germination (35.2 to 12 %) and growth of root length (153.3 to 47.1 %) and shoot length (66.8 to 23.0%) of the vegetables (*Impoea aquatic*, *Cucumis sativus*, *Cucurbita moschata*, *Citrullus lanatus* and *Vigna sesquipedalis*). A thin layer chromatography (TLC) examination of chloroform extract of *B. monosperma* showed four distinct spots (hexane: ethyl acetate::5:1). Three compounds were isolated by preparative TLC. To determine the structures of these compounds NMR, IR and Mass spectroscopic studies will be done in the due course.
Key words: Biological activity, ornamental plant, chemical investigation, *Butea monosperma.*

Introduction

Plants have always been a rich source of biologically active compounds (e.g. morphine, cocaine, digitalis, quinine, tubocurarine, ni cotine, and muscarine) (Wikipedia). These biologically active compounds offer a vast, virtually untapped reservoir of chemical compounds with many potential uses. Many of these compounds are useful drugs in themselves (e.g. alkaloids, morphine and quinine), and others have the important value for agricultural application like growth regulator, insecticide, pesticide, herbicide, etc. The plants are a vast reservour of compounds with a wide range of biological activities. There are about 2000 plant species reported to possess pest control properties. The plant product includes oil, extracts, dried leaves, fruits, seeds, rhizomes etc. The strongest inhibitory effect of aqueous extracts from *Eupatarium adenophorum* on wheat seed germination, radical and plumule growth were reported by Tripathi *et al*. (1981). The extracts of Leaves, stems and radicals of lantana plant have the inhibitory effects growth of some plant species (Wadhwani and Bhardwaja 1981). Tripathi et al., (1981) reported that the strongest inhibitory effect of aqueous extracts of *Eupatorium adenophorum* on wheat seed germination, radical and plumule growth. Bhuyan and Deka (1999) reported that increased germination of *Phaius tankervillae* in nitch and nitsch medium and germination could be further enhanced to 99% by supplementing the medium with coconut water or pineapple juice. Rice (1974) reported that secondary compounds are either bi-products of metabolism or waste products stored in the vacuoles to prevent deleterious effects on the producing plant. 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). One of these uses is in agriculture to manage crops growth with less risk than with synthetic compounds that are toxicologically and environmentally undesirable. Roy et al. (2006) reported that china box (*Hibiscus rosa sinensis*) leaves extract inhibited germination and reduced root and shoot lengths of country bean (*Lablab niger*), lady's finger (*Hibiscus esculentus*), swamp cabbage (*Impoea aquatica*) and yardlong bean (*Vigna unguiculata*). And in another paper (2008), the aqueous extract of leaves of *B. monosperma* was used as positive growth regulator on the same vegetable crops. This extract significantly enhanced germination, root and shoot lengths of these crops. Sarker et al. (2010) also reported that aqueous extract of indian dillenia (*Dillenia indica*) significantly increased germination; radical and plumule lengths growth of the same vegetables crops. But the aqueous extract of leaves of wood apple (*Aegle marmelos*) significantly decreased germination, root and shoot growths of barnyard grass (*Echinochloa crussgali*), spiny amaranth (*Amaranthus spinosus*) and green amaranth (*Amaranthus viridis*) was reported by Jalal et al. (2010). Plant growth regulator is an essential factor in agriculture. Therefore, the experiment was done to check the biological activity of leaves of some ornamental plants and to isolate biological active compounds.

Materials and Methods

An easy bioassay experiment was conducted at the lab of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur from May 2009 to April 2010. For this assay leaves of parrot tree (*B. monosperma*), arabian jasmine (*Jasminum sambac*), china box (*Murraya exotica*) and indian medlar (*Mimusops elengi*) were used. The leaves were extracted and applied on the seeds of swamp cabbage (*I. aquatic*), cucumber (*C. sativus*), pumpkin/squash (*C. moschata*), water melon (*C. lanatus*), and yardlong bean (*V. sesquipedalis*) crops to observe germination and growth performances.

Extract preparation: For preparation of aqueous extracts of above mentioned plants, 100 gm of fresh leaves was cut into smaller pieces and blended them using 500 mL water to make aqueous slurry. The slurry was filtered through screen first, and then Whatmann filter paper No.1 to remove waste. After removing waste the aqueous extracts were used to check of biological activity.

