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Effect of Vermicompost extracts on in-vitro germination and growth of *Withania somnifera* (L.) Dunal

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Abstract
Seed germination in *Withania somnifera* is low and erratic under natural conditions therefore in the present study we pre-soaked the seeds of *W. somnifera* for 12h in Vermicompost leachate (aerated and non-aerated), Vermicompost tea (aerated and non-aerated) and distilled water (DW). Treated seeds were germinated *in vitro* on filter papers moistened with respective pre-soaking treatments. Results indicated that seeds treated with Vcom-T_a resulted in highest germination (70%) and biomass/seedling (0.42g). Seed germination and biomass/seedling in different treatments was Vcom-T_a > Vcom-T_{na} > Vcom-L_{na} > Vcom-L_a > DW. Carbon content was significantly increased in Vcom-L_{na}, Vcom-L_a and Vcom-T_a treatments as compared to control. Nitrogen content was maximum in Vcom-T_a seedlings but there was a non significant difference between control, Vcom-T_a and Vcom-L_{na}. We observed more root hair proliferation in Vcom-L_{na} and Vcom-T_a treated seedlings. Therefore, the use of such organic treatments can be extended to cultivate *W. somnifera* under natural conditions.

Keywords: Vermicompost extract; *Withania somnifera*; *in vitro*, propagation

1. Introduction
Millions of people in the developing countries are dependent on medicinal plants as primary healthcare need due to easy accessibility, affordability, cultural and religious customs [1]. *Withana somnifera* also known as Ashwagandha or Indian ginseng is a valuable herb used traditionally in India and its neighbouring countries from thousands of years [2]. Various scientific studies have confirmed its immense therapeutic potential to treat a number of ailments including cardiovascular, neuronal, inflammatory, stress, diabetes, cancer and immunodeficiency [1, 3-5]. Therapeutic activity of this plant can be attributed to high content of secondary metabolites in its leaves and roots. Ashwagandha is mainly propagated through seeds but seed germination is erratic and low and seedling mortality is high under natural environmental conditions [6, 7]. Germination is a crucial phase in the life cycle of a plant that determine plant survival, growth and stand establishment[8]. Better and synchronized germination is a prerequisite for better productivity. One simple strategy to achieve increased seed germination is seed pre-soaking or seed priming [9]. This approach includes hydropriming, osmo conditioning, hormonal priming etc. [10, 11]. Although chemical seed pre-treatments (KNO_3, KH_2PO_4, K_3PO_4, H_2O_2, SA, PEG) are being widely used to enhance the seed germination of several crop plants [12, 13]. However, studies have reported that inorganic salt solutions accumulate inside the seed during pre-soaking and reduce the osmotic potential of seed thereby adversely affect the development of embryo [14]. Major constraints in the use of synthetic treatments are their high cost and non-affordability by poor farmers along with environmental concerns associated with their disposal [15]. Therefore, some traditional non-chemical pre-treatments can be preferred to improve the quality, uniformity, efficacy and eco-friendly propagation of medicinal plants. Vermicompost is prepared by the activity of earthworms on organic wastes routinely generated in our surroundings and been used in several horticultural studies as an excellent plant growth promoter [16]. It is a rich source of nutrients and plant growth regulators that signify its potential as a viable option to increase the production of industrial plant products [17, 18]. Vermicompost leachate and tea contain earthworm secretions, microbial load and soluble nutrients and these have been reported to have positive influence on germination, plant growth and development [19, 20].
In the present study, we examined the effect of aerated and non-aerated Vcom-L and Vcom-T on germination and seedling biomass of *W. somnifera* in an *in vitro* experiment.

2. Materials and Methods

2.1 Preparation of Vcom-L and Vcom-T

Vcom-L was collected as the liquid that leached from the organic waste during vermicomposting of cattle dung with the help of *Eisenia fetida*. For the collection of Vcom-L, cattle dung (1 kg) was mixed with vegetable waste (2kg) in a steel bin (bottom with holes) and 100 clitellate earthworms were released into it. Before the release of earthworms the mixture was pre-composted for 7 days to remove the toxic volatile gases. A water reservoir was attached on the top of bin for continuous dripping of water into the waste. Vcom-L was collected in another bin placed below the bin containing waste (Fig. 1). For Vcom-T extraction, 10g Vcom was mixed in 100 ml distilled water and the mixture was continuously stirred for 12h on a stirrer. Then the mixture was filtered through filter paper for use. Non aerated Vcom-L (Vcom-L_{na}) and Vcom-T (Vcom-T_{na}) were stored in containers closed with lid while aerated Vcom-L (Vcom-L_a) and Vcom-T (Vcom-T_a) were produced by continuously aerating the liquids for 12h prior to use.

