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Enhancing Seed Germination of *Chlorophytum borivilianum* Sant. Et Fernand. with PGRs, Steroidal Hormones and Zinc

R. Kaur, S. Arora and A.K. Thukral
Department of Botanical and Environmental Sciences,
Guru Nanak Dev University, Amritsar, 143005, Punjab, India

Abstract: The objective of the present study was to improve the seed germination of *Chlorophytum borivilianum* Sant. et Fernand. It is a small perennial and rare herb having aphrodisiac, antiaging, health restorative and health promotional properties. There is a great demand for this herb all over the world. In the field, the plant is grown from the tubers, making the cultivation of this plant costly and labour intensive. The plant sets a large number of seeds, which have a poor germination (8-13%) thus not being preferred by the farmers for cultivation. It, therefore, makes it imperative to understand seed germination studies to improve germination of the seeds of *C. borivilianum* through seed treatment with hormones or chemicals which would also otherwise add to its properties. In the present study seed treatments were given with indole butyric acid, kinetin and 24-epibrassinolide (plant growth regulators) testosterone and cholesterol (steroidal hormones) and zinc (known for vital reproductive functions in man). Characteristics of the fruits, seeds and seed viability were also evaluated. Seed germination and seedling growth of *C. borivilianum* were studied when given various treatments of plant growth regulators, steroids and Zinc (II). The highest germination percentage occurred with 10^{-6} M concentrations of testosterone (36.45%) followed by cholesterol (35.17%) with respect to control (8.5%). The maximum shoot length of seedlings was observed when seeds were treated with 10^{-6} M concentration of testosterone (4.43 cm), cholesterol (4.27 cm) and 24-epiBL (4.25 cm) with respect to control (2.80 cm). The maximum root length of seedling was observed in 10^{-6} M of cholesterol (3.83 cm) and 24-epiBL (3.65 cm) as compared to control (0.87 cm). Maximum fresh weights of seedlings were seen in seeds treated with 10^{-6} M concentrations of cholesterol (28.03 mg), 24-epiBL (26.21 mg) and testosterone (26.16 mg) as compared to control (10.87 mg).

Key words: Cholesterol, 24-epibrassinolide, indol butyric acid, kinetin, testosterone, threatened species

INTRODUCTION

Chlorophytum borivilianum (Vern. Safed Musli) is an important medicinal plant with aphrodisiac properties and its roots are used as a safe herbal viagra. The plant is used in more than 100 ayurvedic formulations (Oudhia, 2001). It is used to cure physical illness and general weakness, used as a revitalizer, as a remedy for diabetes, arthritis, curative for natal

Corresponding Author: Prof. Dr. Ashwani Kumar Thukral, Department of Botanical and Enviroumental Sciences, Guru Nanak Dev University, Amritsar, 143005, Punjab, India Tel: +91-183-9417019459 Fax: +91-183-2258819

and postnatal problems, also used in the treatment of diarrhoea, dysentery, gonorrhea, leucorrhea etc. (Thakur *et al*., 2009). The hypolipaemic/hypocholesteraemic effect of *C. borivilianum* root powder was studied on male albino rats and it was found that administration of 0.75 and 1.5 g of root powder/rat/day for 4 weeks significantly increased high density lipoprotein cholesterol levels and decreased plasma levels and hepatic lipid profiles (Visavadiya and Narasimhacharrya, 2007). The hydroalcoholic extract of tubers alleviated the diabetic potency in rats and dose dependent improvement was found in all the parameters of sexual behaviour (Vyawahare *et al*., 2009).

The annual demand of *C. borivilianum* in India is 3500 tonnes, while its supply is only 500-600 tonnes (Kothari and Singh, 2001). The plant grows in the wild and on account of its growing demand, the world over, it has been over exploited, pushing it to the category of a threatened species on the verge of extinction. The Medicinal Plant Board of India has recognized *C. borivilianum* as the sixth most important herb to be protected and promoted. The government of India is also extending subsidies to the farmers for the cultivation of this herb through National Horticultural Board.

