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Dose-dependent effect of homoeopathic drug *Zinc sulphate* on plant growth using Bacopa monnieri as model system

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ABSTRACT

Background: Zinc is one of the essential micronutrients in plants required in very low quantity for plant growth and development. In higher concentration, it is known to to reduce the rate of photosynthesis, So homoeopathic preparations tested to see it role on plan growth.
Objective: To analyse the effect of homoeopathic preparation of *Zinc sulphate* on plants through *in-vitro* assay using Bacopa monnieri as a model plant system.
Materials and Methods: Six homoeopathic potencies (1X to 6X) of *Zinc sulphate* were used on a decimal scale along with the control (MS basal agar medium). The samples were evaluated by adding fixed amount (100 µl) in the media as well as by dipping the explants in the test sample overnight. At the completion of the incubation period (14 days) the fresh and dry weight, number and length of the roots, number and length of the shoots and the number of leaves were analysed.
Results: It was observed that *Zinc sulphate* showed growth inhibition at potencies from 1X to 5X, whereas at potency 6X, it exhibited growth promotion effect, when compared with the control.
Conclusion: Homoeopathic drug (*Zinc sulphate*) exhibited growth promotion at higher potency (6X) and growth inhibition at lower potencies (1X to 5X) on Bacopa monneiri.

Keywords: Bacopa monnieri, Homoeopathic potencies, In vitro assay, Photosynthesis, Ultra molecular, Zinc sulphate

INTRODUCTION

Micronutrients are essential components for balanced growth and development in plants.[1] Zinc (Zn) is one of the most important essential micronutrients, which is mainly supplied as Zinc sulphate ($ZnSO_4$). It regulates many physiological and a variety of metabolic processes in plants.[2] Zn plays a vital role in the cell division, cell expansion, protein synthesis and also in carbohydrate, nucleic acid and lipid metabolism.[3] Besides this, it also plays an important role in auxin metabolism.[4-7] This micronutrient is also an essential component of zinc finger transcription factors involved in plant morphogenesis and organogenesis.[8,9] However, at much higher concentrations, it can become toxic, thus leading to physiological and morphological disturbances, and eventually decreasing yield. An optimum (3-30 µM) concentration of Zn is required by the plant for growth and development and

excess of Zn reduces photosynthetic rate hindering the plant growth.[10]

The use of homoeopathic preparations in agriculture has started recently. Homoeopathic preparations are being used efficiently for increasing active principles in medicinal plants, plant detoxification for metals such as aluminum and copper, increasing plant growth rate and productivity,[11] plant metabolism[12,13] and control of diseases.[14-16] Based on these reports, the present study was undertaken to explore the applicability of the homoeopathic preparations *Zinc sulphate* in some variables of the plant using Bacopa monnieri[17] as model system for growth and productivity.

MATERIAL AND METHODS

Homoeopathic preparations of Zinc sulphate in decimal scale (1X to 6X) as per the principles of homoeopathy, was provided by Central Council for Research in Homoeopathy (CCRH), Department of AYUSH, Ministry of Health and Family Welfare, Govt. of India, New Delhi. The study was carried out at CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow during 2007-2010.

A fast propagating strain of Bacopa monnieri (BM08), which showed rapid *in vitro* response against different compounds/extracts/drugs as well as to physical stresses was used for evaluating effects of homoeopathic drug *Zinc sulphate* on plants. The testing was carried out by following the standard tissue culture techniques as described earlier.[17]

The powdered globules *Zinc sulphate* samples admixed with lactose of different potencies were dissolved in sterile double distilled water. Evaluation of biological activity was performed by two methods in three replicates for each treatment.

In method A, 100 µl of *Zinc sulphate* sample was mixed with 900 µl of MS basal agar medium and poured into 1.5 ml of graduated micro-centrifuge tube. The twigs (2.5 cm) of *in vitro* grown Bacopa plants were inoculated in three replicates for each treatment.

In method B, the twigs were dipped in drug samples in three replicates for each treatment overnight and inoculated in 1 ml MS basal agar medium poured into graduated micro-centrifuge tube without drug sample.

These inoculated tubes were kept in half transparent desiccators, allowing the air passage through sterile cotton plugs fixed in the opening vents. The tubes were placed so that the medium containing portion of tubes where roots would be initiated was inserted into the stands, which were made of thermocoal sheet. This set up helps to prevent light exposure to the root while the aerial part of plant got sufficient light and aeration. These desiccators were incubated at normal ambient temperature of 25-28°C with a cycle of 14 hours of light and 10 hours of darkness. Data was recorded in terms of root initiation (number and length), shoot elongation (number and length) and increase in number of leaves for every 24 hours from second day of inoculation till 14 days. MS basal agar medium without any drug sample served as control. Controls provided by CCRH for each potency was also included in the evaluation.

