

Full Length Research Paper

Effect of different extracts of callus and plantlets of *Stevia rebaudiana* (Bertoni) on seed germination of some agricultural crops

A. S. Taware^{1*}, S. N. Harke¹, D. S. Mukadam², A. M. Chavan² and S. D. Taware³

¹MGM's, Institute of Biosciences and Technology, MGM Campus, N-6, CIDCO, Aurangabad – 431 001 Maharashtra, India.

²Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India.

³Genome Life Sciences, Labh Chambers, Station Road, Aurangabad, India.

Accepted 8 July, 2010

Biological activity of various extracts from calli grown in presence of different growth hormones and plantlets obtained from micropropagation was observed in laboratory experiment by using *Triticum aestivum*, *Sorghum vulgare*, *Arachis hypogea*, *Glycine max*, *Cajanus cajan*, and *Cicer arietinum* as bioassay materials. The experiment was conducted in sterilized petri dishes for five days at an average temperature of 28°C. The effect of aqueous extracts of different concentrations was compared to distilled water (control). The aqueous extracts of all the samples caused both stimulatory and inhibitory effect on germination, root and shoot elongation. Cold and hot water extracts of calli propagated on 2,4-D singly, combination of 2,4-D with IBA and 2,4-D with GA resulted in significant inhibition for germination of *C. cajan* and *C. arietinum*. Biological activities of the extracts were further examined by employing *Allium cepa* model in which cytogenetically effects of extracts were investigated. No genotoxicity was observed.

Key words: *Stevia rebaudiana* (Bertoni), callus, micropropagation, germination, root length.

INTRODUCTION

Stevia rebaudiana (Bertoni) is a perennial herb which belongs to the daisy family. Being a natural high caloric sweetener due to the stevioside contents in its leaves, it is a major source of high potency sweetener for the growing diabetic population. Stevioside is a glycoside, and has a sweetening power ranging from 100-400 times higher than sucrose (Gujral, 2004; Duke, 1993). The main use of *Stevia* is as a sweetening agent as well as for medicinal and household purposes.

The propagation through seeds is not adequate owing to very low seed germination percentage. Vegetative propagation needs high labor input. Because of these problems large scale production has limitations. Demand

For stevioside is high, hence tissue culture is the only alternative for rapid propagation of *Stevia* plants. Callus culture is also a good possible source for stevioside production.

Callus also contains some metabolites which may show some biological activities. *In vivo* grown *Stevia* has been proved to inhibit the growth of certain bacteria and other infectious organisms hence it is used against wounds, sores and gum disease. The biological activity of *Stevia* compounds has been studied by Tomita et al. (1997). They studied bactericidal activity of a fermented hot-water extract from *S. rebaudiana* Bertoni towards enterohemorrhagic *Escherichia coli* and other food-borne pathogenic bacteria. But effect of *Stevia* on seed germination has not yet been studied. The medicinal properties are attributed to the primary and secondary metabolites synthesized by the plants (Faizi et al., 2003). Hence the objective of the present study is to elucidate potential effects of aqueous extracts of *in vitro* grown and calli of *S. rebaudiana*.

*Corresponding author. E-mail: taware.as@gmail.com.

Abbreviations: IAA, Indole-3- acetic acid; IBA, indole-3-butyric acid; GA, gibberellic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; HW, hot water; CW, cold water.

MATERIALS AND METHODS

Preparation of extracts of *S. rebaudiana*

The dried leaves of *in vitro* and *in vivo* raised *S. rebaudiana* and calli grown on media containing various growth hormones were subjected to different extraction methods, using hot water (HW) and cold water (CW). The following extracts were used for analysis.

- T₀ - Control
- T₁ - Callus grown on MS + 2,4-D + IBA
- T₂ - Callus grown on MS + 2,4-D + GA
- T₃ - Callus grown on MS + 2,4-D + IAA
- T₄ - Callus grown on MS + 2,4-D
- T₅ - Micropropagated plant through lateral bud
- T₆ - Micropropagated plant through callus organogenesis
- T₇ - Commercial *Stevia* powder.

Samples (2.0 g) were collected, dried and subjected to extraction at room temperature for 24 h in different solvents, that is, hot and cold water. The paste was filtered through cheesecloth and was centrifuged (10,000 rpm, 10 min, 25°C). The supernatant obtained after centrifugation was concentrated for biological activity.