Chlorophytyum borivilianum is an herbaceous plant with a condensed stem disc, from which a whorl of leaves originates. Leaves are sessile, 10-40 cm in length and 0.6-3.0 cm in breadth. The inflorescence is a raceme. Flowers are pedicillate with joints. The perianth consists of six white sepals, arranged in two whorls of 3 each. Androecium consists of six stamens, arranged in two whorls and united to the perianth, slightly longer than perianth, bithecous, filaments are glabrous and anthers are yellow, linear and dehisce by longitudinal slits. Gynoecium is tri-carpellary, syncarpous, ovary is superior, adnate at the base of perianth, trilocular (rarely tetralocular) with axile placentation, with two ovules in each locule. Genetic variability in terms of root number, length and diameter was also observed in *C. borivilianum* (Singh *et al.,* 2008). This plant is prone to tuber rot and crown rot diseases (Raghavendra *et al*., 2005) and the storage behaviour of roots also varies from genotype to genotype (Chandra and Kumar, 2007). The dried roots of *C. borivilianum* contain 42% carbohydrates, 8-9% proteins, 3-4% fibres and 2-17% saponins (Bordia *et al*., 1995). Research on this plant in India and elsewhere indicates that saponins account for its medicinal properties. Recently six new steroidal saponins were isolated from the roots of *C. borivilianum* and these saponins showed moderate insecticidal and cytotoxic activity (Acharya *et al*., 2009).

MATERIALS AND METHODS

The mature fruits were collected from *C. borivilianum* plants grown in the Botanical Garden of Guru Nanak Dev University, Amritsar, India, in the month of August and September 2006. The fruits were then sun dried for 6-8 days. Measurements were made on morphological characteristics (colour, shape, number of seeds etc.) fruit length and breadth using vernier caliper (Desai *et al*., 1997). Measurements of seeds for length, breadth, thickness and dry weight were also recorded.

Seed Viability

Seed viability was checked using tetrazolium test (Cotrell, 1947). Seeds were soaked in distilled water for 12 h. Soaked seeds were bisected through the embryo and placed in a 0.1% solution of 2, 3, 5-triphenyl-2H-tetrazolium chloride (TTC) for 3 h. Seeds with pink colour were considered viable.

Seed Treatments and Germination

For germination studies, healthy and uniform size seeds were selected. Seeds were soaked in 10^{-10} M, 10^{-8} M, 10^{-6} M solutions of kinetin, IBA (indole butyric acid), cholesterol, testosterone and 24-epibrassinolide (24-epiBL) and Zn (II) for 12 h and germinated in Petri plates of 10 cm diameter lined with double layered Whatman No.1 filter paper soaked with solutions of the same concentrations. Petri plates were kept in the seed germinator at 30±1°C for 21 days for a photoperiod of 8 h dark and 16 h light and the seedlings were observed for the day of emergence (the day when first radical emerged), percent germination, shoot length, root length and fresh weight. The number of seeds germinated was recorded every day for 150 seeds per treatment in 3 Petriplates each containing 50 seeds (McDonald and Copeland, 1999; Baskin and Baskin, 1998).

Statistical Analysis

The data was analyzed for mean, Standard Deviation (SD) and one way Analysis of Variance (ANOVA) (Bailey, 1995). The differences among the means were compared by Honestly Significant Difference (HSD) using Tukey's test (Daniel, 1991).

RESULTS AND DISCUSSION

Fruit and Seed Morphology

The fruit of *C. borivilianum* is a locilucidal capsule, green to yellow coloured, triquertous to 3-sulcate with average length and breadth of 5.57±0.14 and 6.02±0.25 mm. Each capsule bears 6-12 seeds. The average dry weight of a fruit was 29.8±2.04 mg. The seeds are endospermic, black in colour, look like onion seeds with angular edges. The average length, breadth and thickness of the seeds were 2.72±0.04, 2.46±0.03 and 0.43±0.02 mm. The average dry weight of 100 seeds was 358.6±4.21 mg.

Germination Behaviour of Seeds

Seeds have dormancy of about 8-9 months and germinate in the next growing season. In the present study, the viability of the seeds was found to be about 78.16% and seed germination was 8.5%. Different researchers reported different germination percentages in *C. borivilianum*. About 13% seed germination was reported from Gujarat (Bordia *et al*., 1995). The difference in seed germination may be explained due to its differences in local climate conditions and differences in different ecotypes. In this species, seed germination starts at the beginning of the monsoon and moistened seeds take 12-16 days to sprout. Seeds are grown in a very well prepared seed bed heavily manured with FYM or leaf litter in the first or second week of June and throughout the year and adequate soil moisture is maintained. The seedlings are transplanted in the field during the next June-July season. The germination of *C. borivilianum* seed is hypogeal. Seedling has a long tap root with numerous fibrous lateral roots. It bears all the essential structures of monocotyledonous seedlings like mesocotyl, coleoptile and primary leaf etc.

