Yield and Phytochemical Attributes of Ashwagandha (*Withania somnifera* Dunal) Tubers as Affected by Source of Nutrients

Ewon Kaliyadasa [1], Lalith Jayasinghe [2], and Sriyani Peiris [3]
[1] Department of Export Agriculture, Faculty of Animal Science and Export Agriculture, Uva Wellassa University, Badulla, Sri Lanka
[2] Institute of Fundamental Studies, Kandy, Sri Lanka
[3] Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka
Email: {ewon101k, ulbj2003}@yahoo.com, sepeiris@gmail.com

***Abstract*—Ashwagandha (*Withania sominifera* Dunal) is an imperative medicinal crop in herbal industry since time immemorial which roots are in commercial importance. This herb known for its ability to enhance the resistance to stresses, increases stamina and promotes general wellbeing. The changes in the plant growth and quality of the roots mainly depend on different agronomic practices coupled with stage of harvesting. Plants grown under organic and inorganic sources of nutrients were used up to twelve months of sowing for yield analysis and tubers were analyzed for its alkaloid, total fiber and withaferine A contents upto ten months. Highest values for all above phyto constituents were recorded in tubers of organically grown compared to inorganically fertilized plants except crude fiber content and at six months after sowing is the best stage for harvesting to obtain high quality tubers. This is an important study for biochemical standardization of Ashwagandha tubers in commercial processing.**

***Index Terms*—ashwagandha, withaferine A, organic fertilizer, inorganic fertilizer, fiber, alkaloid**

I. Introduction

Herbal plants are considered as one of the most important sources of medicines since the beginning of human civilization. It is estimated that at least 25% of all modern medicines are derived, either directly or indirectly, from medicinal plants, primarily through the application of modern technology to traditional knowledge. In the case of certain classes of pharmaceuticals, such as antitumoral and antimicrobial medicines, this percentage may be as high as 60% [1], [2]. Ashwagandha belongs to family Solanaceae and also known as Amukkara in Sri Lanka. It is a diverse plant which is native to India and grows throughout the drier and subtropical parts of India. Considering the environmental conditions in these areas, it is clear that dry and intermediate zone in Sri Lanka have the same potential to grow Ashwagandha in commercial scale as this is an extensively used in Ayurvedic medicinal system in Sri Lanka. Ashwagandha has the ability of improving the body capacity to maintain physical effort and helps the body to adapt to various stresses. Further Ashwagandha is specific for a wide range of conditions including arthritic inflammation, anxiety, insomnia, respiratory disorders, nervous disorders, gynecological disorders, male infertility and impotence [3]-[5].

By adopting to a proper agro technological package soil nutrient conditions, shade, moisture and plant diseases can be controlled, to ensure the optimum growth and development of Ashwagandha that helps to remain the pharmacologically active constituents unchanged. All these factors facilitate production of crude drugs of high and uniform quality. The commercial success largely depends on quality [5] and yield of roots which is the product of commerce of Ashwagandha. The standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important for this globally valuable medicinal plant especially under growing conditions in favourable areas where no published data are available. Therefore, it is a timely requirement for an investigation of commercially important phytochemical constituents of Ashwagandha.

The present study was carried out to investigate the effect of source of nutrient on growth and tuber quality in terms alkaloid, crude fiber and withaferine A and determine best harvesting stage of Ashwagandha.

II. Materials and Methods

A. Experimental Location, Methods and Design

The experiment was carried out in a polytunnel under 20% shade that located at the Uva Wellassa University, Badulla, Sri Lanka. The experimental site is situated in the Intermediate 1a agro climatic zone where the average annual rainfall is 2000-2500mm and average annual temperature is 27 ℃. Ashwagandha plant was authenticated and deposited as voucher specimen [PEK 21042013(PDA)] in the National Herbarium, Department of National Botanic Gardens at Peradeniya in Sri Lanka.

Manuscript received August 1, 2017; revised January 18, 2018.

doi: 10.18178/joaat.5.2.144-148

Two different potting media comprised with commercially available organic and inorganic fertilizers were used as treatments. The potting media with organic fertilizer (6 tons/ha; N,P,K composition of compost was 1.38,0.27, and 0.8 percent respectively) was consisted with sand, soil and compost in 1:2:1 ratio and potting media with inorganic fertilizer was with sand and soil in 1:2 ratio with Urea, TSP, MOP at a rate of 40:60:20 kg/ha [6]. The fertilizer mixtures were applied as per treatment as basal application. Basic soil used in both treatments was consisted with 1.38, 0.27 and 0.81 percent of N, P and K respectively. Seeds were sown in poly bags (30*42 cm, 150 gauge black polythene) at a rate of 2-4 seeds per hole and two hole at 10 cm spacing in a single poly bag which at 15cm in each direction . The seedlings were thinned out leaving only one plant per hole after 30 days of planting to maintain required plant population. Completely Randomized Design was used as an experimental design. The two treatments were allocated randomly using four replicates. Each replicate consists of two plants.

