INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

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INFLUENCE OF VERMICOMPOSTS ON THE YIELD AND ALKALOID CONTENT OF *WITHANIA SOMNIFERA*. DUNAL

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ABSTRACT
The impact of vermicomposts *viz.* Cowdung vermicompost, leaf ash vermicompost and poultry feather vermicompost on the yield and alkaloid content of medicinal plant, *Withania somnifera* were assessed and compared with the plants cultivated in the soil amended with chemical fertilizer and the plants cultivated without any fertilizer (control). The plant growth parameters such as shoot length, root length, shoot dry weight, root dry weight, shoot wet weight, root wet weight, shoot: root ratio and the alkaloid withaferin A and withanolide D were significantly increased in the plants cultivated in the soil amended with poultry feather vermicompost.

KEY WORDS: vermicomposts, *Withania somnifera,* withaferin A, withanolide D.

INTRODUCTION

Withania somnifera Dunal (Solanaceae), known in India as Ashwagandha or winter cherry, is one of the most valuable plants of the traditional Indian systems of medicines, is used in more than 100 formulations of Ayurveda, Unani and Sidha and is therapeutically equivalent to ginseng (Sangwan *et al.*, 2004). Phytochemically, the plant is unique in possessing the largest and structurally most diversified set of withanolides (modified steroidal molecules based on an ergostane skeleton), named after the plant. The ethnopharmalogical properties of the plant include adaptogenic, anti-sedative and anti-convulsion activities, and the plant has been employed in the treatment of neurological disorders, geriatric debilities, arthritis and stress and behavior related problems (Dhuley, 2001). Several modern molecular pharmacological studies have demonstrated linkage of these therapeuticcations to one or more withanolides present in the herb (Kaileh *et al.*, 2007). Vermicompost is a product of biodegradation and stabilization of organic materials by interaction between earthworms and microorganisms. It is a finely-divided, peat-like material, with high porosity, aeration, drainage, water holding capacity and microbial activity, which make it an excellent soil conditioner (Edwards, 1998). Addition of different vermicomposts, produced from different sources, like cattle manure, pig manure, food waste, poultry waste etc., increases the rate of germination and growth, and yield of many high value crops (Atiyeh *et al.,* 2000). Vermicompost contains plant-growth regulating materials, such as humic acids and plant growth regulators like auxins, gibberellins and cytokinins, which are responsible for increased plant growth and yield of many crops (Atiyeh *et al*., 2002). These plant growth-regulating materials are produced by action of microbes like fungi, bacteria, actinomycetes etc., and earthworms. Vermicompost provides large particulate surface areas that provide many microsites for microbial activities and for strong retention of nutrients (Shiwei and Fuzhen, 1991). As a result, most nutrients are in available forms such as nitrates, phosphates, and exchangeable calcium and soluble potassium (Edwards, 1998).Further, vermicompost application also suppresses the growth of many fungi, like *Pythium, Rhizoctonia* and *Verticillium*, as a result, many plant diseases are suppressed when vermicompost is applied in ample quantity in the field (Hoitink and Fahy, 1986). Sometimes, vermicompost also controls the population of plant parasitic nematodes (Arancon *et al.,* 2006). Hence, vermicompost exhibits similar effects on growth and yield of plants as shown by soil-applied inorganic fertilizers or plant growth regulators or hormones (Muscolo *et al.,* 1999). However, most research work conducted on the use of vermicompost has only been in the greenhouse conditions, and only a few workers have reported its use and effects under field conditions. The present study is focused on the effect of vermicomposts on yield and alkaloid content of *Withania somnifera*.

MATERIALS AND METHODS

Experimental setup

The experiments were conducted at Pachaiyappa's college, Chennai, India. The medicinal plant, *W. sominifera* was used to assess the manurial quality of different vermicomposts. Thirty earthen pots of approximately 7 kg capacity were filled with 5 kg of clean soil. To loosen the soil, each 5 kg of soil was mixed with 500 g of sand. 500 g half an inch sized brick jelly was spread in each pot at the bottom for easy drainage of excess amount of water. Pots were divided into five groups based on the amendment of different composts namely, Control (control without any compost), CDVC (Cowdung vermicompost + Soil, LAVC (leaf ash vermicompost + soil) PFVC (Poultry feather vermicompost + soil) and CF (Chemical fertilizer + soil). Seeds of *W. sominifera* were procured from the government recognized herbal garden, Irula Tribal Women Welfare society (I.T.W.W.S) of Thandari located at Chengalpattu District, Tamilnadu to conduct the germination and plant growth studies in different compost.