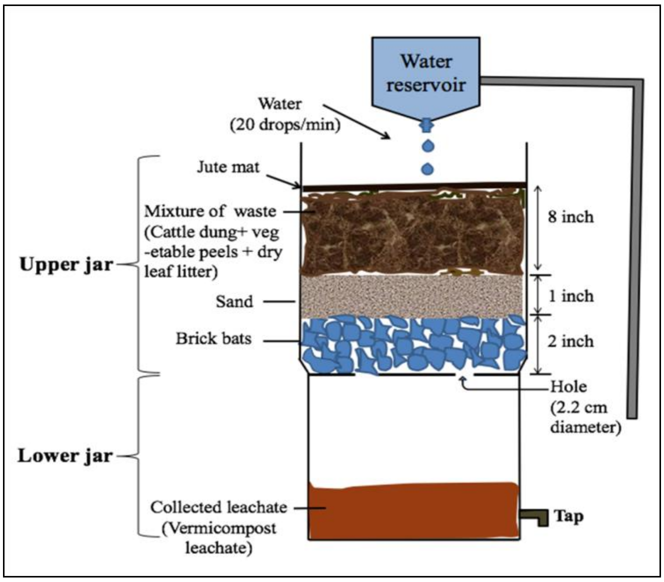

Fig 1: Vermicompost leachate collection unit.

2.2 Plant material, treatments and *in vitro* study

Fresh seeds of Ashwagandha were collected from field grown plants. Seeds were pre-soaked for 12h in DW (control), Vcom-L_a, Vcom-L_{na}, Vcom-T_a and Vcom-T_{na}. Thirty seeds from each soaking treatment were kept in sterilized pertiplates lined with Whatman filter paper # 1 moistened with respective treatments to ensure that the seeds were immersed in treatments. Petriplates with each treatment were incubated in a culture room at a temperature 25±2 ºC in quadruplicate. After 14 days, percent seed germination was recorded as the protrusion of the radical. Seedlings were allowed to develop for 20 days. Six seedlings from each petriplate were randomly selected and shoot length, root length and biomass per seedling was recorded. Root of each seedling from different treatments was scanned under microscope for the intensity of root hair proliferation and photographed.

2.3 Carbon and Nitrogen analysis

Seedlings from each treatment were air dried and grounded to a fine powder. About 2-3 mg of dried powder was combusted at 1000 ºC in a Thermo Fisher 2000 Elemental (CHNS) Analyzer. Calibration curve was prepared using a purified standard of methionine and percentages of carbon and nitrogen were calculated. Vanadium pentoxide (V_2O_5) was used as an oxidation catalyst.

2.4 Statistical analysis

One way analysis of variance (ANOVA) was performed to determine the variation between different treatments. The means were compared by using honestly significant difference at $p<0.005$ (Tukey's test).

3. Results and discussion

Seed pre-soaking releases the germination inhibitors of seed coat and induces the mobilization and solubilization of globulins and synthesis of late embryogenesis proteins [21]. It also enhances the expression of antioxidant enzymes, decreases the level of malondialdehyde and induces α-amylase activity [22, 23]. Pre-soaking enhances speed and synchrony in germination which is essential to improve plant performance and crop productivity [24]. This study was performed to determine the *in vitro* effect of two organic amendments, aerated and non-aerated Vcom-L and Vcom-T on seed germination and seedling growth of *W. somnifera*. Highest and synchronized germination ($p<0.001$) was observed in Vcom-T_a treatment (70.0±1.36%), this was followed by Vcom-T_{na} >Vcom-L_{na} > Vcom-L_a > Vcom-L_{na} > DW (Fig. 2a,b). Organic acids and other metabolites which are produced by the activity of earthworms and microbes have been suggested to play an important role in germination and plant growth [25]. Increase in microbial population in Vcom-T_a due to aeration may be reason of highest germination in Vcom-T_a pre-soaked and treated seeds in the present study. Ingham [26] suggested that aeration enhanced the biological activity

