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Research Article

YIELD AND PHYTOCHEMICAL EVALUATION OF WILD AND CULTIVATED SAMPLES OF ASHWAGANDHA

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ABSTRACT

Ashwagandha (*Withania somnifera* Dunal) is a very important herb in Ayurveda for its wide use in various ailments and health promoting properties. More than 200 Ayurvedic formulations use Ashwgandha as a main ingredient. Because of its versatile medicinal value it is termed as Indian Ginseng. Its annual demand is 7000 tonnes per annum and its actual productions is 1500 tonnes per annum. This demand obviously cannot be met from the wild sources and thus to meet this demand commercial cultivation of ashwagandha is the only route. But, a pertinent question arises with commercial cultivation regarding the medicinal quality owing to the changed conditions in cultivation vis-à-vis wild. The present research work was planned to compare the yield and phytochemical values of cultivated and collected from wild ashwagandha (1) Cultivated by using two factors (a) Chemical fertilizers (N:P:K-20:20:00 Kg/ha) and (b) Organic - (i) Farm yard manure 2 kg/m^2 (ii) Vermi compost 1.3 kg/m^2 and (2) Wild. The study findings suggest- Ashwagandha grown with FYM and Vermi compost is better in terms of yield and both organically grown and wild Ashwagandha are better than other samples in terms of phytochemical parameters. Thus the study suggests medicinal use of organically grown Ashwagandha.

Keywords: Wild, Cultivated, Organic, Ashwagandha, Yield, Phytochemical parameters

INTRODUCTION

Owing to growing global popularity of Ayurveda in the healthcare arena there is a constant rise in the demand for Ayurvedic medicines in the last few decades. Since majority of Ayurvedic medicines are plant based, this growing demand has resulted in a huge pressure on the traditional sources of raw materials i.e. the wild. Obviously, the wild sources are not able to meet the demand of raw material and thus the sector is looking for alternate sustainable sources like cultivated fields. Therefore, many of the high demand medicinal plants used in Ayurveda like Ashwagandha, Chitrak, Safed Musli are now being cultivated on a commercial basis. This has resulted in commercial cultivation of medicinal plants to become a full fledged sector having its own nuances, where yield is the most sought after parameter. Further, due to various factors, farmers are switching to medicinal plants cultivation in place their staple crops. Thus, many medicinal plants like Ashwagandha are being cultivated in agro-climatic conditions which are quite different from that of their natural habitat. The current world herbal trade which stands at US$ 120 billion and is expected to reach US$ 7 trillion by 2050[1]. The domestic market is estimated to be around US$ 2 billion. Both the markets are annually growing at 15-20 %.[2] The volume of demand of medicinal plants in India was 2700 tonnes in 2002 and has grown to 4400 tonnes in 2004-05 with an annual growth rate 21 %.[3] The increasing demand in global and domestic market causes pressure on its sources. At present, 90 % collection of medicinal plants is from the wild; in that 70 % of plants collections involve destructive harvesting, many plants are endangered or vulnerable or threatened.[4] As a result, the raw material supply situation is shaky, unsustainable and exploitative. This results in depletion of resource base, increase adulteration and non-availability of quality herbal drugs for domestic consumption as well as for exports. Ashwagandha, also known as Indian Ginseng, is a highly popular herb and widely used in lot of Ayurvedic formulations, nutraceutical products and other herbal products. The demand of this herb was estimated to be 9127.5 tons per annum in the year 2005[5]. Based on the trend, the current demand of Ashwagandha per annum would be around 12500 tons. Majority of this demand is met from cultivated fields spread across various parts of India.Keeping yield as the important objective, many chemical fertilizers and pesticides are used during the cultivation. These have a probable effect on the ultimate phytochemical and pharmacological profile of the plant. Further, all growers of Ashwagandha do not use one standard package of cultivation

practices. Since Ashwagandha is sourced from various kinds of sources, there is a logical possibility of encountering variations amongst them. Secondly, the purported therapeutic benefits of Ashwagandha as described in classics pertain to the Ashwagandha collected from wild. Therefore, it is essential to study the variations between Ashwagandha collected from wild, cultivated as per the NMPB suggested cultivation practices and cultivated in an organic manner and access which source of Ashwagandha is phytochemically the most potent one.

