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Tuber quality of Ashwagandha (*Withania somnifera* Duanal) affected by different growth conditions

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Abstract

Ashwagandha (*Withania sominifera* Duanal) is an important medicinal herb with increased demand after discovering its anti-stress and sex stimulating properties that are attributed to the presence of biologically active alkaloid compounds. The aim of this study was to elucidate a proper agro technological package that ensures the optimum growth of Ashwagandha to obtain the finest quality without degrading the pharmacologically active constituents. Mixtures of organic and inorganic fertilizers were combined with direct seeding and transplanted as four different treatments in this study. The fresh and dry weights of the tubers were recorded up to 12 months starting from two months after sowing (MAS) while the shoot height, root length, number of leaves, fresh and dry weights of the shoot and the root with a shoot ratio of up to 6 MAS were determined. The results revealed that the growth of Ashwagandha was not affected significantly by the method of planting, type of fertilizer or their combinations during most of the harvests. However, tubers harvested at 6 MAS had the highest recorded dry tuber weight per plant in all four treatments compared to the early harvests where two direct seeded treatments had the best results. Comparison of the phytochemical compounds showed that direct seeding with organic fertilizer had the highest recorded values for alkaloid and withaferine A contents with a lower percentage of fiber compared to the treatments with inorganic fertilizer. In conclusion, direct seeding with organic fertilizer and tubers harvested at 6 MAS are recommended as the best cultivation conditions and harvesting stage to obtain high quality tubers of Ashwagandha, respectively.

Keywords: alkaloids, direct seeding, organic fertilizer, transplanting, withaferine A

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Introduction

Ashwagandha is one of the most valuable herbs in the ayurvedic medical system dating back more than 3000 years in ancient ayurvedic literature. It holds a place in the ayurvedic traditions similar to Korean Ginseng in Chinese therapies and has therefore been often referred to as the Indian Ginseng.

Most of its biological activities have been attributed to the presence of group of compounds referred to as withanolides, one of the major alkaloids. At present, Ashwagandha is very popular among commercial herbalists after the discovery of its sex stimulating properties in males. In addition, this herb has been used in a wide range of conditions as it possesses anti-stress, antioxidant, anti-carcinogenic, anti-aging, cardio protective, hypothyroid, and immuno-modulatory activities (Singh et al., 2010).

Ashwagandha belongs to family Solanaceae and known as Amukkara in Sri Lanka. This important herb is native to India and grows throughout the dry and subtropical parts of India. Though the seeds, leaves and roots of Ashwagandha have various medicinal values the most economically important part is the root. Under Indian growing conditions Ashwagandha roots contain 0.43% alkaloids (Shamaraj et al., 2010), 12.8% starch (Kubsad et al., 2009), 35.65% fiber (Kumar et al., 2011) and minor quantity of oil with an average root yield of 1.848 g $plant^{-1}$ (Kubsad et al., 2009) at maturity. Limited cultivations of Ashwagandha and sharply risen global demand signify the necessity of a proper agro technological package for cultivation of this medicinal plant under different geographical conditions.

The commercial success of Ashwagandha basically depends on yield and quality of tubers, the commercial product. The process of growth and development of tubers and the information on the biochemically active constituents under different growing conditions are scarce. Therefore, the factors affecting its yield and tuber quality need to be studied and optimized for making Ashwagandha cultivation the most remunerative. Thus, it is a timely requirement for an investigation of scientific approach for agro technology of medicinal plants. Considering the medicinal value of the crop, its growing demand and paucity of information on scientific production technology, the present investigation was undertaken with the objective of studying the growth and tuber quality of Ashwagandha as affected by method of planting and type of fertilizer used for investigating the correct harvesting stage.

Materials and Methods

Experimental location and plant material

This study was carried out under 20% shade in a polytunnel in Badulla, Sri Lanka, the area belongs to Intermediate 1 a agro climatic zone where the average annual rainfall and temperature are 2000 - 2500 mm and 27℃, respectively. 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.