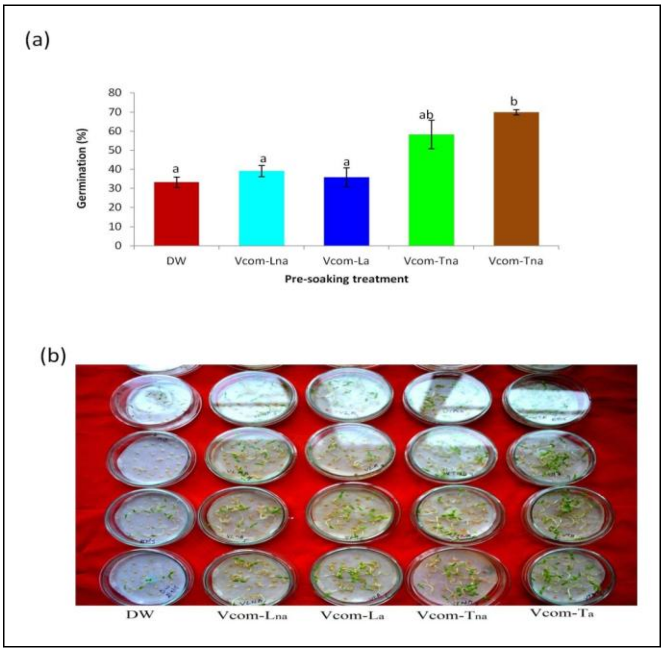

Fig 2: Figure a and b shows the effect of aerated and non-aerated Vcom-L and Vcom-T on the percent seed germination of *W. somnifera.* DW- distilled water, Vcom-L_{na} – non aerated vermicompost leachate, Vcom-L_a – aerated vermicompost leachate, Vcom-T_{na} – non aerated vermicompost tea, Vcom-T_a – aerated vermicompost tea.

of compost teas by increasing the microbial load. Low germination in Vcom-L_a compared to Vcom-T_a treatment may be due to high amount of salts in Vcom-L as suggested by Reigosa *et al.* [27]. Vcom-T_a treated plants had highest shoot length (2.49±0.13 cm) and biomass/seedling (0.42±0.02g). This was followed by Vcom-T_{na} in which shoot length was 2.32±0.12 cm and biomass/seedling was 0.32±0.02g. Vcom-L_a caused increase in shoot length but reduction in biomass in comparison to Vcom-L_{na}. Root length was observed highest (2.56±0.16 cm) in control followed by Vcom-T_a (2.12±0.13 cm) in comparison to other treatments. However, overall, enhancement of growth with Vcom-L_a as well as Vcom-L_{na} was significantly more ($p<0.05$) as compared to control. Growth promoting effect of Vcom-L and Vcom-T treatments can be attributed to the presence of phytohormones of microbial origin such as cytokinin, auxin and gibberellin as reported by Aremu *et al* [28]. The results of the present study are also supported by Keeling *et al*.[29] and Siddiqui *et al*. [30] who observed increased root development and plant growth in oilseed rape and okra after application of Vcom tea. The beneficial effects of Vcom and its by-products are not only limited to the microbial activity or nutrient content but to the soluble humic and fulvic acids which have been reported to stimulate lateral root emergence and proliferation of root hairs [31]. The considerable activity of humic acid molecules have been linked to their interaction with plant growth regulators. This has been explained previously by the evidence on attachment of auxin molecules to humic acids [32]. Auxin play an important role in regulating cell multiplication and enlargement that may possibly be related to the observed dense hairy outgrowths on roots of seedlings treated with Vcom extracts [33].