B. Plant Growth Analysis

Plants from each polybag designated for each month were uprooted manually from the second month of sowing (MAS) up to twelve months and pre-cleaned tubers were used to measure dry tuber weights to determine the yield attributes at two months intervals. Plant materials were cleaned and dried at 40^0C for 48 hours in an oven until constant weight.

C. Phytochemical Analysis

For chemical analysis tubers from each treatment were harvested separately in each month and bulked them from different replicates. Tubers were cleaned and dried at 40^0C for 48 hours in an oven until constant weight, ground and sieved to obtain fine powder for analysis. Total fiber contents were measured as per methodology suggested by Reference [7]. Further total alkaloid content and withaferine A contents were quantified according to Reference [8] and [9] respectively. Sample of imported Ashwagandha tubers available in Sri Lankan market was used as a standard sample to compare the values of two different treatments for each chemical component of this study.

D. Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA-one way) procedure using Minitab statistical package. The means were compared according to Tukey's test to determine the statistical significance between the treatments.

III. Results and Discussion

A. Total Dry Weight

Effect of source of nutrient is not significant for the tuber dry weight, irrespective of the month (P>0.05) during the study period (Fig. 1). Inorganic fertilizer applied plants (T2) showed the highest tuber dry weight until 10 MAS compared to Organic fertilizer applied plants (T1). Tuber yield of Ashwagandha increased with extended period of harvesting. However, at 12 MAS there was a slight reduction in tuber dry weight in both organic and inorganic treatments. The dry tuber weight per plant at 6 MAS was 2.71 g and 2.96 g in T1 and T2 plants in the current study, respectively. All these results were in agreement with the findings [10] who reported that, three of the five divergent accessions of Ashwagandha grown in India, exhibited the root yield per plant in the range of 1.0-2.8 g and 4.8-5.3 g which was harvested at 150 and 210 DAP (days after planting), respectively. As stated above the tuber weights of the present study are also in accordance with the previous findings [3].

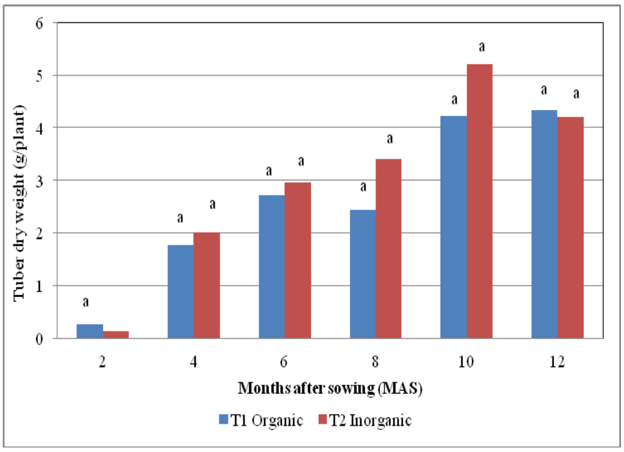

Figure 1. Variation of tuber dry weight as affected by two different nutrient sources T1: organic fertilizer; T2: inorganic fertilizer.
a,b Significantly different between two treatments (P<0.05) (n=8)

B. Total Fiber Content

Fiber content of the tubers is one of the important quality traits for marketing of this medicinal crop. Tubers with less fiber contents are mostly preferred and exploited for commercial purpose. Delayed harvesting might have resulted in higher production of fibrous contents. The variation of total fiber content in tubers was 16.03 to 68.69% (Table I) from 3 MAS to 10 MAS where the significantly lowest total fiber content was recorded in organically treated plants compared to inorganically treated plants until 8 MAS. Fiber content of tubers at 6 and 7 MAS was 36.02% and 40.07%, respectively while that in standard Ashwagandha sample was 36.84% (Table II). This suggests that fiber content of tubers at 6 MAS was comparable to that of commercially available tubers.