Ten seeds per pot were sown and optimum moisture content was maintained. After germination only two seedlings were allowed to grow further to study the physical and chemical parameters.

Plant sampling

Plants were collected and weighed as a whole including root, shoot, leaves and fruits etc., after the specific growth time duration of nine months. This indicated fresh weight of plants. This was done only at the end of the experiment. Pants were harvested after 280 days and were weighed immediately to determine the wet weight of the shoot and root. They were then oven dried at 60^{o} C and the dry weight of shoot, root and root: shoot ratio was determined.

QUANTITATIVE HPLC ANALYSIS OF WITHANOLIDES

Sample preparation

One gram of the finely powdered plant material or commercial product was extracted three times with 3.0 ml of methanol (MeOH) by sonication for 10 min. After centrifugation (5 min at 3000 rev/min), the extracts were combined in a 10-ml volumetric flask and adjusted to the final volume with MeOH. The liquid samples were diluted 1:1 with MeOH. Prior to use, all samples were filtered through a 0.45-mm Nylaflo membrane filter from Gelman (Ann Arbor, MI, USA). Every sample solution was injected in triplicate.

Calibration

Two milligrams of each standard compound were placed in one 5-ml volumetric flask and dissolved in MeOH (stock solution). Five additional calibration levels were prepared by diluting this solution with MeOH. Standard solutions were stored at 48ºC and were stable for at least 30 days (confirmed by re-assaying the solution). Within the range of concentrations injected (400.0–1.6 mg/ml) the detector response was linear. All data were recorded and processed by Millennium 2 software from Waters (Milford, MA, USA).

Analytical method

HPLC analysis was performed on a Waters Alliance 2690 HPLC system, equipped with a 996 photodiode array detector (Waters, Milford, MA, USA). For all separations a Synergi MAX-RP 80 A column (150=4.6 mm, 4 mm particle size) from Phenomenex (Torrance, CA, USA) was used. Standard compounds withaferin-A and withanolides-D were purchased from Astagiri herbal Instutitution, Thambaram, Chennai (India). The mobile phase consisted of water (A) and a mixture of MeOH and reagent alcohol in the ratio of 1:1 (B), which were applied in the following gradient elution: from 65 A/35 B in 25 min to 55 A/45 B. Each run was followed by a 5-min wash with 100 B and an equilibration period of 10 min. The separation temperature was kept constant at 50^{o}C, flow rate and sample volume was set to 1.0 ml/min and 10 µl, respectively. All separations were monitored at 230 nm. Peaks were assigned by spiking the samples with authentic samples of withaferin-A and withanolides-D and comparison of the UV -spectra and retention times.

Accuracy

A recovery experiment was performed to confirm the accuracy of the method. Sample (1.0 g) was spiked with 1.00 ml of the standard stock solution, and then extracted and analyzed under optimized conditions. The recovery rates obtained were 97.00% for withaferin-A and 100.0% for withanolids.

Statistical analyses

All data were expressed as mean ± standard deviation. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 12.0. When there was a significant difference, Tukey's honestly significantly multiple comparison tests were performed by fixing the significance at level of P≤0.05.

RESULTS & DISCUSSION

Plant growth and yield parameters like plant root length, shoot and root wet weight, shoot and root dry weight, root: shoot ratio were significantly influenced in *Withania somnifera* grown in soil amended with vermicomposts than the plants grown in soil amended with chemical fertilizers and control (without any fertilizer) (Table 1).