Seed bioassay

To investigate effect of various extracts on germination, germination tests were conducted for the same cold water and hot water extracts of various samples (Turk et al., 2005). Seeds of different agricultural crops including *Triticum aestivum*, *Sorghum vulgare*, *Arachis hypogaea*, *Glycine max*, *Cajanus cajan*, and *Cicer arietinum* were used. Seeds were treated with cold water and hot water extracts for 4 h and kept for germination. The germination test was carried out in sterile petri dishes of 12 cm in diameter, lined with Whatman No.3 filter paper. The extract of each concentration was added to each petri dish of respective treatment in such an amount just to wet the seeds. The control was treated with distilled water only. 20 seeds of each agricultural crop were placed in the petri dishes and each treatment was replicated 5 times. After five days germination percentage, root length and shoot length were observed. Ratio of germination and elongation were calculated as suggested by Rho and Kil (1986).

$$\text{Relative germination ratio (RGR)} = \frac{\text{Germination of tested plant}}{\text{Mean length of control}} \times 100$$

$$\text{Relative germination ratio (RGR)} = \frac{\text{Germination of tested plant}}{\text{Mean length of control}} \times 100$$

$$\text{Relative elongation ratio (RER) of root} = \frac{\text{Mean length of root of tested plant}}{\text{Mean length of control}} \times 100$$

Onion root tip assay

To evaluate toxicity of cold water extracts of samples toxicity test was performed by using *Allium cepa* as a model (Fiskesjo, 1981). Carefully cleaned onion bulbs were used in this experiment. For each test solution six onions were set up. The bulbs were first allowed to produce roots by keeping in synthetic fresh water (synthetic fresh water was used due to poor quality of tap water, that is, water pipes

are made of copper which is not recommended for experiments) (Rank and Nielsen, 1993). On the next day the bulb with the poorest growth (if any) in each set was discarded, and the rest bulbs were transferred to the test solutions. Bulbs were treated by placing them on the test tubes filled with test solution. On day five, the length of the root bundle of each bulb was measured. Other macroscopic observations like shoot length and root structure were recorded.

After 48 h (two cell cycles) exposure 5 - 6 root tips from each bulb were excised and prepared for the microscopic analysis. The root tips were fixed, and slides were prepared by employing squash technique. The microscopic analysis includes mitotic index and scoring of chromosome aberrations in the late anaphase and early telophase cells. For calculation of mitotic index, one random field was chosen in each slide.

All data were analyzed by using ANOVA, and data were found significantly different at the 0.05 probability level.

RESULTS AND DISCUSSION

The germination percentages of all seeds in cold and hot water extract are seen in Table 1. Low percentage germination and less root length were observed in *Triticum aestivum*, in all cold water extracts, as compared to the control. Cold water extract of the callus developed on combination of 2,4-D and IBA showed lowest germination percentage which is 70%, while cold water extract of callus grown on 2,4-D alone exhibited lowering germination percentage in all used plant seeds (Figure 1). The results of shoot length indicated that the extracts have varying degree of stimulatory and inhibitory effect on shoot length. Inhibition of shoot length of *T. aestivum*, was seen in extracts of callus multiplied on combination of 2,4-D with IBA and 2,4-D with GA. Other cold and hot water extracts showed stimulatory effect on shoot length.

Stimulatory effect on germination percentage and shoot length of *S. vulgare* was found in all cold water extracts. Cold water extract of commercial *Stevia* powder was responsible for highest shoot length. Decrease in root length in case of *S. vulgare* was observed in all cold water extracts as compared to the control.

In hot water extracts, considerable difference in germination percentage was not detected except treatment of extracts of callus grown on 2,4-D with IBA and 2,4-D with GA. Varied degree of root length was observed in hot water extract treatments. Significant difference in germination percentage and root length of *Arachis hypogaea* was not seen in cold water extracts (Table 1). Only extracts of callus grown on 2,4-D alone, plantlet developed by callus and commercial *Stevia* powder showed higher root length as compared to the control. Shoot initiation was not observed in any case. Stimulatory and inhibitory effect was seen in hot water extracts on germination percentage of *A. hypogaea*. Hot water extracts of callus multiplied on 2,4-D and exhibited 100% germination. Highest root length was recorded in hot water extract of callus developed on 2,4-D alone. 100% seed germination for *Glycine max* was detected in cold water extract of callus grown on combination of 2,4-D and GA. Highest

Table 1. Effect of different treatments on seed germination.

Test (cm)	<i>T. aestivum</i>		<i>S. vulgare</i>		<i>A. hypogaea</i>		<i>G. max</i>		<i>C. cajan</i>		<i>C. arietinum</i>	
	CW (%)	HW (%)	CW (%)	HW (%)	CW (%)	HW (%)	CW (%)	HW (%)	CW (%)	HW (%)	CW (%)	HW (%)
T ₀	100	70	70	100	70	90	60	60	30	50	60	50
T ₁	70	80	100	90	60	60	80	100	-	10	20	-
T ₂	80	80	100	100	70	70	100	80	-	10	30	-
T ₃	90	80	100	60	70	80	90	50	70	-	60	-
T ₄	90	90	100	100	80	100	50	90	-	-	-	10
T ₅	80	80	90	100	80	100	60	80	50	60	60	50
T ₆	80	70	90	100	80	90	70	70	80	60	60	50
T ₇	90	100	90	90	80	70	70	80	60	50	80	70

HW = Hot water; CW = cold water.