Treatments and design

Four different treatments were used in this study as direct seeding with organic fertilizer (T1), direct seeding with inorganic fertilizer (T2), transplanting with organic fertilizer (T3), and transplanting with inorganic fertilizer (T4). The potting media with organic fertilizer (6 tons ha^{-1}) was consisted of sand, soil, and compost (N, P, K composition of compost was 1.38, 0.27, and 0.8 percent, respectively) in 1 : 2 : 1 ratio and potting media with inorganic fertilizer was comprised of sand and soil in 1 : 2 ratio with Urea, Triple Superphosphate, and Murate of Potash at a rate of 40 : 60 : 20 kg ha^{-1} (Muthumanickam and Balakrishnamurthy, 1999). The fertilizer mixtures were applied as per treatment as basal application. The soil used in both treatments was consisted of 1.38, 0.27, and 0.81 percent of nitrogen, phosphorous, and potassium, respectively. In direct seeded treatments, seeds were sown in grow bags at a rate of 2 - 4 seeds per hole and two holes were prepared at a 10 cm spacing in a single polybag. The additional seedlings were thinned out leaving only one plant per hole after 30 days of planting to maintain required plant population. Healthy and vigorous, 30-day-old, seedlings maintained in a nursery (topsoil and sand in 2 : 1 ratio as nursery mixture) were established, without damaging the root system, in respective polybags for transplanted treatments.

Completely randomized design (with two factors) was used as the experimental design. The four treatments were allocated randomly with four replicates. Each replicate consisted of two plants.

Harvesting and sample processing

The plants from each polybag designated for each month were uprooted manually from the second month after sowing (MAS)/transplanting up to 12 months and pre-cleaned tubers were used to record fresh and dry tuber weights. Plant height, root length, number of leaves per plant, and fresh and dry weights of shoots were recorded monthly from 2 MAS to determine the growth performances.

Screening for availability of withaferine A

Tubers at 6 MAS from T1 and T2 that recorded the highest tuber dry yields in this study were used to compare the availability of withaferine A with standard withaferine A sample using thin layer chromatography (TLC). One gram (1 g) of sample was stirred over night with 10 mL of 50% aqueous methanol using a magnetic stirrer (BenchMixer) and then centrifuged to obtain aqueous methanol extract. Then, 3 mL of the above extract was partitioned with equal volume of chloroform for three times to obtain the chloroform extract. The solvent was evaporated and the residue was dissolved in methanol for TLC analysis. Methanol extract was spotted on TLC plates (Silica gel GF_{254} precoated) and allowed to run with hexane: ethyl acetate: methanol in a ratio of 2 : 14 : 1. TLC plates were sprayed with vanilline sulphuric acid, heated at 110℃ for 3 minutes and observed under UV light.

Phyto-chemical analysis

Considering the highest dry weight of tuber recorded in this study during the early harvests, tubers from T1 and T2 at 6 MAS were used for chemical analysis after bulking them from different replicates and values were compared with those of imported Ashwagandha tuber samples available in the market as a standard sample. Tubers were cleaned and dried at 40℃ for 48 hours in a dry oven (JSOF-150, JS Research Inc., Korea) until a constant weight was reached. Tuber samples were then ground and sieved to obtain a fine powder for analysis. Total fiber content was analysed as per the method of Maynard (1970). Further, total alkaloid and withaferine A contents were quantified according to the methods described by Harborne (1976) and Chaurasia et al. (2008), respectively.

Statistical analysis

Data were analyzed using one way and two-way analysis of variance (ANOVA) procedures for phytochemicals and growth parameters using Minitab 17 statistical package, respectively. The means were compared using Tukey's test to determine the statistical significance between the treatments.

Results and Discussion

Information on the process of root growth and development, and phytochemical constituents of Ashwagandha is scarce but studies covering these areas are vital to encourage commercial cultivations. Hence, this study is an attempt to discuss the significant findings that enable to enhance the production of a globally important high demanding medicinal herb utilized in new product development in herbal industry.

The establishment of optimum plant population per unit area is the most important parameter to realize the maximum tuber yield of Ashwagandha. Therefore, it is a requisite to study the time and methods of planting to maintain the optimum plant stand (plant density) as both factors are important for boosting yield and quality of dry tubers. Moreover, the tuber quality is governed by alkaloid and starch contents of tubers, which could be achieved by manipulating time of harvest. Generally, Ashwagandha does not require any fertilizer and previous experiments have shown that indigenous cultivars did not respond to fertilizer application. However, several recent studies conducted in different regions of India indicated a positive response of Ashwagandha to nitrogen applications in terms of quality and quantity of its yield (Muthumanickam and Balakrishnamurthy, 1999; Patel et al., 2003; Kubsad et al., 2009). Due to the increased demand for organic and safe products in the market and formulation of various *Ayurvedic* and *Unani* medicines from Ashwagandha tubers, leaves and seeds, it is sensible to cultivate this crop with the application of organic fertilizers. However, considering the need to enhance the production of dry tubers with trade requirements, different agronomic practices that directly affect the final production also need to be attempted.