In seed germination studies it was found that days for the first seedling to emerge do not vary significantly in seeds treated with IBA, Zn (II), testosterone, 24-epibrassinolide (Table 1). Differences in seedling emergence were significant in treatments with kinetin (10^{-10} M, 10^{-8} and 10^{-6} M) and cholesterol (10^{-10} M and 10^{-8} M). When effect of different treatments on seed germination was studied it was found that all concentrations of all treatments were significantly different from each other (Table 2). Maximum seed germination was found 35.17 and 36.45% for 10^{-6} M concentrations of cholesterol and testosterone,

Table 1: Effect of different treatments on seedling emergence of *Chlorophytum borivilianum*

	Days for seedling emergence					
Treatments	Control	10^{-10} M	10^{-8} M	10^{-6} M	F-ratio df $_{(3,8)}$	HSD $p\leq0.05$
IBA	4.50±0.48^{a}	4.33±0.47^{ns}	4.00±0.00^{ns}	4.50±0.48^{ns}	0.33	1.31
Kinetin	4.50±0.48^{a}	3.00±0.00^{b}	4.00±0.00^{c}	3.00±0.00^{bd}	33.33**	0.38
24-epiBL	4.50±0.48^{a}	4.00±0.00^{a}	4.00±0.00^{a}	4.00±0.00^{a}	0.67	0.80
Testosterone	4.50±0.48^{a}	4.00±0.00^{a}	4.00±0.00^{a}	4.33±0.47^{a}	0.44	1.13
Cholestrol	4.50±0.48^{a}	3.00±0.00^{ns}	3.00±0.00^{b}	3.67±0.47^{ns}	6.82*	1.08
Zn (II)	4.50±0.48^{a}	3.67±0.47^{ns}	4.00±0.00^{ns}	3.33±0.47^{ns}	2.22	1.31

Results expressed as Mean±SD, *Asterisks represent the level of significance for ANOVA $p\leq0.05$, **Asterisks represent the level of significance for ANOVA $p\leq0.01$, *Values with the same letter in the same row does not significantly differ at $p\leq0.05$ in Tukey's multiple comparison test

Table 2: Effect of different treatments on % germination of *Chlorophytum borivilianum* after 21 days

	Germination (%)					
Treatments	Control	10^{-10} M	10^{-8} M	10^{-6} M	F-ratio df $_{(3,8)}$	HSD $p\leq0.05$
IBA	8.50±0.43^{a}	14.58±0.51^{b}	16.03±0.00^{c}	15.99±0.36^{cd}	172.55**	1.25
Kinetin	8.50±0.43^{a}	14.49±0.42^{b}	18.68±0.69^{c}	19.70±0.51^{cd}	191.12**	1.67
24-epiBL	8.50±0.43^{a}	16.25±0.48^{b}	18.49±0.48^{c}	27.24±0.87^{d}	340.09**	1.89
Testosterone	8.50±0.43^{a}	18.38±0.45^{b}	28.46±0.50^{c}	36.45±0.49^{d}	1363.85**	1.49
Cholestrol	8.50±0.43^{a}	14.01±0.03^{b}	17.16±0.62^{c}	35.17±0.73^{d}	974.02**	1.68
Zn (II)	8.50±0.43^{a}	18.03±0.09^{b}	18.02±0.01^{bc}	26.28±0.61^{d}	746.12**	1.21

Results expressed as Mean±SD, **Asterisks represent the level of significance for ANOVA $p\leq0.01$, *Values with the same letter in the same row does not significantly differ at $p\leq0.05$ in Tukey's multiple comparison test

Table 3: Effect of different treatments on shoot length of seedlings of *Chlorophytum borivilianum* after 21 days

