

Effects of potentised homeopathic medicines on the germination, growth and photosynthetic activity of *Pisum sativum L.*

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Abstract

The present study evaluates the possible effect of potentised homeopathic medicine, *Arsenicum album* and *Baryta carbonica* on germination, growth and photosynthetic activity of pea seedlings (*Pisum sativum L.*). The seedlings of pea were treated with three different centesimal potencies (8CH, 32CH, 202CH) of both the medicine. With the increase in potency, the morphological parameters viz. length of plumule and radical increased. The photosynthetic pigments (chlorophyll, carotinoids, and pheophytin) also showed the similar result. Hence, a stimulating effect was observed on growth as well as photosynthetic activity at the higher potency as compared to untreated one even after the dilution of drug beyond the Avogadro's number.

Keywords: Arsenicum album, Avogadro's number, Baryta carbonica

INTRODUCTION

Homeopathy has always been a subject of controversy and the debate has been taken up by a meta-analysis published in *The Lancet* [1-5]. The discussion about this form of complementary medicine centers essentially on the specific effects of ultra molecular dilutions (beyond the Avogadro limit), which are judged to be "implausible" according to conventional science, although there is emerging evidence for *in vitro* activity of ultra dilutions [6-11].

Plant-based bioassays represent a relatively simple model on a laboratory scale that allows a large number of experimental repetitions to be performed which is useful for studying the problem of irreproducibility. Botanical trials are not susceptible to the placebo effect nor to ethical problems and rely on almost inexhaustible sources of biological materials [12-13]. Hence, in the current study pea (*Pisum sativum L.*) in hydroponics culture was chosen as an experimental model, as earlier used by many workers [14] and the effect of medicines was observed on various different morphological and photosynthetic activities. The pea seeds were stressed with different centesimal potency of the well known homeopathic remedy, *Arsenicum album* and *Baryta carbonica*.

The primary objective of the current study was to investigate the effect of potentised homeopathic medicines on germination, growth and photosynthetic activity of pea (*Pisum sativum L.*) as compared to the untreated one (distilled water). And the second aim was to determine whether there was a continuous relationship between effect and potency level.

MATERIALS AND METHODS

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Plant Materials

Graded dry seeds of pea (*Pisum sativum* L.) were obtained from National Seed Cooperation, Bhubaneswar (India) and stored in dark at room temperature. Uniform sized seeds were selected and surface sterilized with 0.1% mercuric chloride (HgCl₂) for about five minutes and then was rinsed thoroughly with tap water followed by distilled water. Sixty surface sterilized seeds (three replicates of 20 seed each) were placed in sterilized pteriplates over saturated cotton pads for germination. Twenty milliliters of distilled water (control) and solutions of homeopathic medicines of specific potency were poured into each respective pteriplates. The seeds were allowed to germinate under controlled condition at 25°C in darkness for two days. A seed was considered as normally germinated when the radicle protruded by 2-3 cm without infection and the first leaves were visible [15]. After two days, the germination percentage was calculated by using the following formula:

Germination Percentage = <u>No. of seeds germinated</u> × 100 Total no. of seeds sown

Two-day old uniform surface sterilized germinated seeds were transferred to well aerated Hoagland's nutrient solution (full strength) and Hoagland's solution supplemented with varying potency of homeopathic medicine in hydroponic culture vessels for seedling growth [16]. The seedlings were grown in the growth chamber and the white light was provided (12h photo period) by white fluorescent tubes(36 W Philips TLD) with a photon flux density of 52 μ Em⁻² S⁻¹(PAR). After two weeks, various growth parameters and photosynthetic pigments were analyzed.

Treatments

In the experiment, following treatments were applied to the seeds:

- Distilled water (control, C)
- •Centesimal potency of Arsenicum album (8CH, 32CH & 202CH)

Centesimal potencies were obtained by serial dilution with distill water and successions, starting from mother tincture of respective potency (Dr. Willmar Schwabe India Pvt. Ltd). The dynamization (succession) was made with a ten strong downward strokes and each stroke ending in a jerk. All potencies used in the experiment were freshly prepared and were stored in dark at 4°C.

Analysis of Growth Parameters

The growth parameters like root length, shoot length, fresh matter and dry matter of two weeks old pea seedlings were recorded. The root and shoot were first detached from each other and individual length of root and shoot was measured in centimeter scale. Fresh matter and dry were recorded with the help of an electrical single pan balance. For dry weight the seedlings were kept in oven at 80 °C for a period of three days or more (till constant weight was attained).