Tuber fresh weight

Mean values of tuber fresh weight as affected by method of planting and type of fertilizer over the study period are presented in Table 1. Remarkably, main effects or interaction effects did not significantly affect fresh weight of Ashwagandha tubers during the 12-month period ($p > 0.05$) except in 3, 5, and 11 MAS. At 3, 5, and 11 MAS, a significantly higher tuber fresh weight was obtained in direct seeded plants compared to transplanted plants ($p < 0.05$). Further, out of direct seeded treatments, inorganically grown plants showed the highest tuber fresh weight in many harvests during the study period.

Table 1. Mean values of the tuber fresh weight (g $plant^{-1}$) of Ashwagandha as affected by method of planting and source of fertilizer during the study period (n = 8).

Treatments	Tuber fresh weight (g $plant^{-1}$)										
	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS	11 MAS	12 MAS
Direct seeding (DS)	1.08a	3.53a	6.00a	9.43a	7.59a	7.30a	8.58a	10.58a	16.00a	11.85a	15.51a
Transplanting (TP)	0.52a	1.79b	5.37a	5.50b	6.47a	5.84a	5.71a	9.61a	7.69a	5.08b	20.74a
Pooled SEM ±	0.25	0.37	0.42	0.55	0.85	1.07	1.39	2.04	3.87	2.37	4.16
Organic (O)	0.97a	2.98a	5.29a	7.11a	7.14a	6.21a	7.18a	9.41a	11.83a	11.37a	18.59a
Inorganic (IO)	0.64a	2.34a	6.10a	7.82a	6.91a	6.93a	7.11a	10.76a	11.86a	9.57a	17.67a
Pooled SEM ±	0.26	0.48	0.42	0.91	0.87	1.09	1.49	2.04	4.18	3.11	4.27
DS*O (T1)	1.11a	2.54b	5.27a	9.16ab	7.72a	6.64a	6.84a	8.17a	15.43a	16.48a	15.73a
DS*IO (T2)	1.06a	4.51a	6.73a	9.69a	7.45a	7.96a	10.31a	12.99a	16.57a	15.23a	15.30a
TP*O (T3)	0.82a	2.14b	5.31a	5.05c	6.56a	5.76a	7.52a	10.67a	8.22a	6.27a	21.44a
TP*IO (T4)	0.22a	1.44b	5.43a	5.95bc	6.38a	5.90a	3.90a	8.58a	7.15a	3.90a	20.05a
Pooled SEM ±	0.07	0.26	0.41	0.57	0.91	1.14	1.31	2.07	4.18	2.54	4.49

MAS, months after sowing.
a, b: Means followed by the same letters in a column do not differ significantly by Tukey's test ($p < 0.05$).

Tuber dry weight

Similar to tuber fresh weight, interaction effects were not significant on tuber dry weight ($p > 0.05$). However, the method of planting had a significant effect on tuber dry weight at 2, 3, 5, and 11 MAS ($p < 0.05$; Table 2). Similar to the tuber fresh weight, the highest tuber dry weight was reported in plants with direct seeded treatments up to 11 MAS with significant values at 2, 3, 5, and 11 MAS. However, there was no significant influence of type of fertilizer used on tuber dry weight ($p > 0.05$) during total period of this study.

Tuber dry weight is the most important parameter that determines the economic returns to farmers. However, no attention is generally given to the chemical composition of tubers at harvesting stage. The balance between the yield and chemical composition at harvesting stage would result the maximum benefits to farmers as well as the commercial processors. In the present study, none of the interaction effects between the method of planting and type of fertilizer used was significant on tuber dry weight, irrespective of the harvesting period. Tuber dry weights in direct seeded treatments (T1 and T2), were higher compared to transplanted treatments (T3 and T4) at each harvest, and tuber yield of Ashwagandha increased with extended period of harvesting. The Tubers harvested at 6 MAS recorded the highest root yield compared to early harvestings. The tuber dry weight at 6 MAS was 2.71 g and 2.96 g in T1 and T2 plants in the current study, respectively and the values are in agreement with the findings of Khanna et al. (2006) who reported that three of the five divergent accessions of Ashwagandha grown in India, exhibited the tuber yield in the range of 1.0 - 2.8 g and 4.8 - 5.3 g at 150 and 210 days after planting (DAP), respectively. This suggests that performances of Ashwagandha grown under Sri Lankan conditions are comparable to that grown under Indian conditions with respect to tuber dry yield.