Further, the results of the present study are in agreement with a study who stated that crude fiber content of Ashwagandha dry tubers ranged between 22.0-34.0% and 32.0-38.7% at 150 days after planting (DAP) and 210 DAP, respectively [10]. In addition, the fiber content of tubers recorded in each harvest during the study period showed slight fluctuations in both organically and inorganically treated plants.

TABLE I. MEAN VALUES OF THE CHEMICAL PROPERTIES ANALYSED DURING THE STUDY PERIOD

Chemical property	Treat ment	Month of harvest			
		3MAS	4MAS	5MAS	6MAS
Total fiber content (%)	T1	16.03^{b}	29.04^{b}	32.25^{b}	36.02^{b}
	T2	21.96^{a}	31.21^{a}	40.76^{a}	40.78^{a}
	S.Em±	0.32	0.44	0.41	0.12
Total alkaloid (%)	T1	0.24^{a}	0.13^{b}	0.26^{a}	0.25^{a}
	T2	0.26^{a}	0.20^{a}	0.26^{a}	0.24^{a}
	S.Em±	0.001	0.001	0.002	0.001

Chemical property	Treat ment	Month of harvest			
		7MAS	8MAS	9MAS	10MAS
Total fiber content (%)	T1	40.07^{b}	40.56^{b}	47.96^{a}	68.69^{a}
	T2	41.02^{a}	45.06^{a}	44.05^{b}	67.37^{a}
	S.Em±	0.82	0.65	0.86	0.24
Total alkaloid (%)	T1	0.29^{b}	0.17^{a}	0.30^{a}	0.33^{a}
	T2	0.35^{a}	0.19^{a}	0.26^{b}	0.29^{b}
	S.Em±	0.002	0.001	0.001	0.001

T1: organic fertilizer; T2: inorganic fertilizer. Means followed by the same letter/s in a raw do not differ significantly by Tukey test (P<0.05) n=3

TABLE II. COMPARISON OF MEAN VALUES OF DIFFERENT CHEMICAL PROPERTIES OF COMMERCIALLY AVAILABLE IMPORTED ASHWAGANDHA TUBERS AND TUBERS AT 6 MAS OF THE STUDY

Chemical property	Value in percentage (%)	
	Imported tubers	Tubers at 6MAS
Total fiber	36.84	36.02
Total alkaloid	0.11	0.25
Withaferine A	0.0021	0.0023

C. *Total Alkaloid Content*

Biologically active chemical constituents in Ashwagandha are alkaloids [11] and the medicinal properties of Ashwagandha are attributed to the chemical quality *i.e.* presence of total alkaloids in their roots. Reference [12] found that the alkaloid content in Ashwagandha was greater in late harvested crops (210 days after sowing, DAS) compared to early harvested crops (90 and 150 DAS). Results of the present study are in accordance with the above findings where alkaloid content increased from 0.24 to 0.33% (Table I) in organically treated plants. In inorganically treated plants the range was 0.26-0.35% from 3 MAS to 10 MAS with greater fluctuations at each harvest. At 6 MAS alkaloid content in organically treated tubers was 0.25% and interestingly that in imported Ashwagandha sample was 0.11% (Table II). However, the alkaloid content reported in the present study was lower compared to the values reported by Reference [11] who stated the minimum alkaloid content (0.39%) in the plant roots raised with vermi-compost at 2kg/plot whereas untreated plants recorded an alkaloid content of 0.387%. Reference [10] showed that total alkaloid content exhibited positive correlation to root yield and was not found to be a yield contributing trait. No clear variation pattern of total alkaloid content could be observed in two treatments (Table I) during the study period but drastic fluctuations could be observed in organically treated plants compared inorganically treated plants except few months.

D. *Total Withaferine A Content*

Although many research data are available for the quantification of withanolide in Ashwagandha, there are no published data on withaferine A quantification in tubers. There is a possibility to draw a prediction that withanolide and withaferine A content has positive correlation since withaferine A is the one of two main withanolides in Ashwagandha. Reference [3], reported that the crop harvested at 180 DAS had significantly higher total withanolide content in roots of Ashwagandha (0.560%) compared to others harvested at 120 DAS (0.468%) and 150 DAS (0.498%). Similar observations were made by referenec [13] and reference [14] in Ashwagandha. Further they showed the higher total withanolide yield followed the similar trend as fiber and total alkaloid content that delayed harvesting resulted in higher productions. Findings of this study also agree with above results by showing the same trend in increasing withaferine A (Fig. 1), total alkaloids and fiber content (Table I) in tubers with late harvestings and almost similar withaferine A content to commercially available imported tubers (Table II).