Effect of Different Environmental Factors on Crude Drugs

The main chemically active agents responsible for pharmacological actions of plant products are certain groups or individual substances, which are secondary products of plant metabolism e.g. alkaloids, glycosides, etc. The crude drug which reaches the herbal trade and pharmaceutical manufacturing line pass through various stages viz. environmental conditions, cultivated and wild sources, post-harvest management which affect + vely/- vely on the amount of active constituents present in it. Different habitats and geographical sources expose the plants to different temperature, rainfall, light-hour exposure, soil, atmospheric conditions and environmental conditions. This may result in variations in plant growth, development and metabolism.

Temperature

Temperature is a major factor controlling the development and metabolism of the plant. Although each species has become adapted to its natural environment, plants are able to exist in a considerable range of temperature, thus incurring significant variations in behavior. In general, the formation of volatile oils appears to be enhanced at higher temperatures, albeit very hot days may lead to an excess physical loss of oil.

Rainfall

The important effects of rainfall on vegetation must be considered in relation to annual rainfall, its distribution throughout the year, its effect on humidity and its effects coupled with water holding properties of the soil. Continuous rainfall can lead to a loss of water soluble substances from leaves and roots by leaching e.g. alkaloids in solanaceous plants.

Light- Hour exposure

Plants vary much both in the amount and intensity of the light they require. In certain cases, researchers has shown that light is a factor which helps to determine the amount of glycoside or alkaloids produced. At Gif-Sur-Yvette experiments indicated that with *Datura stramonium* long exposure to intense light brought about a sharp increase in hyoscine content at the time of flowering. (Loughrin *et al*, 1990)[6]. In the wild state the plant is usually found where its shade requirements are met and under cultivation if similar shades are not provided then the plant is supposed to show phytochemical variations.

Atmospheric conditions

It has been reported that *Digitalis lanata* grown in green-houses with CO_2 enriched atmosphere produced 3.5 times of digoxin than the field cultivated plants. (Stuhlfauth *et al,* 1990)[7].

Soil

Different plant species vary enormously in their soil and nutritive requirements. Three important basic characteristics of soil are their physical, chemical and microbiological properties. The basic soil type is modified by the presence of humus, organic fertilizers, chalk, lime, inorganic matters, etc. Variations in particle size result in different soils ranging from clay, via sand to gravel. Particle size is one factor governing water-holding capacity and some plants which produce mucilage e.g. *Chlorophytum borivilianum* Sant. and F. as a water-retaining material contain less mucilage when grown on soil with high moisture content.

Geographic conditions

The effects of altered geographic conditions is illustrated by the report that when *cannabis* seeds grown in England rich in cannabidiol (CBD) and devoid of tetrahydrocannabinol (THC) were cultivated in Sudan, these produced THC in first generation. (Traese and Evans)[8]. It is quite obvious that plants under cultivation in field get different conditions from the plants growing in wild in reference to temperature, rainfall, light-hour exposure and soil. Therefore both sources are susceptible to grow same plant species with altered morphology and phytochemical behavior. Thus, for successful cultivation with higher yield and optimum efficacy, it is mandatory to study the conditions under which the plant flourishes in wild and reproduce these conditions or improve upon them. Small changes in ecosphere can affect plant products e.g. satisfactory rubber trees grown in wild in the Amazon basin but cleared areas converted to rubber plantations have been a failure. Since cultivated fields and wild sources are not the same with respect to geo-climatic parameters and the geo-climatic conditions do play a role on the plant behaviour and its phytochemical profile, it is logical to expect variation in pharmacological behaviour of medicinal plant materials collected from wild and cultivated fields.