Shoot height

Shoot height was highly influenced by treatment combinations of method of planting and type of fertilizer used during early harvests until 3 MAS ($p < 0.05$) but not significantly different after 4 MAS (Table 3). All four treatments showed a gradual increment in shoot height over the data recorded period. The combination of direct seeding and inorganic fertilizer

Table 2. Mean values of the tuber dry weight (g plant^{-1}) of Ashwagandha as affected by method of planting and source of fertilizer during the study period (n = 8).

Treatments	Tuber fresh weight (g plant^{-1})										
	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS	10 MAS	11 MAS	12 MAS
Direct seeding (DS)	0.20a	0.80a	1.90a	2.66a	2.83a	3.05a	2.99a	3.45a	4.81a	5.54a	4.29a
Transplanting (TP)	0.04b	0.43b	1.62a	1.67b	2.28a	2.48a	1.98a	3.12a	2.33a	1.91b	6.95a
Pooled SEM ±	0.03	0.09	0.19	0.21	0.49	0.45	0.45	0.57	1.21	0.84	1.52
Organic (O)	0.17a	0.70a	1.71a	2.19a	2.66a	2.63a	2.44a	3.11a	3.59a	4.12a	5.74a
Inorganic (IO)	0.08a	0.53a	1.81a	2.15a	2.45a	2.90a	2.47a	3.46a	3.56a	3.34a	5.48a
Pooled SEM ±	0.05	0.11	0.19	0.28	0.49	0.46	0.49	0.57	1.30	1.08	1.60
DS*O (T1)	0.27a	0.85a	1.78a	2.61a	2.71a	2.87a	2.44a	2.68a	4.23a	5.82a	4.33a
DS*IO (T2)	0.13a	0.75a	2.02a	2.71a	2.96a	3.23a	3.40a	4.21a	5.20a	5.23a	4.21a
TP*O (T3)	0.06a	0.55a	1.63a	1.68a	2.62a	2.38a	2.44a	3.53a	2.74a	2.37a	7.14a
TP*IO (T4)	0.03a	0.30a	1.60a	1.67a	1.94a	2.58a	1.52a	2.71a	1.92a	1.45a	6.76a
Pooled SEM ±	0.02	0.09	0.20	0.22	0.51	0.48	0.45	0.56	1.30	0.90	1.64

MAS, Months after sowing.
a, b: Means followed by the same letters in a column do not differ significantly by Tukey's test ($p < 0.05$).

(T2) had the highest shoot height until 4 MAS and then highest shoot height was reported in direct seeded and organically grown plants (T1; Table 3).

Root length

The treatment combination had no significant effect on root length up to 6 MAS (Table 3). At 2 MAS, T2 produced the highest root length among the combinations and significantly different with compared to T4 (Table 3). The type of fertilizer used had no significant effect on root length during the study period. When the method of planting is considered, the direct seeded plants showed significantly higher root length at 2 and 4 MAS compared to transplanted plants. Irrespective of different treatments used, a gradual increment in root length was observed till 4 MAS followed by a reduction in root length at 5 MAS and then an almost constant at the end of data recorded period.

Number of leaves

Table 4 indicates a similar trend as in root length for the number of leaves, where no significant difference between treatment combinations can be observed after 2 MAS. However, each treatment showed the highest number of leaves per plant at 4 MAS and thereafter it decreased. This suggests that the plants undergo senescence of leaves due to mutual shading of lower leaves with the growth. At 5 MAS onwards, all treatments recorded an almost constant number of leaves per plant. The type of fertilizer significantly influenced on this parameter at 4, 5, and 6 MAS where the highest number of leaves per plant was recorded in inorganically grown plants compared to its counterpart. In contrast, method of planting had no significant effect on number of leaves per plant during the study period, except at 2 MAS where direct seeded plants showed the highest value.