T₁: Callus grown on MS + 2,4-D + IBA; T₂, Callus grown on MS + 2,4-D + GA; T₃, Callus grown on MS + 2,4-D + IAA; T₄, Callus grown on MS + 2,4-D; T₅, Micropropagated plant through lateral bud; T₆, Micropropagated plant through callus organogenesis; T₇, Commercial *Stevia* powder.

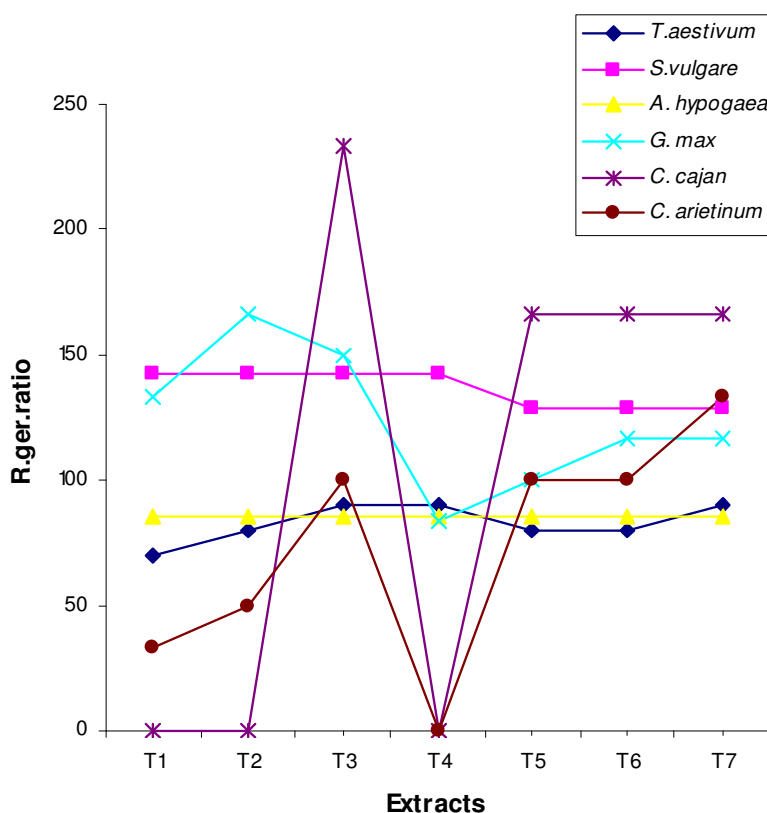


Figure 1. Effect of cold water extracts on seed germination. T₁: Callus grown on MS + 2,4-D + IBA; T₂, Callus grown on MS + 2,4-D + GA; T₃, Callus grown on MS + 2,4-D + IAA; T₄, Callus grown on MS + 2,4-D; T₅, Micropropagated plant through lateral bud; T₆, Micropropagated plant through callus organogenesis; T₇, Commercial *Stevia* powder.

root length in *G. max* was seen in the treatment of cold water extract of callus grown on 2,4-D. All the cold water extracts showed stimulatory effect on root length as compared to the control. Hot water extract of callus multiplied on combined 2,4-D and IBA showed 100%

germination while highest root length was observed in treatment of callus extract multiplied on combination of 2,4-D and IAA.

Inhibitory effect was seen for *Cajanus cajan* in the cold water extracts of callus multiplied on combination of 2,4-