Importance of Desa pariksa in Ayurveda

The concept of bhumidesa or habitat described in Ayurveda; Susruta has explained importance of bhumipariksha in context of collection of plant products in chapter (Su.Su:37)[9]. The effect of ecological conditions on properties of plants were decipherable from the opinion of Caraka, which states that plants of Himalayas are qualitatively better than those of Vindhyas. (ca.su.25/40)[10]. The concept of vanya and gramya varieties of some medicinal plants like masha, mentioned in nighantus possesses different medicinal properties and it reveals a clandestine notion of effect of ecosphere on plants. Thus, with changing trend it can be rationally postulated that cultivated field is an extended type of bhumidesa that should be taken into consideration while ascertaining drug quality. Ayurveda attributes mahabhautik configuration and composition of a drug to its pharmacological properties. The plants under cultivation and the plants growing in wild get different light hours (agni), soil conditions (prithvi), climatic conditions (vayu) and water supply (jala) which may foster a change in mahabhautik composition and consequently alteration in medicinal properties and values.

Opportunities of Ashwagandha cultivation

Ashwagandha, also known as Indian Ginseng, is a highly popular herb and widely used in lot of Ayurvedic formulations (more than 200), nutraceutical products and

other herbal products[11]. Its annual demand is 7000 tonnes but its actual production is 1500 tonnes per year[12]. The demand of this herb was estimated to be 9127.5 tons per annum in the year 2005[13]. Based on the trend, the current demand of Ashwagandha per annum would be around 12500 tons. The global interest in this plant and the high demand for its roots provide ample scope to cultivate this plant on commercial scale. Other opportunities for cultivation include Present price for roots is attractive, crop gives economically remunerative returns in comparison to traditional crops, ease of cultivation under rain fed condition. Due to increasing demand and low production rates causes pressure on its wild germ plasm to overcome this cultivate Ashwagandha in commercial manner.

Need of the Study

As stated above, Ashwagandha sourced from various different types of sources having different geo-climatic and ecological conditions are available for the use of end users. Plant growth and development, and often the nature and quantity of the secondary metabolites, are affected by temperature, rainfall, daylight, biotic interactions, etc (Traese and Evans)[8]. Medicinal plants in high demand and which are unavailable in the wild to meet the demand requires being cultivated. Often cultivation results in improved quality raw material because of selective control and improvement of certain factors of plant growth. But, the fundamental principle in determining the cultivation practices is the observation of conditions in wild where in the plant is flourishing and replicating them in field. Any change in these conditions can have an effect on the phytochemical and pharmacological behavior of the plant. Since Ashwagandha is sourced from various kinds of sources, there is a logical possibility of encountering variations amongst them. Secondly, the purported therapeutic benefits of Ashwagandha as described in classics pertain to the Ashwagandha collected from wild. Therefore, it is essential to study the variations between Ashwagandha collected from wild, cultivated as per the NMPB suggested cultivation practices and cultivated in an organic manner and access which source of Ashwagandha is phytochemically the most potent one.

Aims and Objective

- To cultivate Ashwagandha under 2 types of cultivation practices, one by following NMPB suggested PoP's and second, in an organic manner, in the Herbal Garden (Dhanwantari Upavan) of NIA, Jaipur, India.
- To explore and find out a wild source of Ashwangandha.
- To collect the roots from the above three sources.
- To undertake phytochemical analysis of these three samples including PH, Moisture content, Ash values, extractive values and TLC profile by using available protocols.
- To explore phytochemical variations among these three samples.

MATERIALS AND METHODS

The present investigation was carried out at the Research Plot, Dhanvantari upwan, Department of Dravyaguna Vigyan, National Institute of Ayurveda, Jaipur (Rajasthan), India during 2011-2012. The experiment was carried out in randomized block design with factorial concept. The experiment consisting of six treatment combinations, comprising of two inorganic fertilizer level *viz.*, control (A_0) and 20-20-00 kg NPK/ha (A_1) and three organic manures level *viz.*, control (B_0), FYM @ 2 kg/m^2 (B_1) and Vermicompost @ 1.3 kg/m^2 (B_2) and no application of inorganic fertilizer and organic manure i.e. control. Six combinations were A_0B_0 (No application of chemical fertilizers and organic manure i.e. Control), A_0B_1 (application of FYM), A_0B_2 (application of Vermi compost), A_1B_0 (application of chemical fertilizers), A_1B_1 (application of chemical fertilizers and FYM) and A_1B_2 (application of chemical fertilizers and Vermi compost). All the combinations were replicated four times. The cultivar Jawahar Ashwagandha-20 was used for the experiment. The land was ploughed, harrowed and leveled to bring the soil to fine tilth after receiving pre-monsoon rains. The field was divided into plots for different treatments. Quantity of inorganic fertilizers and organic manure to be applied to ashwagandha was calculated as per the treatments. Nitrogen and phosphorus were applied in the form of urea and DAP respectively. Inorganic fertilizers and organic manures were applied and well mixed with the soil of respective plots before the sowing of the seeds. Certified seeds were sown in line on 19th August 2011. Organically growing seeds were treated with Tricoderma and inorganically growing seeds were treated with Bavistin at the time of sowing. Total five rows per plot and seven plants per row were maintained. The fully matured crop was harvested on 29th March, 2012 when the leaves were drying out and berries yellow red in color. The data of all the characters studied were subjected to statistical analysis.