	Shoot length (cm)					
Treatments	Control	10^{-10} M	10^{-8} M	10^{-6} M	F-ratio df $_{(3,8)}$	HSD $p\leq0.05$
IBA	2.80±0.13^{a}	3.35±0.07^{b}	3.67±0.00^{c}	3.75±0.12^{cd}	180.84**	0.25
Kinetin	2.80±0.13^{a}	2.30±0.08^{ns}	2.30±0.08^{ns}	2.34±0.01^{ns}	2.16	ns
24-epiBL	2.80±0.13^{a}	2.80±0.08	3.87±0.12^{b}	4.25±0.42^{bc}	35.62**	0.72
Testosterone	2.80±0.13^{a}	3.70±0.08^{b}	3.82±0.05^{bc}	4.43±0.06^{d}	495.77**	0.19
Cholestrol	2.80±0.13^{a}	3.40±0.04^{b}	3.72±0.12^{c}	4.27±0.02^{d}	343.43**	0.21
Zn (II)	2.80±0.13^{a}	2.60±0.08^{b}	2.78±0.06^{bc}	2.91±0.03^{cd}	58.81**	0.19

Results expressed as Mean±SD, **Asterisks represent the level of significance for ANOVA $p\leq0.01$, *Values with the same letter in the same row does not significantly differ at $p\leq0.05$ in Tukey's multiple comparison test

Table 4: Effect of different treatments on root length of seedlings of *Chlorophytum borivilianum* after 21 days

	Root length (cm)					
Treatments	Control	10^{-10} M	10^{-8} M	10^{-6} M	F-ratio df $_{(3,8)}$	HSD $p\leq0.05$
IBA	0.87±0.02^{a}	1.70±0.04^{b}	1.78±0.00^{bc}	1.88±0.04^{cd}	217.33**	0.140
Kinetin	0.87±0.02^{a}	1.60±0.08^{b}	1.63±0.05^{bc}	1.70±0.08^{bcd}	75.95**	0.200
24-epiBL	0.87±0.02^{a}	2.79±0.04^{b}	2.89±0.06^{bc}	3.65±0.36^{d}	84.56**	0.580
Testosterone	0.87±0.02^{a}	2.47±0.13^{b}	2.85±0.11^{c}	3.20±0.07^{d}	246.68**	0.300
Cholestrol	0.87±0.02^{a}	2.80±0.04^{b}	2.82±0.02^{bc}	3.83±0.02^{d}	3688.61**	0.092
Zn (II)	0.87±0.02^{a}	1.58±0.02^{b}	1.68±0.06^{bc}	1.83±0.08^{cd}	120.54**	0.180

Results expressed as Mean±SD, **Asterisks represent the level of significance for ANOVA $p\leq0.01$, *Values with the same letter in the same row does not significantly differ at $p\leq0.05$ in Tukey's multiple comparison test

respectively. Effect of different treatments on shoot length of seedlings of *C. borivilianum* was found to be significant in all concentrations except at 10^{-10} M concentration of 24-epiBL (Table 3). Testosterone and cholesterol enhanced the shoot length at all concentrations. 24-epiBL at 10^{-6} M and 10^{-8} M concentrations also increased the shoot length of seedlings. Root length of seedlings was also significantly affected by different treatments. Maximum root length was observed in all concentration of cholesterol, testosterone and 24-epiBL (Table 4). Similar trend was also noticed in seedling biomass (fresh weight), where maximum

Table 5: Effect of different treatments on seedling biomass of *Chlorophytum borivilianum* after 21 days

	Fresh wt. (mg)					
Treatments	Control	10^{-10} M	10^{-8} M	10^{-6} M	F-ratio df $_{(3,8)}$	HSD $p\leq 0.05$
IBA	10.87±0.13^a	16.10±0.79^b	17.25±2.40^{bc}	20.63±0.00^d	34.82**	3.94
Kinetin	10.87±0.13^a	10.87±0.13	13.50±0.48^b	14.16±0.03^{bc}	43.01**	1.49
24-epiBL	10.87±0.13^a	20.49±0.39^b	24.20±0.58^c	26.21±0.18^d	700.93**	1.16
Testosterone	10.87±0.13^a	22.72±0.40^b	20.82±0.48^c	26.16±0.76^d	349.01**	1.59
Cholestrol	10.87±0.13^a	21.44±0.90^b	24.22±0.63^c	28.03±0.07^d	352.51**	1.78
Zn (II)	10.87±0.13^a	18.53±0.41^b	19.23±0.19^{bc}	19.27±0.76^{bcd}	167.10**	1.43

Results expressed as Mean±SD, *Asterisks represent the level of significance for ANOVA $p\leq 0.05$, **Asterisks represent the level of significance for ANOVA $p\leq 0.01$, *Values with the same letter in the same row does not significantly differ at $p\leq 0.05$ in Tukey's multiple comparison test

biomass was contributed by cholesterol, testosterone and 24-epiBL in all concentrations. IBA also significantly enhanced the seedling biomass (Table 5).