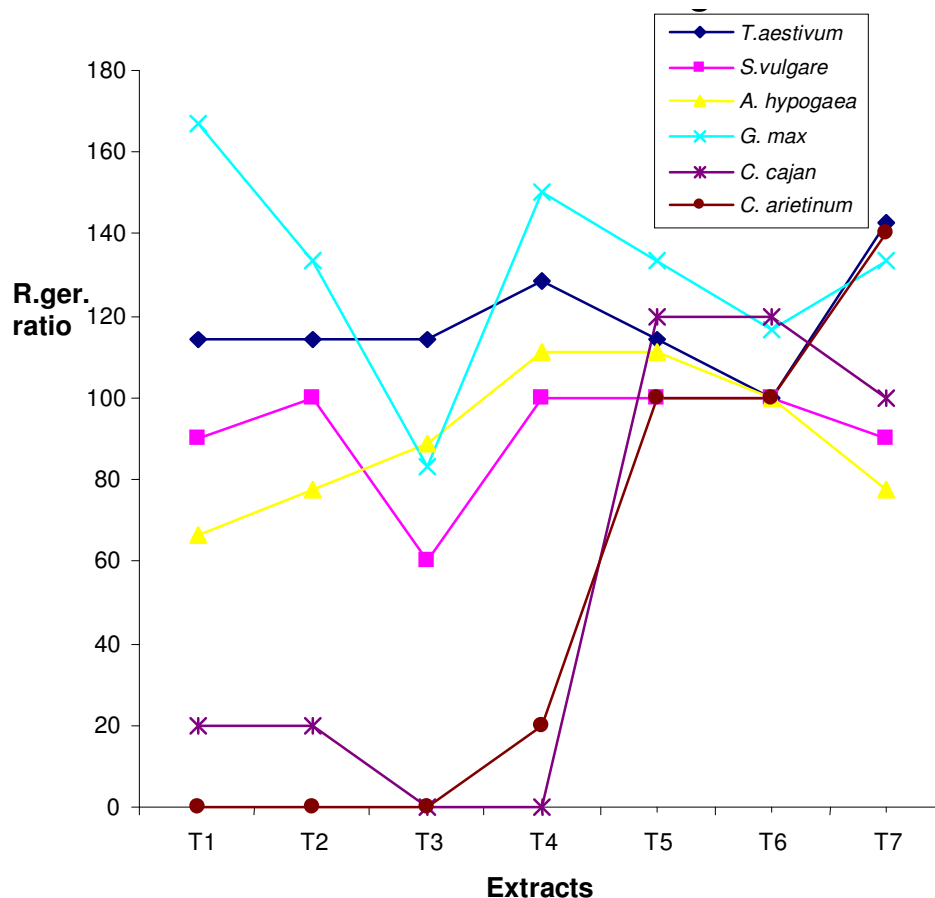


Figure 2. Effect of hot water extracts on seed germination. T1: Callus grown on MS + 2,4-D + IBA; T2, Callus grown on MS + 2,4-D + GA; T3, Callus grown on MS + 2,4-D + IAA; T4, Callus grown on MS + 2,4-D; T5, Micropropagated plant through lateral bud; T6, Micropropagated plant through callus organogenesis; T7, Commercial *Stevia* powder.

D with IBA and 2,4-D with GA. The remaining extracts proved to be stimulative for germination percentage. Shoot initiation was observed in the treatment of cold water extracts of callus produced on combination of 2,4-D with IAA, micropropagated through lateral bud and commercial *Stevia* powder. The cold water extracts of callus grown on 2,4-D with IAA, micropropagated through lateral bud, callus-originated plant and commercial *Stevia* helped root initiation, but the length was not as much as the length found in the control.

The hot water extract treatment did not yield acceptable results in germination percentage. Germination percentage was decreased by 10% in extracts of callus multiplied on 2,4-D with IBA and 2,4-D with GA (Figure 2). Total inhibitory effect was seen in the extract of callus grown on 2,4-D with IAA and 2,4-D alone. Hot water extract of micropropagated through lateral bud and callus-originated plant showed significant increase in shoot length. Effect of hot water extracts on root length was not at considerable level.

Both stimulatory and inhibitory effect was observed in

germination percentage of *C. arietinum*. In the treatment of cold water extract of callus grown on 2,4-D with IBA and 2,4-D with GA, a decrease (up to 20 and 30%, respectively) in percentage germination was seen. An increase in germination percentage was detected with commercial *Stevia* extract. Shoot initiation was observed in the treatment of extract of callus multiplied on 2,4-D with IAA and callus-originated plant. Results of root length were not satisfactory for *C. arietinum* (Table 2). In the hot water extract of callus multiplied on combination of 2,4-D with IBA, 2,4-D with GA and 2,4-D with IAA, complete inhibition of seed germination was observed. Only 10% seed germination occurred in the hot water extract of 2,4-D alone. Hot water extracts of plantlets obtained through shoot proliferation and callus organogenesis showed only 50% seed germination which was equal to the control. Increased seed germination of 70% was found with commercial *Stevia* extract. Results of shoot length were not significant. Shoot initiation was observed only in hot water extract of callus-originated plantlets (Table 3 and Figure 5). Stimulatory effect on

Table 2. Effect of different treatments on root length.