Biologically activity checking by petridish method: Petridish experiment was done for observation of germination percentage; shoot and root growth of the vegetable crops. For this experiment, clean petridish with two sheets of filter papers was used to grow crops. Fifteen mL of each aqueous extract was poured in each petridish, while water was used as control with five times replications. Twenty five seeds were put on each petridish and kept them to grow for 7 to 15 days. After sprouting the

seeds, germination percentages were recorded each day by counting the sprouted seeds. The root and shoot lengths were also recorded by measuring with a ruler at the end day of the experiment for statistical analysis.

Statistical analysis and identification of effective aqueous extract: For identification of effective extract, statistical analysis was done on different parameters (such as, germination percentages, root and shoot lengths) using the computer software MSTAT-C. After analyzing the data, it was identified that the aqueous extract of *B. monosperma* was the biologically active extract. This biological active extract was used for further chemical investigation to identify bioactive compounds.

Chemical investigation: For isolation of bioactive compounds from the effective extract, 2.5 kg of fresh leaves of *B. monosperma* was dried and grinded to make powder. Hundred g of powder from the stock was soaked in 250 mL chloroform for 72 hours with regular interval of shaking to dissolve compounds in chloroform. It was filtered through Whatmann filter paper No.1, and the filtrate was then evaporated by using Thin Film Rotary Evaporator under reduced pressure to get crude. A very small amount of this crude was used to develop color of compounds on normal phase silica thin layer chromatography (TLC). After preliminary TLC test, the identified compounds were separated individually from the crude mixture by preparative TLC. Thirty mg of crude was dissolved in chloroform and spotted on this plate with capillary tube. This plate was then put in the tank containing the solvent system (hexane:ethyl acetate::5:1) to develop bands of compounds. The band of the each compound was cut and dissolved in ethyl acetate and stirred by magnetic stirrer and then filtered through Whatmann filter paper no. 1. The filtrate was then evaporated by rotary evaporator under reduced pressure to get purified compounds. These compounds were then stored in refrigerator for further NMR, IR, mass analyses to elucidate the structure.

Results and Discussion

The aqueous extracts of ornamental plants leaves were applied to observe their biological activity like germination inhibition or activation; root and shoot length growth inhibition or enhancement. The results of biological activity of the extracts are written as follows.

From the Table 1, it is showed that the aqueous extract of *B. monosperma* significantly enhanced germination and growth root and shoot length of the crops. The germination percentages of I. *aquatica*, *C. sativus*, *C. moschata*, *C. lanatus*, and *V. sesquipedalis* were recorded 90.0, 82.0, 41.0, 72.4 and 72.8 %, respectively, which were 12% for *I. aquatica*, 26% for *C. sativus*, 27.8% for *C. moschata*, 20.4% for *C. lanatus* and 35.2% for *V. sesquipedalis* enhancement in comparison with control. This extract also increased root and shoot lengths of the seedlings. The root lengths of the seedlings were recorded 2.3, 7.5, 7.6, 7.5 and 4.4 cm, and the shoot lengths were 7.7, 14.5, 13.6, 7.5, and 19.7 cm, respectively. The root and shoot lengths of the crops seedlings increased 47.47, 47.06, 153.33, 56.25, and 57.14 %; and 66.81, 57.61, 44.68, 22.95, and 34.01 %, respectively. The same result was recorded by Sona *et al.* (2008) and observed the aqueous extract of *B. monosperma* enhanced germination percentage (4 to 17.33%), root length (17.34 to 97.57%) and shoot length (13.03 to 87.92%) of *L. niger*, *H. esculetus*, *I. aquatica* and *V. unguiculata.* Similar results were also reported in Sarker et al. (2010) about the growth increasing effect of leaf extract of indian dilenia on seed germination and seedling growth of country bean, yard long bean, okra and swamp cabbage.

Table 1. Biological activity of aqueous extracts of plants leaves on vegetable crops

a) Effects on *I. aquatica*

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)
Water /control (Tc)	82.0 AB	2.0 B	4.6 B
B. monosperma (T1)	90.0 A	2.9 A	7.7 A
J. sambac (T2)	73.0 AB	1.2 C	5.0 B
M. exotica (T3)	66.6 B	1.1 C	3.2 C
M. elengi (T4)	82.0 AB	1.4 BC	4.9 B
$\delta_{\overline{x}}$	5.421	0.119	0.086

b) Effects on *C. sativus*

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)
Water /control (Tc)	56.0 B	5.1 AB	9.2 B
B. monosperma (T1)	82.0 A	7.5 A	14.5 A
J. sambac (T2)	59.0 B	5.6 AB	11.2 AB
M. exotica (T3)	18.0 C	3.3 B	11.0 AB
M. elengi (T4)	86.6 A	4.2 B	5.3 C
$\delta_{\overline{x}}$	2.927	0.476	0.561

c) Effects on *C. lanatus*

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)
Water /control (Tc)	52.0 AB	4.8 B	6.1 B
B. monosperma (T1)	72.4 A	7.5 A	7.5 A
J. sambac (T2)	41.6 B	3.4 B	3.7 C
M. exotica (T3)	42.6 AB	4.2 B	4.4 C
M. elengi (T4)	25.6 B	0.2 C	0.0 D
$\delta_{\overline{x}}$	7.453	0.223	0.336