The variation of withaferin A content was 0.0011 to 0.0089% (Fig. 2) in the period of 2 MAS to 10 MAS in the two treatments. The highest withaferin A content, 0.0089% was recorded in organically treated plants at 10 MAS during the study period.

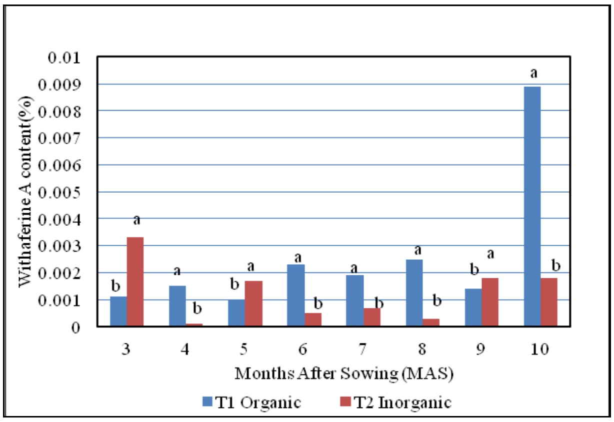

Figure 2. Variation of total withaferine A content as affected by two different nutrient sources T1: organic fertilizer; T2: inorganic fertilizer. ^{a,b} Significantly different between two treatments (P<0.05) n=3

None uniform variation pattern of withaferin A content could observe in two treatments (Fig. 2) during the study period but remarkable fluctuations could observe in inorganically treated plants compared organically treated plants. During 6, 7 and 8 MAS the variation of withaferin A content among organically treated plants were very low and almost similar values were recorded.

In this study highest percentage of withaferine A and alkaloid contents were recorded in tubers which organically treated compared to inorganically treated during most harvests particularly at the harvest of 6MAS though no significant differences between organically and inorganically treated plants for alkaloid percentage. The application of organic fertilizers created the conducive micro environment along with beneficial free-living soil bacteria such as rhizobacteria or plant growth promoting *rhizobacteria* which lives in close association of plant roots and enhance plant growth by its ability to fix atmospheric nitrogen, production of indole acetic acid, siderophore, nitrate and single molecules resulting in an increased mineral up take in the plant roots [15]. Further these beneficial microorganisms have also increased the nitrogen availability in soil through biological nitrogen fixation resulting in the promotion of cell division and synthesis of organic compounds in leaves, ultimately increasing the bio mass and root yield [16] and consequent increase in total alkaloid content in various plant parts including roots [16]. Those reasons might cause the increment of withaferine A and alkaloid contents in tubers of organically treated compared to inorganically treated during the most harvests of current study. Moreover, the application of inorganic nutrients may not significantly influence the various economic traits in contradiction due to the fact that biosynthesis of secondary metabolites is under genetic control to influence plant growth and seed yield in various responsive crops including Ashwagandha.

IV. Conclusion

Chemical composition of Ashwagndha has great influence on determining its stage of harvesting due to its commercial exploitation. With the results of this study, 6 months after sowing is the best stage for harvesting by emphasising the chemical composition that resulted highest values for withaferine A (0.0023%), alkaloid (0.25%) and lower value of fiber (36.02%) in organically treated plants over inorganically treated plants. Further higher total alkaloid content, almost similar withaferine A and lower total fiber content were recorded in tubers at 6 months after sowing compared to imported tubers available in market will be a great success in commercial exploitation.

Acknowledgment

The authors wish to thank Uva Wellassa University of Sri Lanka for the funds provided by the University Research Grant, Associate Professor. Piyal Marasinghe, Officer In-charge, Medicinal Garden, Haldunmulla, Sri Lanka who kindly donated Ashwagandha seeds and support given by Industrial Technology Institute, Colombo in chemical analysis.

References

[1] J. B. Calixto, "Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents)," *Braz. J. Med. Biol. Res.*, vol. 33, pp. 179–189. 2000.

[2] N. J. Sucher and M. C. Carles, "Genome-based approaches to the authentication of medicinal plants," *Planta Medica*, vol. 74, no. 6, pp. 603–623, 2008.

[3] V. S. Kubsad, Y. B. Palled, and C. P. Mansur, "Effect of spacing and fertilizer levels on physiological parameters in relation to productivity of ashwagandha (Withania somnifera)," *Indian. J. Agric. Sci.*, vol. 79, pp. 501-505, 2009.