RESULTS

The mean values of horticultural parameters of Ashwagandha grown organically and chemically are furnished in Table 1.

Phytochemical Parameters

The Physicochemical analysis and the observation made during the Physicochemical analysis of Ashwagandha Root samples of wild and cultivated according to different parameters is discussed below and all the result perceived was in accordance with the standards laid down in Ayurvedic Pharmacoepia of India Vol. –I, 2002.

Table 1: Observations of Cultivated Varieties for Horticultural Parameters

Treatments	Root length cm	Root diameter cm	Root fresh wt. g	Root dry wt. g	Seed yield g/ plant
Factor-1					
A_0	26.63	4.34	20.17	8.61	11.17
A_1	21.28	4.21	29.23	6.68	12.33
S. E.	1.47	0.18	0.71	0.41	0.45
C.D. at 5%	4.44	0.53	2.15	1.23	1.37
	S	NS	S	S	S
Factor-2					
B_0	19.75	3.35	20.63	3.82	10.75
B_1	26.33	4.43	25.93	4.27	11.63
B_2	25.80	5.05	27.68	3.80	12.88
S. E.	1.20	0.14	0.58	0.33	0.37
C.D. at 5%	3.63	0.43	1.75	1.00	1.12
	S	S	S	S	S
Interaction (A X B)					
A_0B_0	25.05	3.18	14.20	8.65	10.50
A_0B_1	31.95	4.58	22.90	8.83	11.25
A_0B_2	22.90	5.28	23.40	8.35	11.75
A_1B_0	14.45	3.53	27.05	7.78	11.00
A_1B_1	20.70	4.29	28.95	7.25	12.00
A_1B_2	28.70	4.83	31.95	5.00	14.00
S. E.	2.08	0.25	1.07	0.58	0.64
C.D. at 5%	S	NS	3.04	NS	NS

A_0B_0 - No application of chemical fertilizers and organic manure (Control), **A_0B_1 -** Application of FYM, **A_0B_2 -** Application of Vermi compost, **A_1B_0** Application of chemical fertilizers, **A_1B_1 -** Application of chemical fertilizers and FYM, **A_1B_2** - Application of chemical fertilizers and Vermi compost

Table 2: Observations of Wild Ashwagandha Sample for Horticultural Parameters

Wild Sample Parameters	Observations
Root Length (Cm)	18 Cm
Root Diameter (Cm)	4 Cm
Root Fresh Weight (g)	15.44 g
Root Dry Weight (g)	12.7 g
Seed Yield/Plant (g)	12 g

Table 3: Observations of comparison between wild and cultivated ashwagandha sample for horticultural parameters

sample / Observations parameters	A_0B_0	A_0B_1	A_0B_2	A_1B_0	A_1B_1	A_1B_2	Wild
Root length (cm)	25.05	31.95	22.90	14.45	20.70	28.70	18
Root diameter(cm)	3.18	4.58	5.28	3.53	4.29	4.83	4
Root fresh wt (g)	14.20	22.90	23.40	27.05	28.95	31.95	15.44
Root dry wt (g)	8.65	8.83	8.35	7.78	7.25	5.00	12.7
Seed yield/plant (g)	10.50	11.25	11.75	11.00	12.00	14.00	12