TABLE 1. Effect of vermicomposts and chemical fertilizer on root length, shoot and root wet and dry weight (g) of *Withania somnifera*

Treatments**	Shoot length (cm)	Shoot wet weight (g)	Shoot dry weight (g)	Root length (cm)	Root wet weight (g)	Root dry weight (g)	Root : shoot ratio
C	73.20 ± 0.28	38.24 ± 1.23^{a}	11.82 ± 0.28^{a}	16.42 ± 1.24^{a}	15.73 ± 0.48^{a}	6.47 ± 0.48^{a}	0.55 ± 0.036^{a}
CDVC	109.30± 0.67	56.47 ± 1.54^{c}	17.84 ± 0.52^{d}	22.46 ± 1.53^{c}	32.45 ± 0.62^{c}	13.26 ± 0.54^{d}	0.74 ± 0.034^{c}
LAVC	104.50 ± 0.62	57.36 ± 1.52^{c}	16.45 ± 0.45^{c}	20.60 ± 1.60^{c}	34.54 ± 0.67^{d}	12.34 ± 0.65^{c}	0.75 ± 0.048^{c}
PFVC	116.80 ± 0.68	63.27 ± 1.20^{d}	19.67 ± 0.67^{e}	25.54 ± 1.40^{d}	42.35 ± 0.82^{e}	15.43 ± 0.74^{e}	0.78 ± 0.042^{c}
CF	81.20 ± 0.34	42.58 ± 1.52^{b}	15.46 ± 0.46b	19.45 ± 1.36^{b}	21.56 ± 0.56^{b}	10.46 ± 0.46^{b}	0.68 ± 0.038^{b}

C –control, CDVC – cowdung vermicompost, LAVC- leafash vermicompost, PFVC – poultryfeather vermicompost, CF -chemical fertilizer.

All values are the mean and standard deviation of six replicates.

**denotes significance at 1% level.

Mean values designated with different superscripts indicate that differences between treatments are significant according to the Tukey's honestly significantly different (HSD) multiple comparison test (P< 0.05).

Such influence of vermicompost on plant growth of *Withania somnifera* may be attributed to better availability of plant growth regulators and humic acid in vermicompost, which is produced by the increased activity of microbes (Arancon *et al.,* 2006). Studies of various researchers on assessment of growth and yield in a variety of medicinal plants including*, Chlorophytum borvallianum* (Paturd *et al.,* 2002), *Ocimum basilicum* (Munnu *et al.,* 2002), *Capsicum annum (*Norman *et al*., 2003) *Pelargonium graveolens* (Ram *et al*., 2003), *Phyllanthus amarus* (Annamalai *et al.,* 2004), *Aloe vera* (Saha *et al.,* 2005) and *Andrographis paniculata* (Vijaya *et al*., 2008) grown in the soil fertilized with vermicompost revealed significant growth promotion as indicated from the increase in the height of shoot, collar width, root length, leaf area, root shoot ratio, number of flowers, number of branches, alkaloids, steroids, germination percentage and yield. Venkatesh *et al*. (1998) found that application of inorganic fertilizers and vermicompost resulted in greater availability and plant content of macro nutrients compared to application of inorganic fertilizers without vermicompost.

Experiments to assess growth, and yield in a variety of plantations including neem seedlings (Biradar, 1998), mulberry (Murakar, *et al*., 1998), turmeric (Vadiraj, *et al*. 1998), paddy (Vasanthi and Kumaraswamy, 1999), grapes (Venkatesh, *et al*., 1997), chickpea (Sahni, *et al*., 2007) and strawberry (Singh, *et al*., 2008) revealed significant growth promotion as indicated from the increase in the height of shoot, collar width, root length, leaf area, root shoot ratio, number of leaves, canes for vine, number of branches for vine, germination percentage and yield. Results of the present study demonstrate that vermicomposting procedure is an important factor in the conversion and releasing of nutrients in the soil. The study clearly shows that action of earthworm accelerates and promotes biodegradation of organic wastes and converts into high manurial quality biofertilizer rich in macro and micronutrients in the form readily available to plants. The comparison of growth induced in the experimental plant, *W. somnifera* revealed that vermicompost promotes growth greatly than the chemical fertilizers in terms of the physical parameters of growth analysed in the study. Poultry feather-vermicompost seems to promote growth better than other composts. At the end of 280 days study period, *W. somnifera* was recorded root length (19-26cm) , shoot wet weight (42-63g/plant), root wet weight (21-42g/plant), shoot dry weight (15-20g/plant), root dry weight (10-15g/plant), root: shoot ratio (0.68-0.78) relative to growth and yield of these features in the plants raised statistically significant *($p \leq 0.05$)* in the control (Table1). The plants fertilized with chemical fertilizer registered slightly low rate of growth as it could be inferred from low level of growth comparable to the plants amended with vermicompost.