Test (cm)	<i>T. aestivum</i>		<i>S. vulgare</i>		<i>A. hypogaea</i>		<i>G. max</i>		<i>C. cajan</i>		<i>C. arietinum</i>	
	CW	HW	CW	HW	CW	HW	CW	HW	CW	HW	CW	HW
T ₀	5.98 (0.75)	4.81 (1.79)	4.85 (0.61)	3.17 (1.04)	1.38 (0.96)	2.88 (0.32)	2.31 (1.2)	2.2 (1.29)	2.5 (0.78)	2.66 (0.82)	3.36 (1.24)	2.92 (0.81)
T ₁	2.44 (0.33)	2.35 (0.43)	1.93 (0.22)	1.4 (0.74)	1.2 (0.88)	1.0 (0.74)	3.4 (1.48)	3.15 (1.28)	-	0.1	0.11	-
T ₂	2.28 (0.65)	2.18 (0.64)	1.03 (0.60)	1.53 (0.90)	1.07 (0.80)	0.88 (0.54)	3.14 (1.29)	2.05 (1.11)	-	Just in.	0.13 (0.05)	-
T ₃	3.30 (0.53)	5.22 (1.17)	2.26 (1.11)	5.95 (1.7)	1.62 (0.69)	3.7 (0.67)	6.56 (1.4)	10.5 (0.8)	2.04 (1.19)	-	3.36 (1.42)	-
T ₄	3.32 (0.9)	3.16 (1.21)	3.38 (1.04)	4.89 (1.14)	4.73 (1.50)	4.25 (1.47)	9.6 (1.14)	5.55 (0.52)	-	-	-	-
T ₅	3.85 (0.66)	4.22 (0.55)	2.1 (1.68)	2.95 (1.70)	1.91 (0.98)	2.35 (1.22)	2.71 (1.10)	7.26 (1.8)	1.88 (0.61)	2.33 (0.74)	2.83 (0.74)	3.20 (0.42)
T ₆	3.88 (0.54)	4.48 (0.55)	3.05 (1.47)	2.79 (0.90)	2.41 (1.25)	3.0 (0.97)	3.14 (1.73)	4.18 (0.96)	2.31 (1.24)	3.28 (0.95)	2.71 (0.99)	3.62 (1.22)
T ₇	5.0 (0.9)	4.25 (1.00)	3.96 (1.61)	0.55 (0.05)	3.2 (1.46)	2.91 (1.84)	4.03 (1.22)	1.78 (0.42)	2.60 (0.41)	2.78 (0.4)	3.85 (0.5)	4.5 (0.75)

HW = Hot water; CW = cold water.

Table 3. Effect of different treatments on shoot length.

Test (cm)	<i>T. aestivum</i>		<i>S. vulgare</i>		<i>A. hypogaea</i>		<i>G. max</i>		<i>C. cajan</i>		<i>C. arietinum</i>	
	CW	HW	CW	HW	CW	HW	CW	HW	CW	HW	CW	HW
T ₀	3.01 (0.34)	4.45 (1.71)		0.63 (0.1)	-	-		-		0.6 (0.17)	-	-
T ₁	2.5 (0.56)	2.21 (0.64)	0.77 (0.31)	0.18 (0.09)	-	-	-	-	-	-	-	-
T ₂	1.57 (0.78)	2.57 (0.48)	0.24 (0.15)	0.2 (0.09)	-	-	-	-	-	-	-	-
T ₃	3.5 (0.66)	4.88 (1.18)	0.84 (0.37)	2.06 (1.13)	-	-	8.3	-	1.15 (0.54)	-	0.7	-
T ₄	5.01 (1.76)	3.9 (1.37)	1.41 (0.53)	4.45 (1.49)	-	-	-	-	-	-	-	-
T ₅	4.08 (0.90)	4.06 (1.18)	1.2 (0.88)	0.4 (0.12)	-	-	-	-	1.5 (0.14)	1.53 (0.35)	-	-
T ₆	3.21 (1.17)	4.95 (0.89)	1.12 (0.5)	1.28 (0.57)	-	-	-	-	-	2.6 (0.42)	1.26 (0.20)	1.45 (0.58)
T ₇	4.7 (1.21)	3.95 (1.0)	2.54 (1.32)	3.76 (1.3)	-	0.8	-	-	2.44 (0.47)	-	-	-

HW = Hot water; CW = cold water.

root length was observed. Increased root length was seen in the extracts of shoot-originated and callus-originated plants, and commercial *Stevia*, but stimulatory effect of cold water extract of callus grown on MS+2,4-D was seen only in case of *Arachis hypogaea* and *G. max* (Figure 3). The hot water extract of callus grown on MS+ IAA+ 2,4-D proved to be stimulatory for *G. max* only (Figure 4).