Table 4: Observations of quantitative examination of inorganic matters

Name of Samples	Ph	LOD In %	% of Total Ash	% of Acid Insoluble Ash	% of Water Soluble Ash	Chl % of Extract	N-Hexane % of Ext.	Acetone % Of Ext.	Methanol % of Ext.	Water % of Ext.
A_0B_0	5.33	6.9 %	6.26 %	0.46 %	1.048 %	0.1553 %	0.4034 %	0.8783 %	16.9227 %	22.5675 %
A_0B_1	5.40	6.9 %	6.34 %	0.53 %	0.978 %	0.1663 %	0.4195 %	0.8620 %	18.9487 %	20.8854 %
A_0B_2	5.32	5.2 %	6.5 %	0.35 %	1.006 %	0.2652 %	0.4108 %	0.8471 %	20.7835 %	21.1049 %
A_1B_0	5.23	5.3 %	5.18 %	0.49 %	1.578 %	0.4687 %	0.4279 %	0.7066 %	21.1955 %	22.999 %
A_1B_1	5.30	6.1 %	5.14 %	0.44 %	1.052 %	0.3153 %	0.4130 %	0.7763 %	20.9226 %	20.7279 %
A_0B_0	5.41	6.9 %	5.38 %	0.49 %	0.99 %	0.1598 %	0.4531 %	0.7677 %	19.6978 %	22.0345 %
WILD	5.50	5.4 %	6.52 %	0.36 %	0.984 %	0.4303 %	0.5033 %	0.8992 %	21.7264 %	23.6082 %

Table 5: Observations of Quantitative Examination of Inorganic Matters and TLC Profiles

Samples	% of alkaloid	n-Hexane ext. TLC R_f Value	Chl. ext. TLC R_f Value	Acetone ext. TLC R_f Value	Methanol ext. TLC R_f Value	Aqueous ext. TLC R_f Value
A_0B_0	1.13 %	0.15, 0.35, 0.53	0.15, 0.43, 0.53, 0.69, 0.76, 0.84, 0.92	0.07, 0.15, 0.21, 0.27, 0.36, 0.46, 0.61, 0.75, 0.86	0.07, 0.15, 0.21, 0.43, 0.53, 0.66, 0.76, 0.82, 0.92	0.07, 0.15, 0.21, 0.35
A_0B_1	1.28 %	0.15, 0.35, 0.53	0.15, 0.43, 0.53, 0.69, 0.76, 0.84, 0.92	0.07, 0.15, 0.21, 0.27, 0.36, 0.46, 0.61, 0.75, 0.86	0.07, 0.15, 0.21, 0.43, 0.53, 0.66, 0.76, 0.82, 0.92	0.07, 0.15, 0.21, 0.35
A_0B_2	1.13 %	0.15, 0.35, 0.53	0.15, 0.43, 0.53, 0.69, 0.76, 0.84, 0.92	0.07, 0.15, 0.21, 0.27, 0.36, 0.46, 0.61, 0.75, 0.86	0.07, 0.15, 0.21, 0.43, 0.53, 0.66, 0.76, 0.82, 0.92	0.07, 0.15, 0.21, 0.35
A_1B_o	1.03 %	0.15, 0.35, 0.53	0.15, 0.43, 0.53, 0.69, 0.76, 0.84, 0.92	0.07, 0.15, 0.21, 0.27, 0.36, 0.46, 0.61, 0.75, 0.86	0.07, 0.15, 0.21, 0.43, 0.53, 0.66, 0.76, 0.82, 0.92	0.07, 0.15, 0.21, 0.35
A_1B_1	1.17 %	0.15, 0.35, 0.53	0.15, 0.43, 0.53, 0.69, 0.76, 0.84, 0.92	0.07, 0.15, 0.21, 0.27, 0.36, 0.46, 0.61, 0.75, 0.86	0.07, 0.15, 0.21, 0.43, 0.53, 0.66, 0.76, 0.82, 0.92	0.07, 0.15, 0.21, 0.35
A_1B_2	1.22 %	0.15, 0.35, 0.53	0.15, 0.43, 0.53, 0.69, 0.76, 0.84, 0.92	0.07, 0.15, 0.21, 0.27, 0.36, 0.46, 0.61, 0.75, 0.86	0.07, 0.15, 0.21, 0.43, 0.53, 0.66, 0.76, 0.82, 0.92	0.07, 0.15, 0.21, 0.35
Wild	1.28 %	0.15, 0.35, 0.53	0.76, 0.84, 0.92	0.07, 0.15, 0.21, 0.27, 0.36, 0.46, 0.61, 0.75, 0.86	0.07, 0.15, 0.21, 0.43, 0.53, 0.66, 0.76, 0.82, 0.92	0.07, 0.15, 0.21, 0.35