The analysis of *W. somnifera* treated with vermicomposts and chemical fertilizer confirmed the presence of withaferin A and withanolide D in all parts of the plant (Table 2). Alkaloid content of withaferin A was maximum in PFVC (0.68%) treated plant followed by LAVC (0.35%), CDVC (0.25%), CF (0.22%) and C (0.16%). Whereas withanolide D was high in PFVC (0.53%) followed by CDVC (0.43%), LAVC (0.35%), CF (0.28%) and C (0.26%). Based on higher values, withaferin A and withanolide D could be ranked in the following order PFVC>LAVC>CDVC>CF>C and PFVC>CDVC>LAVC>CF>C. *Withania somnifera* is an important medicinal plant and a major source of alkaloid and steroidal lactones (withanolides) which are regularly used in pharmaceutical industries (Rakesh and Alok, 2003). In the present study withaferin A and withanolide D were high in the plants grown in the soil amended with vermicomposts than in the plants grown in soil amended with chemical fertilizer and control. Supratik *et al*. (1998) also reported that the application of organic fertilizer increased the alkaloid content of *Withania somnifera* than plants grown on inorganic fertilizer. Paturde *et al.* (2002) have shown that vernicompost had the potential to increase the steroid content in *Chlorophytum borivalianum.*

TABLE 2. Withaferin A and withanolide D of *Withania somnifera* grown in various treatments

Treatments	% of withaferin A	% of withanolide D
C	0.156	0.26
CDVC	0.253	0.43
LAVC	0.349	0.35
PFVC	0.680	0.53
CF	0.223	0.28

C -control; CDVC – cowdung vermicompost; LAVC-leafash vermicompost;
PFVC – poultryfeather vermicompost; CF -chemical fertilizer.
All values are the means of three replicates.

The overall effects of the application of PFVC, CDVC and LAVC in the experiments reported in this investigation in terms of increased germination, increased plant growth, number of flowers and fruits and high level of alkaloid content are economically critical, since *Withania somnifera* is a valuable medicinal plant used in the pharmaceutical industries to prepare drugs to treat ulcers, venom toxins, skin diseases, rheumatism, inflammation, parkinson's disease etc. The increased rate of germination, growth and flowering of *Withania somnifera* could be associated with greater nutrient quality of the vermicomposts. It could also have been due to other biological effects such as increase in the beneficial enzymatic activities, increased populations of beneficial microorganisms, on the presence of biologically active plant growth–influencing substances such as plant growth regulators or plant hormones in the vermicomposts. However, the increased yield and alkaloid content of *W. somnifera* in the present study could not be explained on the basis of availability of macronutrients in soil amended with vermicompost because the nutrient content were equalized at the time of planting and analysis of soil samples after planting and at subsequent sampling at different intervals confirmed that the concentration of macronutrients in all pots were statistically the same. This confirms the findings of earlier workers who postulated that increased yield and production of alkaloid content resulted from earthworm activity in the vermicompost might have influenced the plant yield indirectly, which might have helped in attaining higher alkaloid content of

many medicinal plants (Annamalai *et al*., 2004; Saha *et al*., 2005). Thus, our study also indicated that the growth and yield of *W.somnifera* and can be well correlated with the plant alkaloid withaferin A high in the vermicompost. Experimental data presented herein provides a sound basis that vermicomposting is a suitable technology for conversion of different types of organic wastes viz. cowdung, leafash and poultry feather into value added material. On the basis of results observed in the present study poultryfeather vermicompost, cowdung vermicompost and Leafash vermicompost are recommended for the cultivation of the medicinal plant *Withania somnifera.* However further field studies are required to explore the utility of the vermicomposts in various combinations.

CONCLUSION

The present study revealed that substitution of vermicompost with chemical fertilizers is quite useful grown in *W.somnifera* for higher growth, yield, alkaloid compound, and it helps in getting higher marketable alkaloid compound withaferin A and withanolide D of firmer alkaloid with attractive colour and good quality attributes. However, the vermicomposts can vary according to agro climatic condition of the production region, but poultryfether- vermicompost is sufficient for higher productivity of better quality of *W. somnifera.*

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