Quantified variables

Initiation of roots

Root initiation was observed every 24 hours till the 14th day. The observations were recorded as roots number and root length in centimeter (cm).

Shoot length

Shoot length was measured by measuring the distance between base of the stem and shoot apex.

Number of leaves

Increase in the number of leaves was recorded at every 24 hours till the 14th day.

Fresh weight

After completion of incubation period (14 days), the fresh weight of plants was determined by weighing on analytical balance. The initial weight of explants was subtracted from final weight.

Dry weight

After completion of incubation period (14 days), the plants were kept in incubator at 37°C for dry weight determination by weighing on analytical balance.

Statistical Analysis

The data for all variables was analysed for mean values and standard error. Statistical analysis was performed by using one way Analysis Of Variance (ANOVA) and was considered at $P < 0.05$ and $P < 0.01$ level of significance.

RESULTS

The treatment of *Zinc sulphate* in different potencies (1× to 6×) to the plant using Bacopa monnieri as

a model system showed different results in *in vitro* assay [Figure 1]. When zinc sulphate was applied at 1× to 5× potencies, there was a decrease in fresh and dry weight, number and length of roots, length and number of shoots and number of leaves in both the methods of treatment, that is the drug was mixed in the medium and when explant was dipped in the drug. But at 6× potency, root number, root length, number of leaves, fresh and dry weight of plant increased significantly ($P < 0.01$) in both the methods [Tables 1 and 2].

DISCUSSION

Since there is decrease in number as well as length of root and shoot, number of leaves, fresh and dry weight of *Bacopa* plants at 1× to 5× potencies, it is assumed that these potencies of *Zinc sulphate* are more than the desired that is required for plant growth and development because *Zinc sulphate* is known to cause phytotoxicity beyond certain concentration.[18,19] However, at potency 6× there is an increase in number and length of root and shoot, number of leaves, fresh and dry weight of plants. This might be due to the concentration of Zinc at 6× potency is equivalent to that of the desired concentration of the element for normal plant growth and development. However, potencies more than 6× are needed to be evaluated for conclusive interpretation. *Sulphur* is an essential macronutrient and required in large amount.[20] Considering the very low concentration of *Sulphur* in 6× potency, it would not have any effect on plant growth and development. Therefore, only Zinc was considered since it is an essential micronutrient in plants required in very low amount (3-30 µM) for plant growth and development, which plays a fundamental role in many physiological and biochemical processes, such as structural and functional integrity of bio-membranes, photosynthesis,

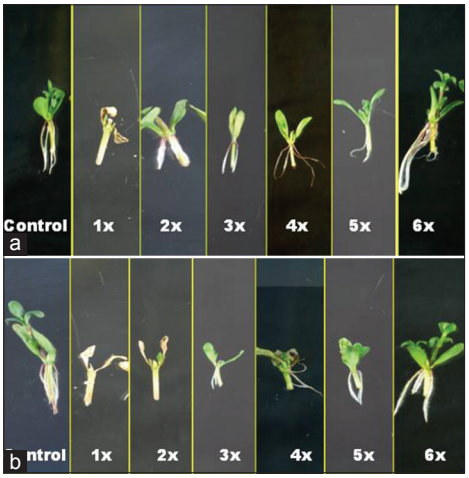

Figure 1: Dose-dependent effect of *Zinc sulphate* on plant growth using *Bacopa monnieri* (a) 100 µl of drug mixed in the MS medium, (b) Explant dipped in the drug

Table 1: Effect of *Zinc sulphate* in different potencies on Bacopa monnieri using method A

Growth parameter	Control	Potency					
		1X	2X	3X	4X	5X	6X
Root no	3±0.474	0±0	2±0.474	3±0.474	3±0.474	2±0.474	4±0.474**
Root length (cm)	2±0.471	0±0	1.5±0.236	1.5±0.236	1.5±0.236	1.5±0.236	2.5±0.236**
Shoot no	2±0.472	1±0.472	2±0.472	1±0.472	2±0.472	1±0.472	3±0.472
Shoot length (cm)	3±0.472	2±0.472	3±0.472	2±0.472	2.5±0.472	2±0.472	3.5±0.472
Leaves no	8±0.943	2±0.943	6±0.943	2±0.943	6±0.943	4±0.943	10±0.943**
Fresh mass (g)	0.0987±0.009	0.0387±0.001	0.063±0.004	0.055±0.001	0.072±0.004	0.066±0.004	0.112±0.010**
Dry mass (g)	0.007±0.001	0.002±0.001	0.004±0.001	0.002±0.001	0.008±0.002	0.008±0.002	0.012±0.003