In the earlier work, seed germination effects were evaluated or the plant extracts in which stimulatory as well as inhibitory effects were reported. In one of the study carried out by Maharjan et al. (2007), inhibitory effect of aqueous extracts of *Parthenium hysterophorus* was seen in cereals. Similarly, Baruah et al. (1994) explained that chloroform extract of the aerial parts of *Eupatorium adenophorum* Spreng acts as a germination inhibitor for

Allium cepa, *Raphanus sativus*, and *Cucumis sativus* seeds. Further studies done by Bogatek et al. (2006) indicated the inhibitory effect of two cultivars of sunflower (*Helianthus annuus* L.) Lech and Ogradowy on mustard (*Sinapis alba*) seeds. According to studies of Paudel and Gupta (2008), essential oils of Eucalyptus, camphor and lemon-grass exhibited significant reduction in germination of *P. hysterophorus*. Cold and hot water extracts

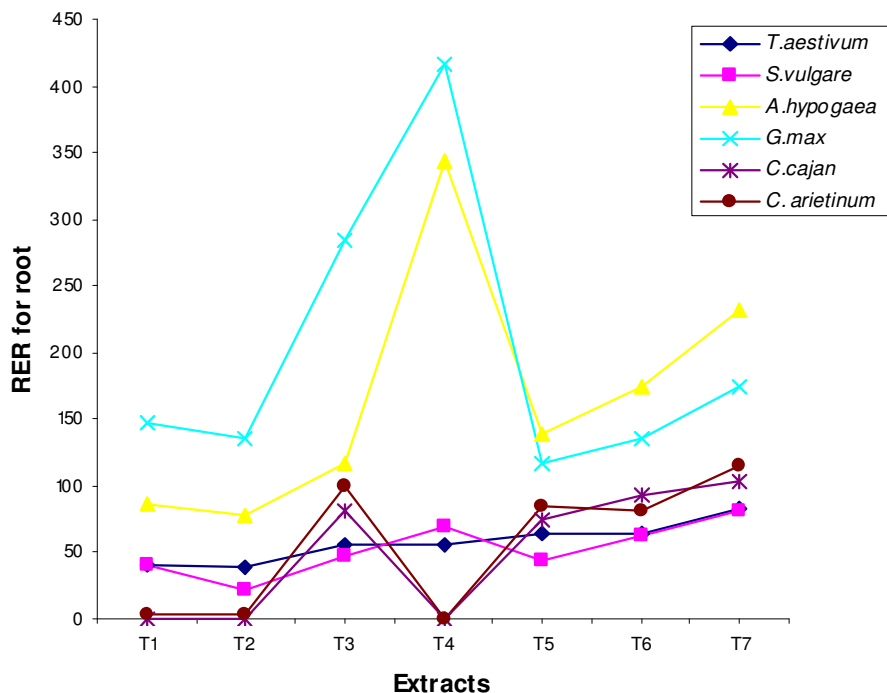


Figure 3. Effect of cold water extracts on root length. T1: Callus grown on MS + 2,4-D + IBA; T2, Callus grown on MS + 2,4-D + GA; T3, Callus grown on MS + 2,4-D + IAA; T4, Callus grown on MS + 2,4-D; T5, Micropropagated plant through lateral bud; T6, Micropropagated plant through callus organogenesis; T7, Commercial *Stevia* powder.