The inference that can be drawn from the present study is that plant growth regulators IBA, kinetin, 24-epiBL, testosterone and cholesterol and Zn (II) can significantly enhance the seedling growth. These substances are also found in plants and it has been observed that certain steroids if applied exogenously to plants stimulated cell division (Geuns, 1978), pollen germination (Ylstra *et al*., 1995) and growth and flowering (Kopcewicz, 1970; Kopcewicz and Porazinski, 1974; Bhattacharya and Gupta, 1981; Gregory, 1981; Shore *et al*., 1992; Hayat *et al*., 2001). Steroids may also increase the metabolism of plants by synthesizing more nucleic acids, proteins and enzymes (Cerana *et al*., 1983; Kalinich *et al*., 1985; Dogra and Thukral, 1994). Germination percentage and seedling growth were significantly increased by 10^{-6} M concentration of cholesterol and 17 β-estradiol in 10 days old seedlings of *T. aestivum* (Dogra and Thukral, 1991). Bhattacharya and Gupta (1981) studied the effect of steroid hormones on growth and apical dominance of sunflower. Many studies has shown that substances like brassinolide, can regulate plant growth (Franck-Duchene *et al*., 1998; Hayat *et al*., 2001; Shore *et al*., 1992), the division of isolated protoplasts (Oh and Clouse, 1998), the rate of photosynthesis (Guang-Jian *et al*., 1998), sugar uptake (Nakajima and Toyama, 1999), uptake and translocation of K^+ and PO_4^- ions (Jin-Xin *et al*., 1995). Brassinosteroids are reported to increase the crop yield, stress tolerance (Sharma and Bharadwaj, 2007) and disease resistance (Ohri *et al*., 2005) in many crops. 24-epiBL significantly affected the biomass, growth and free proline concentration in *Spirulina platensis* under salt stress (Saygideger and Deniz, 2008). Zinc is an essential element for both plants and animals and it plays an important role in several plant metabolic processes, activates number of enzymes involved in protein synthesis and carbohydrate, nucleic acid and lipid metabolism (Marschner, 1986; Pahlsson, 1989). Similarly Kinetin and IBA have been used by many researchers for root production in different plants (Romanov *et al*., 2000; Ozelbaykal and Gezeral, 2005). Large scale adventitious roots of *Echinacea purpurea* were produced using IBA for the production of secondary metabolites in airlift bioreactor (Wu *et al*., 2007).

CONCLUSION

Chlorophytyum borivilianum is a medicinal herb, getting extinct because of it's over exploitation from its natural habitat. So, cultivation of this important medicinal plant should be encouraged and for this establishment of good seedling stand is prerequisite for the improvement of yield and quality. The present study establishes that steroids, PGRs and Zn (II) can significantly enhance germination and seedling characteristics of *C. borivilianum* by inducing the metabolic activity of embryos required for germination. The most effective treatments were 10^{-6} M concentrations of testosterone, cholesterol and 24-epiBL which enhanced germination and seedling growth several times more than control. The study further reveals that the seeds of *C. borivilianum* can be used for seedling production at commercial level by giving the above said treatments. This will also help in the *ex-situ* conservation of *C. borivilianum*.

REFERENCES

Acharya, D., A.C. Mitaine-Offer, N. Kaushik, T. Miyamoto and T. Paululat *et al*., 2009. Cytotoxic spirostane-type saponins from the roots of *Chlorophytum borivilianum*. J. Natural Prod., 72: 177-181.

Bailey, N.T.J., 1995. Statistical Methods in Biology. 3rd Edn., Cambridge University Press, Cambridge, UK.