Contd.

d) Effects on *C. moschata*

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)
Water /control (Tc)	13.2 CD	3.0 BC	9.4 AB
B. monosperma (T1)	41.0 A	7.6 A	13.6 A
J. sambac (T2)	8.4 D	1.5 BC	2.9 BC
M. exotica (T3)	17.6 C	0.4 C	0.8 C
M. elengi (T4)	27.4 B	4.8 AB	7.4 ABC
$\delta_{\bar{x}}$	1.364	0.637	1.259

e) Effects on *V. sesquipedalis*

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)
Water /control (Tc)	37.6 B	2.8 B	14.7 B
B. monosperma (T1)	72.8 A	4.4 A	19.7 A
J. sambac (T2)	70.6 A	1.5 C	3.3 D
M. exotica (T3)	63.4 A	1.3 C	4.7 CD
M. elengi (T4)	23.4 B	2.0 BC	5.9 C
$\delta_{\bar{x}}$	3.866	0.139	0.251

Table 2. R_f values of the detected compounds of *B. monosperma*

Hexane: ethyl acetate	Detected compound	Rf value
5:1	P1	0.85
	P2	0.65
	P3	0.44
	P4	0.18

Rahman (2009) also observed the same result and experimented on *Hibiscus esculentus* seeds by treating with the aqueous extracts of *B. monosperma*, *Mussaenda erythrophylla, Polyanlthia longifolia, Thuja orientali and Hiptage madablota*. He observed that the extract of *B. monosperma* increased germination percentage, root and shoot lengths of *H. esculentus*. The aqueous extract of another plant, *J. sambac* (T_2) reduced germination, root and shoot lengths of these crops. The germination were 73, 59, 8.4, 41.6, 70.6%; 1.2, 5.6, 1.5, 3.4, and1.5 cm for root length; and 5.0, 11.2, 2.9, 3.7 and 3.3 cm for shoot length of *I. aquatic*, *C. sativus*, *C. moschata*, *C. lanatus*, and *V. sesquipedalis*, respectively, compared with control. For other two ornamental plants, *M. exotica* and *M. elengi* also reduced the germination percentages, root and shoot lengths of these crops in comparison with control.

According to the result, it is concluded that the plant, *B. monosperma* contains biological active compounds which were responsible for germination and growth enhancement. Chloroform crude extract of *B. monosperma* was used to purify the bioactive compounds.

Purification of bioactive compounds: Very small amount of crude extract was used to develop spots fornumber of compounds using normal phase TLC (Fig. 1A). The TLC of chloroform crude extract showed four compounds at hexane: ethyl acetate (5:1 v/v), which were designated as P_1, P_2, P_3 and P_4, respectively, and their polarity order (non-polar to polar) were from P_1 to P_4. The R_f values of the compounds are in the Table 2. The R_f is calculated dividing by the distance traveled by the solvent front to the distance traveled by the compound. The higher R_f value indicates the most non-polar compound and lower R_f value indicates the most polar compound. These four compounds were separated by performing preparative TLC (Fig. 1B). Three compounds (P_1, P_2, and P_3) were purified by carrying out further preparative TLC (Fig. 1C) and the compound P_4 was unpurified due to its complex mixture. The purity of the compounds was confirmed by running further TLC compared with the original crude (Fig. 1D).