Shoot fresh weight

Treatment combinations had significant effects on fresh shoot weight during first 3 MAS. Among four treatments direct seeded plants recorded the significantly highest fresh shoot weight compared to transplanted plants up to 3 MAS, irrespective

Table 3. Mean values of shoot height (cm) and root length (cm) of Ashwagandha as affected by method of planting and source of fertilizer during the study period (n = 8).

Treatments	Shoot height (cm)					Root length (cm)				
	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
Direct seeding (DS)	18.31a	42.42a	64.50a	83.63a	104.38a	11.42a	14.77a	18.88a	15.88a	16.44a
Transplanting (TP)	7.56b	28.43b	66.25a	75.88b	88.25b	7.19b	13.74a	16.00b	14.13a	15.29a
Pooled SEM ±	1.26	3.17	2.47	2.47	3.06	0.92	0.68	0.86	0.73	0.69
Organic (O)	13.20a	32.99a	62.88a	80.50a	95.63a	9.43a	14.16a	17.00a	14.13a	15.07a
Inorganic (IO)	12.68a	37.85a	67.88a	79.00a	97.00a	9.18a	14.35a	17.88a	15.88a	16.66a
Pooled SEM ±	2.39	4.00	2.31	2.86	4.31	1.22	0.71	1.00	0.73	0.65
DS*O (T1)	17.38a	36.45ab	60.00a	85.50a	104.50a	10.60ab	13.95a	18.25a	15.00a	15.63a
DS*IO (T2)	19.25a	48.38a	69.00a	81.75a	104.25a	12.23a	15.58a	19.50a	16.75a	17.25a
TP*O (T3)	9.03b	29.53b	65.75a	75.50a	86.75a	8.25ab	14.36a	15.75a	13.25a	14.50a
TP*IO (T4)	6.10b	27.33b	66.75a	76.25a	89.75a	6.13b	13.13a	16.25a	15.00a	16.08a
Pooled SEM ±	1.27	2.94	2.33	2.61	3.28	0.91	0.68	0.91	0.70	0.66

MAS, Months after sowing.
a, b: Means followed by the same letters in a column do not differ significantly by Tukey's test ($p < 0.05$).

of the type of fertilizer used ($p < 0.05$). Moreover, at 6 MAS, type of fertilizer used had a significant effect on fresh shoot weight; inorganically grown plants recorded the highest weight compared to organically grown plants ($p < 0.05$; Table 4).

Shoot dry weight

Mean values of shoot dry weight as affected by the method of planting and type of fertilizer used are presented in Table 5. The interaction effect was significant on shoot dry weight at 2 and 6 MAS ($p < 0.05$). Main effect of method of planting had a significant effect on shoot dry weight throughout the study period where direct seeding had the highest shoot dry weight. Type of fertilizer used had a substantial effect on shoot dry weight at 6 MAS.

Table 4. Mean values of number of leaves and shoot fresh weight (g plant^{-1}) of Ashwagandha as affected by method of planting and source of fertilizer during the study period (n = 8).

Treatments	Number of leaves per plant					Shoot fresh weight (g plant^{-1})				
	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
Direct seeding (DS)	11.63a	21.13a	50.38a	29.13a	28.63a	8.54a	17.11a	29.68a	62.86a	100.36a
Transplanting (TP)	8.13b	19.25a	53.88a	32.50a	32.00a	2.18b	10.21b	32.98a	65.24a	98.14a
Pooled SEM ±	0.87	1.17	4.15	3.90	3.69	0.78	1.42	2.70	3.48	2.71
Organic (O)	9.63a	19.50a	45.50b	24.88b	24.63b	5.13a	12.50a	30.64a	60.34a	95.15b
Inorganic (IO)	10.13a	20.88a	58.75a	36.70a	36.00a	5.59a	14.82a	32.01a	67.76a	103.35a
Pooled SEM ±	1.09	1.19	3.37	3.25	3.06	1.43	1.88	2.75	3.22	2.26
DS*O (T1)	10.50ab	19.00a	42.00a	23.50a	23.00a	7.33a	13.34b	24.82a	54.46a	96.43a
DS*IO (T2)	12.75a	23.25a	58.75a	34.75a	34.25a	9.74a	20.87a	34.54a	71.26a	104.30a
TP*O (T3)	8.75ab	20.00a	49.00a	26.25a	26.25a	2.93b	11.67b	36.46a	66.23a	93.88a
TP*IO (T4)	7.50b	18.50a	58.75a	38.75a	37.75a	1.43b	8.76b	29.49a	64.25a	102.40a
Pooled SE M ±	0.86	1.08	3.50	3.44	3.23	0.73	1.00	2.34	2.86	2.40

MAS, Months after sowing.
a, b: Means followed by the same letters in a column do not differ significantly by Tukey's test ($p < 0.05$).