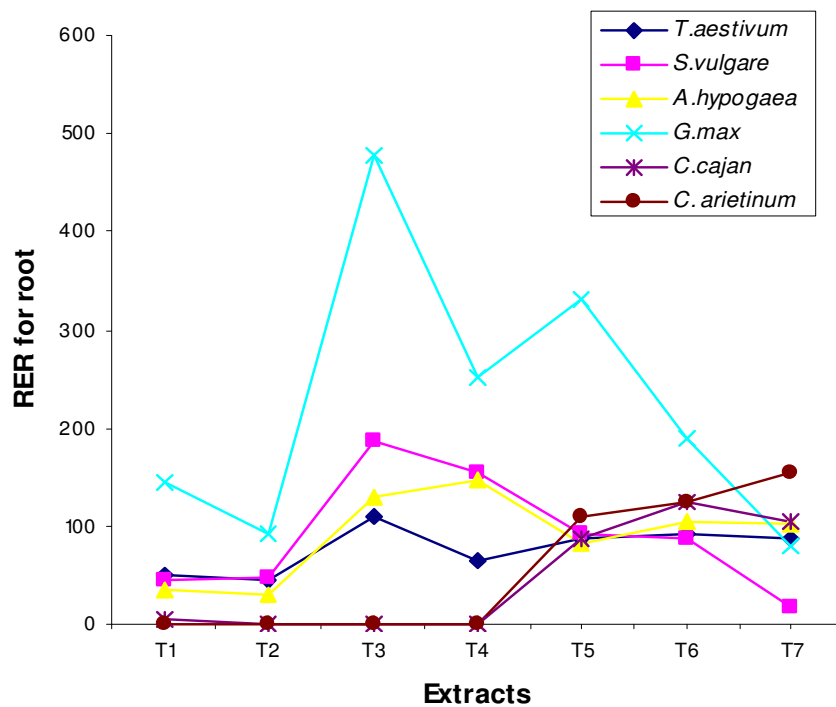


Figure 4. Effect of hot water extracts on root length. T1: Callus grown on MS + 2,4-D + IBA; T2, Callus grown on MS + 2,4-D + GA; T3, Callus grown on MS + 2,4-D + IAA; T4, Callus grown on MS + 2,4-D; T5, Micropropagated plant through lateral bud; T6, Micropropagated plant through callus organogenesis; T7, Commercial *Stevia* powder.

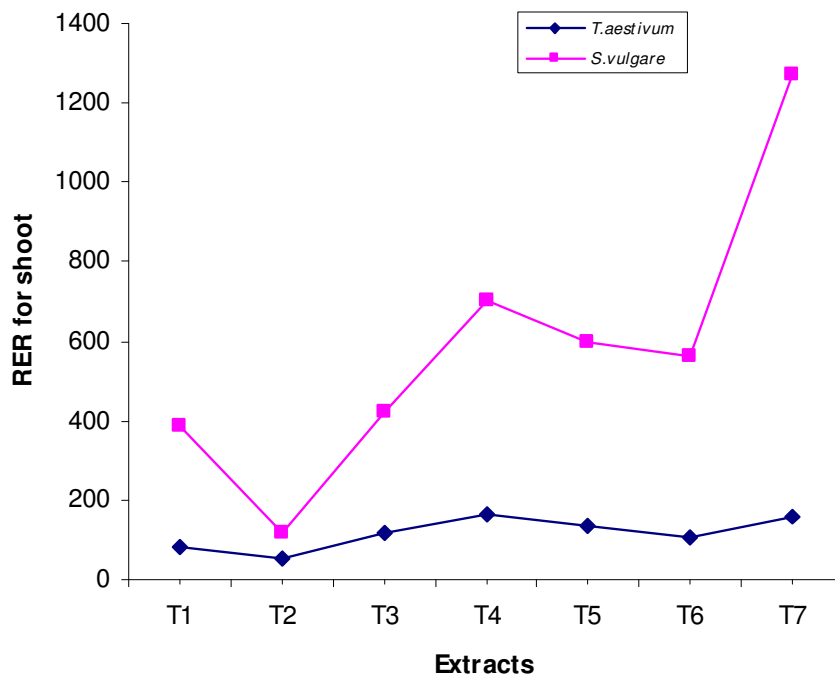


Figure 5. Effect of cold water extracts on shoot length. T1: Callus grown on MS + 2,4-D + IBA; T2, Callus grown on MS + 2,4-D + GA; T3, Callus grown on MS + 2,4-D + IAA; T4, Callus grown on MS + 2,4-D; T5, Micropropagated plant through lateral bud; T6, Micropropagated plant through callus organogenesis; T7, Commercial *Stevia* powder.

Table 4. Effect of cold water extract on onion.

Extract	Shoot length (cm)	Root length (cm)	MI
T ₀	3.2	7.8	36.8
T ₁	-	2.1	24.1
T ₂	-	6.1	31.3
T ₃	-	0.2	15.5
T ₄	1.5	1.9	20.0
T ₅	2.9	Browning	-
T ₆	-	-	-
T ₇	-	2.1	23.2

T1: Callus grown on MS + 2,4-D + IBA; T2, Callus grown on MS + 2,4-D + GA; T3, Callus grown on MS + 2,4-D + IAA; T4, Callus grown on MS + 2,4-D; T5, Micropropagated plant through lateral bud; T6, Micropropagated plant through callus organogenesis; T7, Commercial *Stevia* powder.

of calli multiplied on 2,4-D singly, combination of 2,4-D with IBA and 2,4-D with GA caused significant inhibition of germination in *C. cajan* and *C. arietinum*. Water extracts of these calli may contain some inhibitory compounds rendering seed germination impossible. These kinds of studies on calli were not carried out earlier; hence this study was undertaken for the first time to understand chemical behavior of calli.