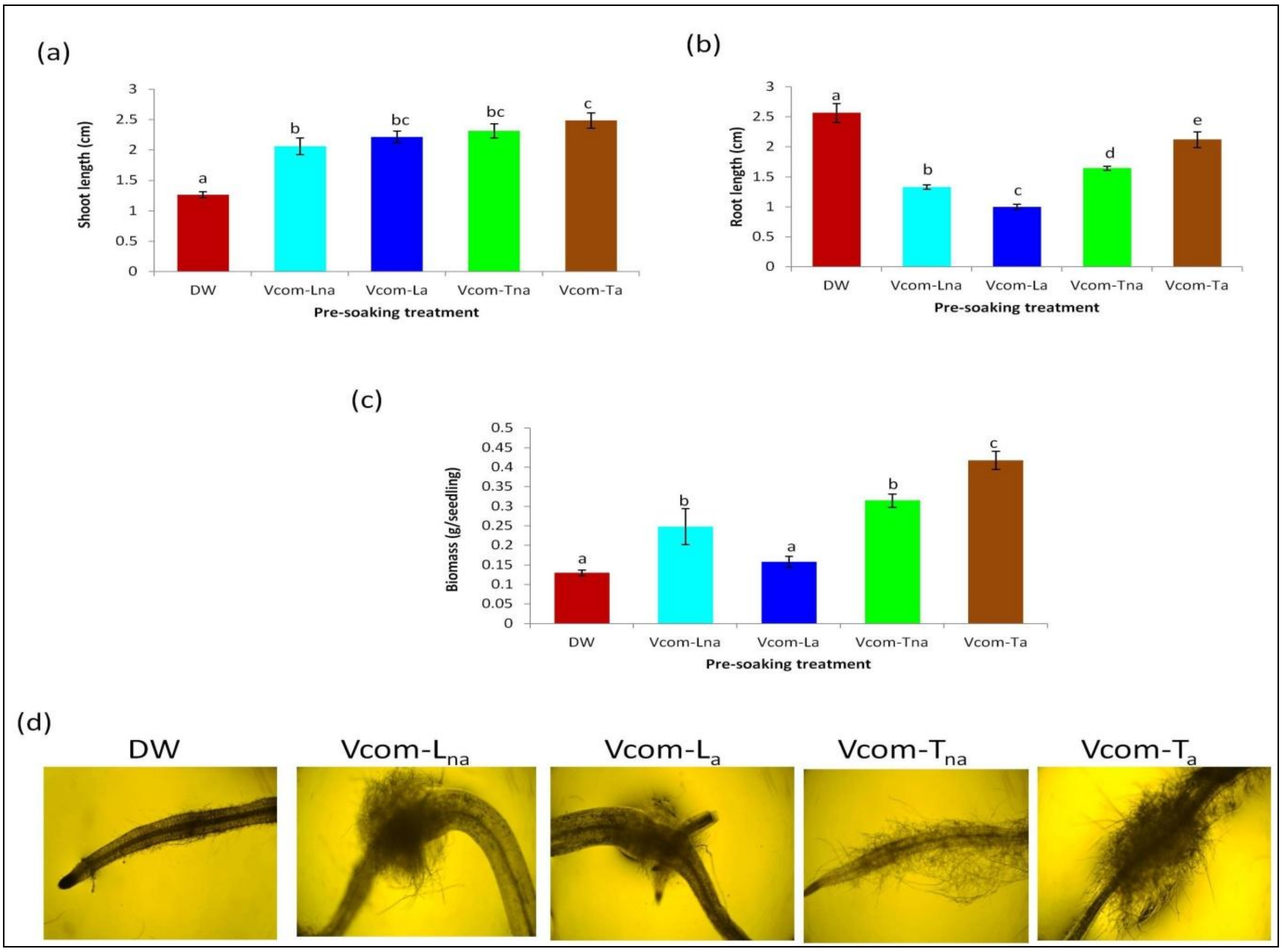

Fig 3: Effect of aerated and non aerated Vcom-L and Vcom-T on the shoot length (a), root length (b), biomass (c) and root hair proliferation of seedlings of *W. somnifera*. DW- distilled water, Vcom-L_{na} – non aerated vermicompost leachate, Vcom-L_a – aerated vermicompost leachate, Vcom-T_{na} – non aerated vermicompost tea, Vcom-T_a – aerated vermicompost tea.