Table 5. Mean values of shoot dry weight (g plant^{-1}) and shoot: root ratio of Ashwagandha as affected by method of planting and source of fertilizer during the study period (n = 8).

Treatments	Shoot dry weight (g plant^{-1})					Shoot : root ratio				
	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
Direct seeding (DS)	0.84a	21.13a	3.17a	4.45a	5.99a	0.22a	0.34a	0.46a	0.46a	0.40a
Transplanting (TP)	0.26b	19.25a	1.62b	4.25a	4.65b	0.19 a	0.27a	0.38a	0.43a	0.39a
Pooled SEM ±	0.11	1.17	0.44	0.31	0.42	0.04	0.06	0.02	0.08	0.06
Organic (O)	0.63a	19.50a	2.49a	4.16a	5.22a	0.21a	0.34a	0.43a	0.43a	0.41a
Inorganic (IO)	0.47a	20.88a	2.30a	4.53a	5.43a	0.19a	0.28a	0.41a	0.46a	0.38a
Pooled SEM ±	0.15	1.19	0.53	0.31	0.49	0.04	0.06	0.03	0.08	0.06
DS*O (T1)	0.89a	19.00a	2.88a	3.78a	5.89a	0.26a	0.37a	0.48a	0.46a	0.38a
DS*IO (T2)	0.78ab	23.25a	3.46a	4.73a	6.09a	0.17a	0.31a	0.44a	0.46a	0.41a
TP*O (T3)	0.35ab	20.00a	2.09a	4.55a	4.55a	0.17a	0.30a	0.39a	0.39a	0.42a
TP*IO (T4)	0.17b	18.50a	1.14a	4.34a	4.77a	0.21a	0.25a	0.38a	0.47a	0.35a
Pooled SEM ±	0.12	1.08	0.45	0.31	0.46	0.04	0.06	0.03	0.08	0.07

MAS, Months after sowing.
a, b: Means followed by the same letters in a column do not differ significantly by Tukey's test ($p < 0.05$).

Shoot : root ratio

Interestingly, none of the interaction effects or main effects were not significantly affect on shoot : root ratio during the study period (Table 5; $p < 0.05$). Increased shoot : root ratio was observed up to 4 MAS, followed by gradual reduction and then constant ratio.

Results of the present study revealed that there is no significant effect of either main effects (method of planting and source of fertilizer) or their interaction effects on growth performances of Ashwagandha during most of the months. Growth parameters such as shoot height, root length, number of leaves per plant, shoot dry weight and tuber dry weight were significantly different at 2 MAS between direct seeded and transplanted treatments but not affected by type of fertilizer used. The reason might be the transplant shock that led to poor performances in transplanted plants compared to direct seeded plants and with time no such significant difference could be observed in many of the treatments. Researches conducted with different agronomic practices reported that six months after planting is the best stage to harvest Ashwagandha (Kubsad et al., 2009; Shamaraj et al., 2010). At 6 MAS the highest root length (17.25 cm), fresh shoot weight (104.30 g $plant^{-1}$), dry shoot weight (7.80 g $plant^{-1}$) and tuber dry weight (2.96 g $plant^{-1}$) were recorded in T2 plants whereas shoot height (104.5 cm) and tuber fresh weight (7.72 g $plant^{-1}$) were highest in T1 plants. The highest number of leaves per plant (37.75) and the highest root: shoot ratio (0.42) were reported in T4 and T3 plants, respectively. The second highest values for above parameters were observed in either T1 or T2 depends on their first highest response. Shamaraj et al. (2010) stated that root length (14.04 cm), root shoot ratio (0.103), fresh tuber weight (27.94 g $plant^{-1}$) and dry tuber weight (6.20 g $plant^{-1}$) were at maximum at maturity and depending on the date of sowing in the year these values could range between 10.69 - 14.96 cm, 0.064 - 0.117, 20.61 - 27.84 g $plant^{-1}$ and 4.29 - 5.58 g $plant^{-1}$ for the above parameters, respectively. Kubsad et al. (2009) reported the highest values for root length (39.0 cm), plant height (65.8 cm) fresh tuber weight (8.295 g $plant^{-1}$) and dry tuber weight (2.720 g $plant^{-1}$ for Ashwagandha at 180 days after sowing (DAS). The result of the present study is in agreement with most of the findings of Kubsad et al. (2009) but not with Shamaraj et al. (2010).