Baskin, C.C. and J.M. Baskin, 1998. Seeds: Ecology, Biogeogrphy and Evolution of Dormancy and Germination. Academic Press, San Diego, California.

Bhattacharya, B. and K. Gupta, 1981. Steroid hormone effects on growth and apical dominance of sunflower. Phytochemistry, 20: 989-991.

Bordia, P.C., A. Joshi and M.M. Simlot, 1995. Safed Musli: Medicinal and Aromatic Plants. In: Advances in Horticulture, Chadha, K.L. (Ed.). Malhotra Publishing House, New Delhi, pp: 429-451.

Cerana, R., A. Bonetti, M.T. Marre, G. Romani, P. Lado and E. Mrre, 1983. Effect of brassinosteroid on growth and electrogenic proton extrusion in Azuki bean epicotyls. Physiol. Plant, 59: 23-27.

Chandra, R. and D. Kumar, 2007. Studies on storage behaviour of *Chlorophytum borivilianum* genotypes. Indian J. Hortic., 64: 2-2.

Cotrell, H.J., 1947. Tetrazolium salt as a seed germination indicator. Nature, 159: 748-748.

Daniel, W.W., 1991. Biostatistics: A Foundation for Analysis in the Health Sciences. 5th Edn., John Wiley and Sons Inc., New York.

Desai, B.B., P.M. Kotecha and D.K. Salunkhe, 1997. Seeds Handbook: Biology, Production, Processing and Storage. Marcel Dekker Inc., New York.

Dogra, R. and A.K. Thukral, 1991. Effect of Steroid and Plant Hormones on Some Germination Aspects of *Triticum aestivum*. In: New Trends in Plant Physiology, Dhir, K.K., I.S. Dua and K.S. Chark (Eds.). Todays and Tomorrows Printer, New Delhi, India.

Dogra, R. and A.K. Thukral, 1994. Protein, nucleic acids and some enzyme activities in maize plant as affected by presowing seed treatment with steroids. Indian J. Plant Physiol., 37: 164-168.

Franck-Duchene, M., Y. Wang, S.B. Tahar and R.N. Beachy, 1998. *In vitro* stem elongation of sweet pepper in media containing 24-epibrassinolide. Plant Cell Tissue Organ Culture, 53: 79-84.

Geuns, J.M.C., 1978. Steroid hormones and plant growth and development. Phytochemistry, 17: 1-14.

Gregory, L.E., 1981. Acceleration of plant growth through seed treatment with brassinosteroids. Am. J. Bot., 68: 586-589.

Guang-Jian. L., G.U. Ming-Liang, Y.Y. Li and L. Shao, 1998. Effect of DA-6 and BR+GA_3 on growth and photosynthetic rate in spinach. Acta Horticult. Sinica, 25: 356-360.

Hayat, S., A. Ahmad, A. Hussain and M. Mobin, 2001. Growth of wheat seedlings raised from the grains treated with 28-homobrassinolide. Acta Physiol. Plant., 23: 27-30.

Jin-Xin, D., M.A. Gou-Rui., H. Su-Qing., Y. Meng-Zhao and J.X. Ding *et al*., 1995. Studies on physiological effects of epiBR on cucumber (*Cucumis sativus* L.). J. Zhejiang Agric. Univ., 21: 615-621.

Kalinich, J.F., N.B. Mandava and J.A. Todhunter, 1985. Relationship of nucleic acid metabolism to brassinolide induced responses in beans. J. Plant Physiol., 120: 207-214.

Kopcewicz, J., 1970. Influence of estrogens on the flower formation in *Cichorium intybus* L. Die Naturwissenschaften, 57: 136-136.

Kopcewicz, J. and Z. Porazinski, 1974. Effect of growth regulators, steroids and estrogen fraction from sage plants on flowering of a long-day plant, *Salvia splendens*, grown under non-inductive light conditions. Biol. Plant., 16: 132-135.

Kothari, S.K. and K. Singh, 2001. Evaluation of Safed musli (*Chlorophytum borivilianum* Santapau and Fernandes) germplasm. J. Med. Aromatic Crops, 10: 147-149.

Marschner, H., 1997. Mineral Nutrition of Higher Plants. 2nd Edn., Academic Press, The Netherlands, ISBN: 0-12-473543-6.