Mean±SE of plant n=3 **$P < 0.01$

Table 2: Effect of *Zinc sulphate* in different potencies Bacopa monnieri using method B

Parameter	Control	Potency					
		1X	2X	3X	4X	5X	6X
Root no	4±0.472	0±0	2±0.472	2±0.472	1±0.472	1±0.472	5±0.472**
Root length (cm)	1.5±0.236	0±0	1.5±0.236	0.5±0.204	1.0±0.472	1.0±0.472	2.0±0.472
Shoot no	2±0.472	1±0.472	2±0.472	1±0.472	2±0.472	1±0.472	2±0.472
Shoot length (cm)	2.5±0.472	2±0.472	2.25±0.472	2±0.472	2.5±0.472	2±0.472	3.0±0.472
Leaves no	6±0.943	2±0.943	4±0.943	2±0.943	4±0.943	4±0.943	8±0.943*
Fresh mass (g)	0.0783±0.002	0.0377±0.001	0.066±0.002	0.050±0.003	0.063±0.004	0.070±0.004	0.103±0.004**
Dry mass (g)	0.004±0.001	0.002±0.001	0.003±0.001	0.002±0.001	0.0038±0.002	0.005±0.001	0.008±0.002

Mean±SE of plant n = 3 **$P < 0.01$, *$P < 0.05$

auxin biosynthesis and metabolism, response to oxidative stress, apoptosis inhibition and phosphate transport.[21-24] Zinc is also an essential component of enzymes and transcription factors involved in plant morphogenesis and organogenesis.[8,9] Nevertheless, these observations can be substantiated by the fact that homoeopathic drugs also contain the starting material even at higher potencies[25] and they increase plant metabolism by increasing production and allocation of carbon skeletons that would be used for maintenance and growth.[26] It was reported previously by Godoy[27] and Bonato *et al*.,[28] that, in Homoeopathy, the same drug frequently causes different effects, depending on the applied dynamisation. Variable effects of different dynamisations of homoeopathic drug on plant Cerotoma tingomarianus have been reported.[29] Such behaviour of homoeopathic drugs is still insufficiently explained. But it is believed to be related to existing rhythmic movements in nature, and also to the law of similitude that occurs between the applied homoeopathic solution and the organism that receives it.[30,27] In homoeopathic science, it is also believed that when there is a similitude between the homoeopathic solution and the organism, the vital energy responds, opposing the energy of the medication to a text of its own energy.[29] However, when there is no similitude between the homoeopathic solution and the organism, there can be disorder in the metabolic system of the plant, resulting in several negative growth and developmental aspects of the plant. Therefore, the observations made in the present investigation are of great importance with special reference to the use of homoeopathic formulations in agriculture for higher productivity.

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पृष्ठभूमि: ज़िन्क पौधों के लिये एक आवश्यक सूक्ष्म मात्रिक पोषक तत्व है जिसकी पौधों में वृद्धि एवं विकास के लिए कम मात्रा में आवश्यकता होती है। यह उच्चसान्द्रण पर प्रकाश संश्लेषण की दर को घटा देता है। इस कारण पौधों के उचित वृद्धि व विकास प्रदान करने के लिये होम्योपैथी उपयोग की जा सकती है।

उद्देश्य: इस अध्ययन का उद्देश्य बृह्मी (बकोपा मोनेरी) का मॉडल पौधों के रूप में 'इन विट्रो' अध्ययन द्वारा होम्योपैथिक दवा जिंक सल्फेट का पौधों की वृद्धि व विकास में प्रभाव का विश्लेषण करना।

विधियाँ: जिंक सल्फेट की 6 होम्योपैथी पोटेंसी (1X से 6X), एक नियन्त्रित पोटेंसी के साथ उपयोग की गईं। नमूनों का मूल्यांकन माध्यम में एक निश्चित मात्रा (100 यूएल) मिलाकर व एक्सप्लान्ट को जाँच नमूनों में रातभर डुबोकर किया गया। सहेजन काल (14 दिन) पूर्ण होने के उपरान्त, ताज़ा व शुष्क भार, जड़ों की संख्या व लम्बाई, तने की संख्या व लम्बाई और पूर्ण संख्या का भी विश्लेषण किया गया।

परिणाम: जिंक सल्फेट की 1X से 5X पोटेंसी से वृद्धि में रूकावट बकोपा पौधों में पाई गई। जबकि 6X पोटेंसी में, नियंत्रित नमूने की तुलना में वृद्धि पाई गई।

निष्कर्ष: होम्योपैथिक औषधि जिंक सल्फेट ने उच्च 6X पोटेंसी में बकोपा मोनेरी में वृद्धि दिखाई। परन्तु निम्न पोटेंसियों (1X से 5X) में वृद्धि में रूकावट दिखायी।