Onion root tip assay was used to evaluate genotoxic effect of the extracts. Lowest mitotic index was observed

in the treatment with extract of callus grown on combination of 2,4-D and IAA. According to Okpuzor et al. (2003), some of the plants like *Cassia alata*, *Adenopus breviflorus*, *Cnestis ferruginea*, and *Microdemis puberula* exhibited very low level genotoxicity when tested by using *Allium* assay. In the present work, genotoxic effect was not observed in any of the extract, but they exhibited somewhat physiological actions such as reduction in root length (Table 4). Browning of root tip was also detected in the water extract of those that were micropropagated



Figure 6. Effect of water extracts of calli and tissue cultured plants on onion. A. Growth of onion bulb in control, B. Growth of onion bulb in extract of callus grown on 2,4-D + IBA, C. Growth of onion bulb in extract of callus grown on 2,4-D + GA, D. Growth of onion bulb in extract of callus grown on 2,4-D + IAA, E. Growth of onion bulb in extract of callus grown on 2,4-D, G. Growth of onion bulb in extract of initiated plantlet through lateral bud, H. Growth of onion bulb in extract of callus originated plantlet, I. Growth of onion bulb in extract of commercial *Stevia*.

through lateral bud and callus originated plantlets (Figure 6). This may be because of presence of some inhibitory substances in water extracts. Our results are in accordance with Schwartzman et al. (1977) who showed non-toxicity of *Stevia* extract.

In all the experiments we performed, micro-propagated plants and commercial *Stevia* extracts exhibited some-what similar effects. The stimulatory and inhibitory actions of different calli grown with different hormones may be due to the different compounds present in distinct extracts. The difference in chemical composition of calli may be as a result of different hormones used. The present study might throw some light for further studies on

this issue.

REFERENCES

- Duke J (1993). *Stevia rebaudiana*. CRC handbook of alternative cash crops. CRC Press, Boca Raton, FL, pp. 422-424.
- Baruah NC, Sarma JC, Sarma S, Sharma RP (1994). Seed germination and growth inhibitory cadinenes from *Eupatorium adenophorum* spreng, J. Chem. Ecol. 20(8): 1885-1892.
- Bogatek R, Gniazdowska A, Zakrzewska W, Oracz K, Gawronski SW (2006). Allelopathic effects of sunflower extracts on mustard seed germination and seedling growth, *Biologia Plantarum*, 50(1): 156-158.
- Faizi S, Rasool N, Rashid M, Ali Khan RA, Ahmed S, Khan SA, Aqeel Ahmad A, Bibi N, Ahmed SA (2003). Evaluation of the antimicrobial property of *Polyalthia longifolia* var. pendula: isolation of a lactone as the active antibacterial agent from the ethanol extract of the stem. *Phytother. Res.* 17(10): 1177-1181.
- Fiskesjo G (1981). Benzo(a) pyrene and N-methyl-N' nitro-N-nitrosoguanidine in *Allium* Test. *Hereditas.*, 95: 155-162.
- Gujral SR (2004). *Stevia* 0% Calorie, 100% Sweet, 100% Nature. *Science Tech. Entrepreneur*, 12(10).
- Maharjan S, Shrestha BB, Jha PK (2007). Allelopathic effects of aqueous extract of leaves of *Parthenium hysterophorus* L. on seed germination and seedling growth of some cultivated and wild herbaceous species. *Scientific World*, 5(5): 33-39.
- Okpuzor J, Omidiji O, Otaru K, Odeyemi M (2003). Impairment of cell division on exposure of onion root tips *Allium cepa* var. Milan coppery, to some medicinal plant extracts.

- (<http://www.abstracts.aspb.org/pb2003/public/P28/1299.html>).
- Paudel VR, Gupta VNP (2008). Effect of some essential oils on seed germination and seedling length of *Parthenium hysterophorus* L., *Ecoprint*, 15: 69-73.
- Rho BJ, Kil BS (1986). Influence of phytotoxin from *Pinus rigida* on the selected plants. *J. Nat. Sci.* 5: 19-27.
- Rank J, Nielsen MH (1993). A modified *Allium* test as a tool in the screening of the genotoxicity of complex mixtures. *Hereditas*, 11: 49-53.
- Schvartzman J, Krimer D, Moreno AR (1977). Cytological effects of some medicinal plants used in the control of fertility. *Experientia*, 33: p. 663.
- Tomita T, Sato N, Arai T, Shiraishi H, Sato M (1997). Takeuchi, M., and Kamio, Y., Enterohemorrhagic *Escherichia coli* 0157:H7 and other food-borne pathogenic bacteria. *Microbiol. Immunol.* 41(12): 1005-1009.
- Turk MA, Lee KD, Tawaha AM (2005). Inhibitory effects of aqueous extracts of black mustard on germination and growth of radish. *Res. J. Agric. Biol. Sci.* 1(3): 227-231.