[4] R. R. Kumar, L. P. A. Reddy, J. C. Subbaiah, A. N. Kumar, H. N. N. P. Kadri, and B. Bhukya, "Genetic association among root morphology, root quality and root yield in ashwagandha (Withania somnifera)," *Genetika*, vol. 43, pp. 617 -624, 2011.

[5] R. R. Kumar, L. P. A. Reddy, A. N. Kumar, K. Komaraiah, S. Purnanand, and K. P. Sastry, "Root textural quality in ashwagandha (Withania somnifera) as influenced by crop growth periods and morphotypes," *Ind. Crop. Produc.*, vol. 34, pp. 1231-1234, 2011.

[6] D. Muthumanickam and G. Balakrishnamurthy, "Optimum stage of harvest for ex traction of total Withanolides in ashwagandha (Withaniasomnifera Dunal.)," *J. Spices and Aromat. Crop.*, pp. 95-96, 1999.

[7] M. A. Joslyn, *Methods in Food Analysis*, Academic Press, 1970.

[8] J. B. Harborne, *Phytochemical Methods*, London: Chapman and Hall, 1973, pp. 49-188.

[9] N. A. Chaurasia, G. C. Uniyal, P. Lal, L. Misra, N. S. Sangwan, R. Tuli, and R. Singh, "Analysis of withanolides in root and leaf of withania somnifera by HPLC with photodiode array and evaporative light scattering detection," *Phytochem. Anal.*, vol. 19, pp. 148–154, 2008.

[10] P. K. Khanna, A. Kumar, A. Ahuja, and M. K. Kaul, "Biochemical composition of roots of Withania somnifera (L.) dunal," *Asian J. Plant Sci.*, vol. 5, pp. 1061-1063, 2006.

[11] G. Singh, P. K. Sharma, R. Dudhe, and S. Singh, "Biological activities of Withania somnifera," *Annals of Biological Res.*, vol. 1, no. 3, pp. 56-63, 2010.

[12] K. V. Patel, D. H. Patel, S. Patel, and S. Sriram, "Effect of seed rate and crop duration on root yield and quality of ashwagandha," *J. Med. Aromat. Plant Sci.*, vol. 25, pp. 54-57, 2003.

[13] L. S. Kahar, S. S. Tomar, M. A. Pathan, and K. B. Nigam, "Effect of sowing dates and variety on root yield of ashwagandha," *Indian J. Agric. Sci.*, vol. 16, no. 7, p. 495, 1991.

[14] B. R. Baraiya, G. A. Tiwari, and V. K. Sanakia, "Alkaloid concentration in different parts of growing crop of ashwagandha (Withania somnifeaa Dunal) at different growth intervals," *J. Med. Aromat. Plant Sci.*, vol. 27, pp. 439-442, 2005.

[15] Y. Bashan and G. Holguin, "Azospirillum plant relations: Environmental and physiological advances (1990-1996)," *Can. J. Microbiol.*, vol. 43, pp. 103-121, 1997.

[16] M. C. Singh, N. Kumar, and V. Kumar, "Response of bio-organic nutrition on growth, yields and quality of Ashwagandha (Withania somnifera Dunal.)," *Hortflora Res. Spectrum*, vol. 1, no. 3, pp. 208-214, 2012.

Ewon Kaliyadasa was born in Ratnapura, Sri Lanka in October 1978. She obtained PhD in Biological Sciences from Durham University, United Kingdom in 2015; M.Phil in Crop Science in Postgraduate Institute of Agriculture, University of Peradeniya, Sri Lanka in 2012; M.Sc in Crop Science in Postgraduate Institute of Agriculture, University of Peradeniya, Sri Lanka in 2007; B.Sc Agriculture (Honors) specialization in Crop Science from Faculty of Agriculture, University of Ruhuna, Sri Lanka in 2005. Her major field of study is medicinal herb production and plant stress signaling

She works as a SENIOR LECTURER (GR II) in the Faculty of Animal Science and Export Agriculture, Uva Wellassa University, Sri Lanka since September 2013.
Dr. Kaliyadasa is a member of Society of Biology (SoB), member of Society for Experimental Biology (SEB), associate member of Sri Lanka Association for Improving Higher Education Effectiveness (SLAIHEE) and Life Member of Sri Lanka Association for the Advancement of Science (SLAAS). Further obtained Associate Fellowship of the Higher Education Academy (AFHEA) in recognition of attainment against UK Professional Standards Framework for teaching and learning support in higher education. Further Awarded Commonwealth Scholarship by Commonwealth Scholarship Commission in UK to pursue a PhD at Durham University, United Kingdom in 2011.