Root of Vcom-T_a treated seedlings showed intense hair proliferation than other treatments and control (Fig. 3d). In our study (Fig. 4a,b) seedlings grown with Vcom-T_a treatment had highest Carbon (43.34±0.97%) and Nitrogen (5.77±0.22%) content compared to other treatments. Carbon content of seedlings with Vcom-L_a (37.49±0.72) and Vcom-L_{na} (37.08±0.92) treatment was more than Vcom-T_{na} (35.26±0.24%) and control. However, Nitrogen content of seedlings with Vcom-L_a (4.62±0.17%) and Vcom-T_{na} (4.47±0.02) treatment was less than Vcom-L_{na} (5.42±0.27%) and Vcom-T_a (5.77±0.22%) and control (5.22±0.12%). High tissue carbon and nitrogen content indicates biomass accumulation and increase in growth and development of young stages due to the presence of high amount of soluble nitrogen, phosphorus and potassium in Vcom-T and Vcom-L [34].

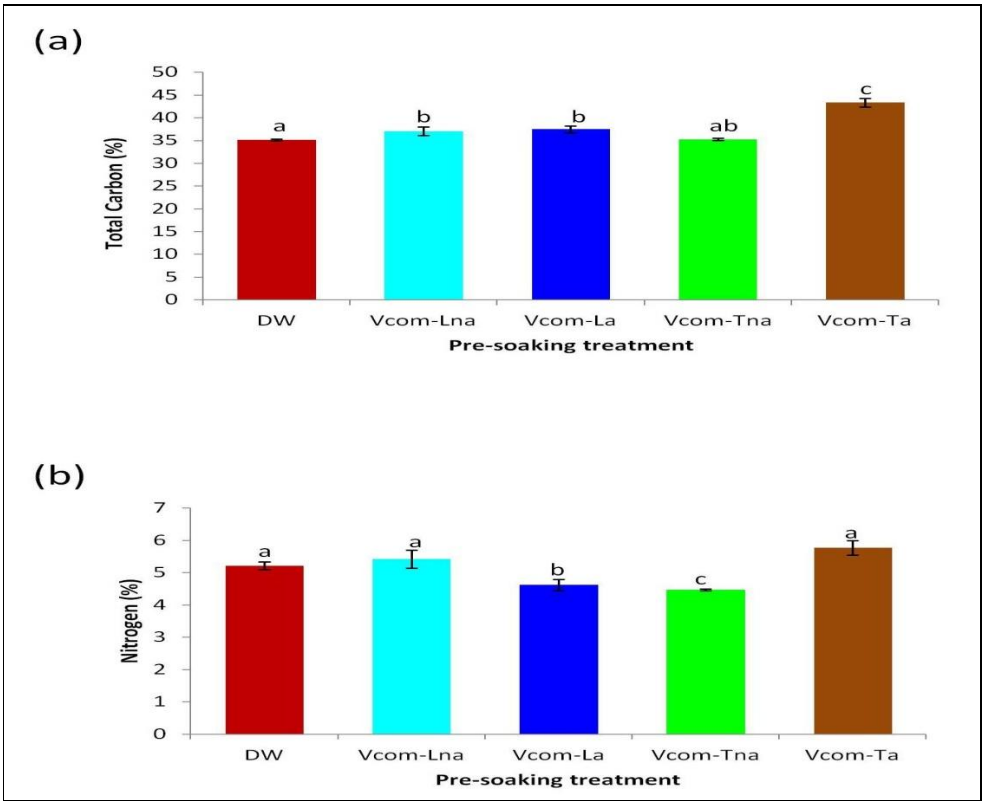

Fig 4: Effect of aerated and non aerated Vcom-L and Vcom-T on carbon (a) and nitrogen (b) content of seedlings of *W. somnifera.* DW- distilled water, Vcom-L_{na} – non aerated vermicompost leachate, Vcom-L_a – aerated vermicompost leachate, Vcom-T_{na} – non aerated vermicompost tea, Vcom-T_a – aerated vermicompost tea.

4. Conclusion
The data clearly show that Vcom-T and Vcom-L treatments enhanced the germination and seedling biomass compared to control. But prominent effect was observed with Vcom-T_a treatment. However aeration had no effect on the biological activity of Vcom-L. These organic preparations can be used in place of chemical treatments for the eco-friendly propagation of *W. Somnifera.*

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