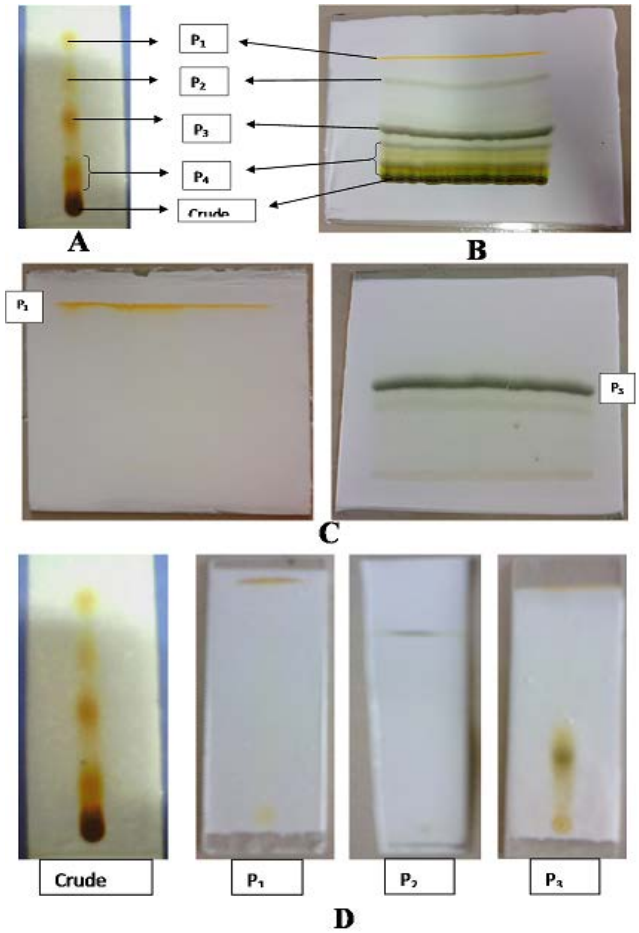

Fig. 1. (**A**) Normal phase TLC crude extract Solvent Ratio: Hexane: ethyl acetate::5:1, (**B**) Preparative TLC Solvent Ratio: Hexane: ethyl acetate::5:1, (**C**) Purification by running further reparative TLC Solvent Ratio: Hexane: ethyl acetate::5:1, and (**D**) Conformation of purified compounds compare with original crude Solvent system: Hexane: ethyl acetate::5:1.

On the preparative TLC, each band of the individual compound was cut and dissolved in ethyl acetate and

filtered with Whatmann filter paper no. 1, and then individual compound was isolated after evaporation.

The aqueous extract of leaves of *B. monosperma* enhanced germination and growth of root and shoot lengths of *I. aquatica*, *C. sativus*, *C. moschata*, *C. lanatus*, and *V. sesquipedalis*. This study indicates that the leaves of *B. monosperma* might contain biological active compounds for this kind of germination and growth enhancement activity.

References

Bhuyan J and P.C. Deka. 1999. Seed culture of *Phaius tankervillae*. Indian Forester. 125: 910-912.

Kohata, K., Y. Yamauchi, T. Ujihara and H. Hprie. 2004. Growth Inhibitory Activity of Tea-Seed saponins and Glyphosate to Weed Seedlings. J. Janick (cd.), Alexandria, 38(4): 267-270.

Rahman S. 2009. Growth Regulatory Activities of Aqueous extract of Some fruit Leaves on Pot Soil with emphasis on Chemical Investigation. M.S. Thesis, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. pp. 66-69.

Rice, E.L. 1974. Allelopathy. Academic Press. Inc. New York. N. Y. p: 353.

Roy B., Alam, M.R., Sarker, B.C. Rahman, M.S., Islam, M.J., Hakim, M.A. and R.I Mahmood. 2006. Effect of aqueous extracts of some weeds on germination and growth of wheat and jute seeds with emphasis on chemical investigation. J Biol. Sci. 6: 412-416.

Roy, S.R., Roy, B., Sarker, B.C., Roy, P.K. and Miah, M.Y. 2006. Inhibitory activity and chemical investigation of some ornamental plants. Journal of Science and Technology 4:100-104.

Roy, S.R., Roy, B., Sarker, B.C., Uddin, M.N. and Joha, M.S. 2008. Growth regulatory activities of aqueous extracts of five ornamental plants. Journal of Science and Technology 6:121-125.

Sarker, B.C., Shil, P., Roy, B., Zoha, M.S. and Uddin, M.N. 2010. Biological activity of some fruit leaves and chemical investigation on *Dillernia indica* leaves. J. Agrofor. Environ. 4(2): 15-20.

Tripathi, R. S., Singh, and R. S. and Rai, J.P.N. 1981. Allelopathic potential of *Eupatorium adnophorum* a dominant rederal weed of Meghalya. Proceedings of the Indian National Science Academy. 47: 458-465.

Wadhani, V and T.N. Bhardwaja. 1981. Effect of *Lantena camera* extract on ferspare germination. Experienlia. 37: 245-247.

Jalal S., B. Roy, B. C. Sarker, M.N. Uddin and Sona R. Roy. 2010. Growth Inhibitory Effect of Ten Fruit Leaves and Chemical Investigation of Wood Apple Leaves. Journal of Agroforestry and Environment. 4(1): 101-104.