Availability of withaferine A in Ashwagandha tubers

TLC fingerprints for two selected treatments, T1 and T2 were used up to 11 MAS months period against a standard sample of withaferine A. This comparison clearly confirmed the availability of withaferine A, in both T1 and T2 samples except in 3 MAS with different intensities of its colour (Fig. 1). Further, TLC fingerprinting revealed the presence of a fairly high content of volatile constituents in both treatments in addition to an identical spot as that of standard withaferine A.

Phytochemical comparison of Ashwagandha tubers

Tubers from T1 and T2 at 6 MAS were analysed and compared with commercially available imported Ashwagandha sample for its chemical properties (Table 6). The lowest total fiber content and the highest withaferine A content were recorded in T1 compared to T2 and the values were significantly similar to those of commercial sample ($p < 0.05$; Table 6). Interestingly, similar alkaloid contents were detected in T1 and T2 which were significantly higher than those of commercial tubers (Table 6).

Biologically active chemical constituents in Ashwagandha are alkaloids (Singh et al., 2010) and the medicinal properties of its roots are attributed to the chemical quality *i.e.* presence of total alkaloids in tubers. Tuber quality of Ashwagandha in terms of alkaloid, moisture and fiber contents is governed by the stage of the crop growth starting from flower initiation to physiological maturity (Shamaraj et al., 2010). Patel et al. (2003) found that the alkaloid content in Ashwagandha was higher in late harvested crop (210 DAS) compared to early harvested crop (90 and 150 DAS). Results of this study revealed that at 6 MAS, alkaloid content of tubers from organically grown plants was higher (0.26%) than that from inorganically grown plants (0.24%). Interestingly alkaloid content in standard Ashwagandha sample was 0.13%. However, the alkaloid content of tubers observed in present study was lower compared to the values reported by Kumar et al. (2012) who detected a minimum alkaloid content of 0.39% in tubers from plants raised with vermi-compost at 2 kg plot^{-1} whereas untreated plant