McDonald, M.B. and L.O. Copeland, 1999. Seed Science and Technology Manual. Scientific Publisher, Jodhpur, India.

Nakajima, N. and S. Toyama, 1999. Effects of epibrassinolide on sugar transport and allocation to the epicotyl in cucumber seedlings. Plant Prod. Sci., 3: 165-171.

Oh, M.H. and S.D. Clouse, 1998. Brassinolide affects the rate of cell division in isolated leaf protoplasts of *Petunia hybrida*. Plant Cell Rep., 17: 921-924.

Ohri, P., S.K. Sohal, R. Bhardwaj and U.R. Khurma, 2005. Effect of 28-homobrassinolide and 24-epibrassinolide on the development of *Meloidogyne incognita*. Int. J. Nematol., 15: 43-47.

Oudhia, P., 2001. My experiences with wonder crop safed moosli. International Seminar on Medicinal Plants and Quality Standardization, 2001 Chennai, India.

Ozelbaykal, S. and O. Gezerel, 2005. The effects of the different doses of IBA (indole butyric acid) on the rooting performances in the reproduction of Gemlik and Domat olive trees by using the green twig procedure in the ecology of Cukurova Region. J. Central Eur. Agric., 4: 481-484.

Phalson, A.M., 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. Water Air Soil Pollut., 47: 287-319.

Raghavendra, V.B., T.L. Kumar and H.S. Shetty, 2005. Compatibility of Phyton with common fungicide and their role on the yield of safed musli. World J. Agric. Sci., 1: 62-64.

Romanov, G.A., N.P. Aksenova, T.N. Konstantinova, S.A. Golyanovskaya, J. Kossmann and L. Willmitzer, 2000. Effect of indole-3-acetic acid and kinetin on tuberisation parameters of different cultivars and transgenic lines of potato *in vitro*. Plant Growth Regul., 32: 245-251.

Saygideger, S. and F. Deniz, 2008. Effect of 24-epibrassinlide on biomass, growth and free proline concentration in (Cyanophyta) under NaCl stress. Plant Growth Regul., 56: 219-223.

Sharma, P. and R. Bharadwaj, 2007. Effect of 24-epibrassinolde on growth and metal uptake in *Brassica juncea* L. under copper stress. Acta Physiol. Plant, 29: 259-263.

Shore, L.S., Y. Kapulnik, B. Ben-Dor, Y. Fridman, S. Wininger and M. Shemesh, 1992. Effects of estrone and 17â-estradiol on vegetative growth of *Medicago sativa*. Physiol. Plant, 84: 217-222.

Singh, A.K., H.P. Singh, S.P. Singh and A. Kalra, 2008. Genetic variability and correlation studies for selection criteria in safed musli (*Chlorophytum borivilianum*, Santapau). Commun. Biometry Crop Sci., 3: 67-71.

Thakur, G.S., M. Bag, B.S. Sanodiya, M. Debnath and A. Zacharia *et al*., 2009. *Chlorophytum borivilianum*: A white gold for biopharmaceuticals and neutraceuticals. Curr. Pharm. Biotechnol.,

Visavadiya, N.P. and A.V.R.L. Narasimhacharya, 2007. Ameliorative effect of *Chlorophytum borivilianum* root on lipid metabolism in hyperlipaemic rats. Clin. Exp. Pharmacol. Physiol., 34: 244-249.

Vyawahare, N.S., V.G. Kagathara, A.D. Kshirsagar, R. Rajendran, M.N. Patil, A.A. Jagtap and S.S. Sadar, 2009. Effect of hydroalcoholic extract of *Chlorophytum borivilianum* tubers in alleviating the diabetic impotency in streptozotocin induced male diabetic rats. Pharmacognosy Res., 1: 314-319.

Wu, C.H., H.N. Murthy, E.J. Hahn and K.Y. Pack, 2007. Large scale cultivation of adventitious roots of *Echinacea pururea* in airlift bioreactor for the production of chichoric acid, chlorogenic acid and caftaric acid. Biotechnol. Lett., 29: 1179-1182.

Ylstra, B., A. Touraev, A.O. Brinkmann, A. Heberle-Bors and A. van Tunen, 1995. Steroid hormones stimulate germination and tube growth of in vitro matured tobacco pollen. Plant Physiol., 107: 639-643.