Estimation of Photosynthetic Pigments

The extraction of chlorophyll pigments was done following the method of Porra et.al [17]. According to which about 0.5 gm of leaves were cut into small pieces and put in 10ml of 80% of acetone and then kept in darkness in refrigerator for 28 hours or more (48 h) at 4 °C. Other pigments such as phaeophytin-a, phaeophytin-b, total phaeophytin and carotenoid were extracted using 80% acetone and estimated following the standard method of Arnon (1949) as amended by Lichtenthaler [18]. The absorbency of extracted liquid was measured at different wave lengths using UV- Visible spectrophotometer (Perkin-Elmer No. Lambda 35).

RESULTS AND DISCUSSION Germination Study

The stimulation of seed germination was noted with the application of potentised homeopathic medicine, *Arsenic album* and *Baryta carbonica* in all potencies as compared to control one (distilled water). Both medicines increased the rate of germination, although minimal differences were evident between the individual potencies (Figure 1). A high stimulating effect was observed at higher potency (202CH) of both the medicines. The result was contradictory to what observed by Gangar and Brizzi [19-20]. But according to Tulip (unpublished), no homeopathically potentised 15X essential mineral nutrient solution statistically increased the rate of corn seed germination when compared to water.



Fig 1. Germination percentage of pea seedling on various treatments

Growth Parameters

With the treatment of potentised homeopathic medicine, a gradual increase in plant growth was observed. A remarkable enhancement of different morphological parameters was observed surpassing the control set. The shoot length substantially increased with the increase in potency. The highest shoot length (21.55 cm) and root length (14.98 cm) was observed in 202 CH potency of *Arsenic album*. Similar findings were reported by Baumgartner et al., (1998). The change in shoot and root growth of the seedlings treated with homeopathic medicine was found to be in the following trend: 202CH AA > 202CH BC > 32CH AA > 32CH BC > 8CH BC > 8CH AA > C.



Fig 2. Variation of Root & Shoot length

This indicates that the drug which is nothing but a water potentisation of the crude substances has a pronounced effect. The effect is selective for a particular potency. It also confirms the homeopathic concepts of potentisation that higher the potency, stronger is the action.

The fresh weight (FW) and dry weight (DW) of the plant samples grown in various potency levels were presented in Figure 3. The FW was increased with the increase in the potency level of the homeopathic medicines but not much effect was observed in case of dry weight.



Fig 3. Impact of homeopathic drugs on Fresh and Dry weight of the seedlings

Photosynthetic Activity

Photosynthetic pigment estimation is one of the important biochemical parameters which were used as the index of production capacity. Figure 4-6 concludes that the pigment contents in plants showed an almost linear increase in response to increase in potency of homeopathic drugs.



Fig 4. Impacts of potentised drug, Arsenicum album (AA) and Baryta carbonica (BC) on the chlorophyll contents pea seedlings. Values are average of ten replicates.

Pigments such as chlorophyll-a, b and total chlorophyll increased with increase in the potency of the medicine. Total chlorophyll content was highest in *Baryta carbonica* at 202CH potency. Other photosynthetic pigments like carotenoid, phaeophytin a, b, and total phaeophytin shows a positive effect on when treated with homeopathic medicines (Figure 5-6). The result indicates that homeopathic drugs at higher potency had a significant stimulatory effect.



Fig 5. Impact of potentised drugs on Pheophytin pigments

The photosynthetic pigment was highest in the seedling when treated with 202 CH potency of *Baryta carbonica*. The trend of increase in photosynthetic content was slightly differing from that which was shown by the growth parameters. Variation of photosynthetic pigments in response to treatment of homeopathic drugs was presented below:

202 CH BC> 32 CH BC> 8 CH BC>202 CH AA>32 CH AA>8 CH AA>C



Fig 6. Overall variation of photosynthetic pigments of two weeks old pea seedlings

CONCLUSIONS

Solutions of potentised homoeopathic medicine demonstrated a biological response, as observed in their effects on germinating seeds, seedlings development and photosynthetic activity of pea seeds. All the treatments induce a significant effect (either increase or decrease) on plant, conforming that both at high and ultra-high potencies can provoke a biological action. The particular process of dynamization thus appears to be as fundamental in the activation of the different behavior of the high and ultra-high dilutions.

Healthy plants models seem a useful approach to investigate basic research questions about the specificity of homeopathic preparations. More investigation with more advanced methods is recommended, especially in the sectors of potentisation techniques, effective potency levels and conditions for irreproducibility.

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