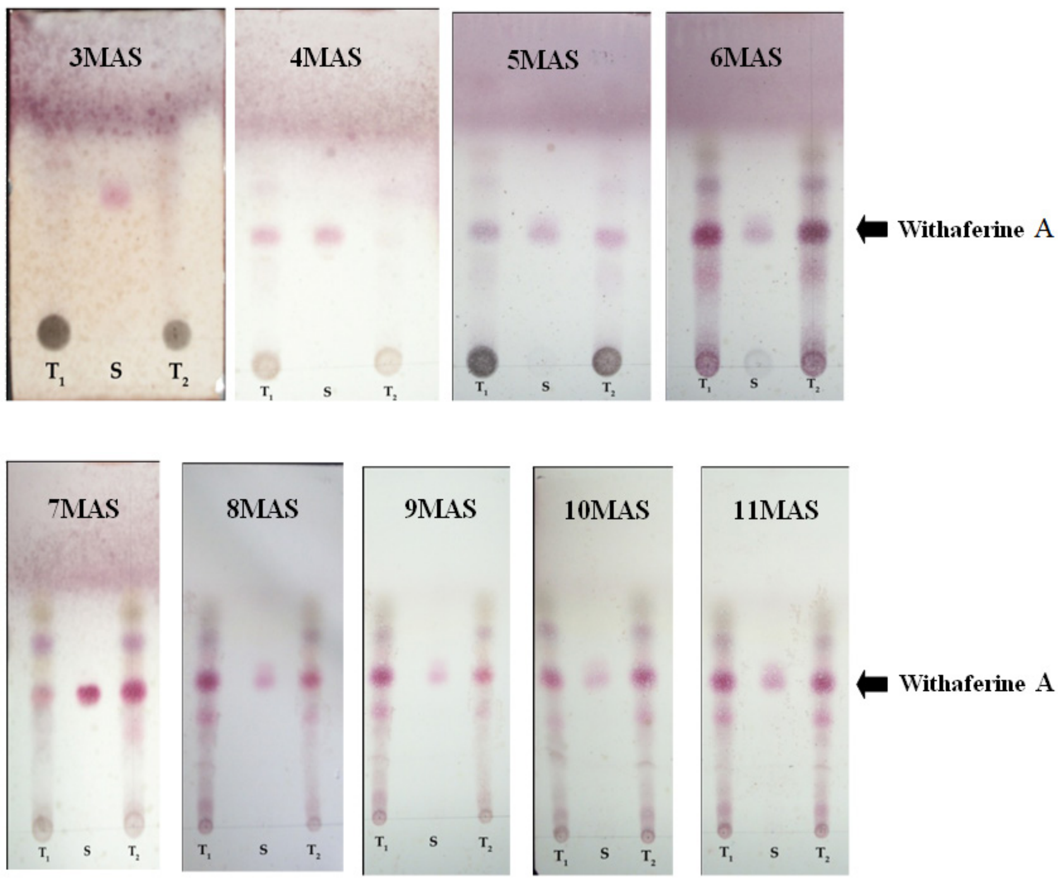

Fig. 1. Comparison of thin layer chromatography (TLC) plates in different months after sowing (MAS) for the availability of withaferin A in selected two treatments with a standard withaferin A sample. T1: direct seeding, organic fertilizer (DS*O); T2: direct seeding, inorganic fertilizer (DS*IO); S: standard withaferine A; TLC eluent: hexane : ethyl acetate : methanol in a ratio of 2 : 14 : 1 was the solvent system; Detect reagent: Vanilline sulphuric acid.

Table 6. Mean values of total fiber, alkaloid and withaferine A contents (%) of tubers from direct seeded Ashwagandha as affected by source of fertilizer at 6 months after sowing.

Sample	Total fiber (%)	Total alkaloid (%)	Withaferine A (%)
Direct seeded organically grown (T1)	36.21a	0.26a	0.0025a
Direct seeded inorganically grown (T2)	40.97b	0.24a	0.0007b
Commercial sample	36.98a	0.13b	0.0021a
Pooled SEM ±	0.12	0.001	0.0001

a, b: Means followed by the same letters in a column do not differ significantly by Tukey's test ($p < 0.05$).

recorded an alkaloid content of 0.387% in their tubers. Shamaraj et al. (2010) also reported 0.43% alkaloids in Ashwagandha tubers used in their study.

Chemical composition in terms of active ingredients of Ashwagandha has a great influence on determining its stage of harvesting due to its commercial exploitation along with the total tuber dry matter content. Analysis of withaferine A content of tubers has immense importance in comparing other chemical properties to decide the best harvesting stage under different growing conditions. Withaferine A content in tubers from organically grown plants was significantly higher compared to that from inorganically grown plants at 6 MAS of the present study and this value is not significantly different to that of commercially available tubers.

Fiber content of the tubers is one of the important quality traits for marketing of this medicinal crop. Tubers with less fiber content are mostly preferred and exploited for commercial purpose. Delayed harvesting might have resulted in higher fiber content in tubers. Total fiber content of tubers at 6 MAS was 36.21% in organically grown plants and it was comparable to that in commercially available Ashwagandha samples (36.98%). However, the same parameter in inorganically grown plants was significantly higher (40.97%) compared with commercial sample and organically grown plants. Further, the values detected in this study were in the range reported by Khanna et al. (2006); 22.0 - 34.0% and 32.0 - 38.7% total fiber contents when analyzed at 150 and 210 DAP, respectively.

Conclusion

In view of both growth performances and basic chemical compositions of Ashwagandha, 6 MAS could be recommended as the best stage of harvesting under commercial cultivation. In addition, direct seeding and organic fertilizer can be recommended as the best method of planting and the best fertilizer to be used, respectively. Tubers from direct seeded, organically grown plants produced the highest contents of withaferine A (0.0025%), and alkaloids (0.26%) and a lower content of fiber (36.02%) than those from direct seeded inorganically grown plants at 6 MAS.

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