The data given in Table 5 shows the total alkaloid % and TLC profile of wild and cultivated samples

DISCUSSION

When the collected wild sample of Ashwagandha observed for Yield parameters including the root length, root diameter, root fresh weight, root dry weight and seed yield which was lower than the cultivated sample. When Comparison drawn between Factor 1 (Chemical fertilizers) and Factor 2 (FYM and Vermi compost) for yield parameters revealed that the samples grown organically shows more significant result regarding yield parameters and the samples grown chemically which was at par with factor 2. But interaction of these factors had shown no significant result on yield parameter. When comparison drawn between Factor-1 (Samples grown organically), factor 2 (Samples grown chemically) and wild sample for yield parameter revealed that the samples grown organically is more superior to wild sample in yield parameter and factor 1 at par to factor 2. This is because lack of selective control and improvement of certain factors of plant growth in wild source. So it was concluded that the factor 1 and factor 2 enhances yield parameters if used alone not in combination as observed by scholar. The data presented in Table 4. Reflect the PH of wild sample having more basic nature than cultivated samples. The moisture content data reveals that there was comparatively more loss on drying in cultivated samples than in wild revealing more water content in this sample. The average total Ash value of the wild sample was higher than cultivated samples and the average acid insoluble ash and water soluble ash value of the cultivated samples are higher than wild sample. The extractive values in various solvents shows the fact that both samples contained most aqueous soluble substances followed by methanol soluble extractives. Least extractive value was observed in chloroform. The wild samples possessed high extractability in each solvent. Table 5 reveals the total alkaloid % is high in wild (1.28 % w/w) and the cultivated variety A_0B_1 (1.28 %) at par with wild. the lowest total alkaloid % is found in cultivated variety A_1B_o (1.03 %) and The T.L.C analysis represented in Table 5 revealed that maximum no. of spots (9) were observed in the acetone and methanol extract in Toluene : Ethyl acetate -10:90 and Dichloromethane: Methanol- 95:5 as mobile solution exposure to Iodine vapour and under spray treatment of Vanillin H_2SO_4 heated at 105^0c for 5 minutes. Wild sample of Ashwagandha showed same R_f value and same intensity that of cultivated Ashwagandha samples in all chromatogram. But in all solvent systems excepting chloroform extract, solvent system chloroform : methanol - 80:20, the cultivated samples registered 4 extra spots from total 7, remaining 3 spots registered same R_f values and intensity as that of wild Ashwagandha sample. Thus, confirm not said that there are some phytochemical variations between wild and cultivated samples; it may be due to incomplete separations of phytoconstituents of wild sample. Thus logically it was proved that phytochemically the wild sample of Ashwagandha enjoyed qualitative and quantitative (High PH, low moisture content, high extractive values, high alkaloid %,) superiority over cultivated samples of Ashwagandha. The above findings suggest that the cultivated sample grown with FYM and organic manures was best in yield parameters were as the wild sample was best in phytochemical parameters but the yield in wild sample was the least this presents a paradoxical situation to select the optimum source of Ashwagandha for therapeutic purposes. considering (1) The not so significant quantitative variation of phytochemicals among the wild and the organically grown ashwagandha and to the virtual possible lack of control in time of harvesting from the wild source the authors suggest Ashwagandha cultivated with FYM and organic manure is the optimal source of Ashwagandha for clinical use.

CONCLUSION

To sum of all the findings and their critical analysis the following conclusion was drawn. Cultivated source is a reality and will be the sustainable source for Ashwagandha. Among the various prevalent pop's Ashwagandha grown with FYM and organic manures seems to be the best in terms of yield criteria under geo climatic conditions of Jaipur, India. Phytochemically both the wild and cultivated Ashwagandha (grown with organic manures) appears to be the best and at par confirming the above suggestion of using the later as an optimal source of Ashwagandha.

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