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SHORT COMMUNICATION

EFFECT OF NITROGEN AND FARM YARD MANURE ON PHYSIOLOGICAL PARAMETERS IN ASHWAGANDHA (*WITHANIA SOMNIFERA*) UNDER VERTISOL SOIL TYPE

AJAY*, P. RAMESH, K. SAMMI REDDY, S. RAMANA AND B. MAJI

Division of Environmental Soil Science, Indian Institute of Soil Science, Nabibagh, Bhopal – 462038, M.P.

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An experiment was conducted to study the effect of nitrogen and farm yard manure (FYM) on some physiological, biochemical parameters and quality of root of medicinal plant ashwagandha at pre-flowering and post-flowering stages. Significant differences in pigment content (chlorophyll and carotenoids) were observed in both pre and post-flowering stages. The total phenol and ortho-dihydric phenol content decreased with the application of nitrogen in pre-flowering stage, but increased with the application of nitrogen at post-flowering stage. The activity of polyphenol oxidase(PPO) showed an increasing trend with the application of nitrogen in pre-flowering stage and mixed trend in post-flowering stage. The highest grade of roots B, C and D were formed at all applied nitrogen levels. The quality of root based on the alkaloid content was found to be better at low nitrogen level (N_0 and N_{20}) and FYM. However, the root yield was maximum 20 kg N ha^{-1}.

Key words: **Alkaloids, ashwagandha, photosynthesis, root grade.**

In Madhya Pradesh more than 80% area is rain fed and the productivity of the traditional crops is at stake due to weather vagaries and mono cropping. Cultivation of medicinal crops in a cropping sequence may help in sustaining the productivity and the profitability of the farmers. Ashwagandha is an important medicinal plant cultivated in North Western Madhya Pradesh (Nigam and Kandalkar 1991). The roots of Ashwagandha and occasionally its leaves and seed are used in ayurvedic and unani medicines. The pharmacological activity of the roots is attributed to the presence of several alkaloids and withaniols, which is effected due to nutritional status of the soil (Maheshwari *et al* 2000). Garcia-Mateos *et. al.* (1996) reported positive correlation between nitrogen level and biochemical fractions including alkaloids. Therefore, an experiment was conducted to study the effect of different doses of nitrogen and FYM on some basic physiological and biochemical aspects and to determine the optimum N requirement for *ashwagandha* in vertisol.

The field experiment was conducted at Indian Institute of Soil Science, Bhopal during kharif 2002 with 4 levels of nitrogen (0, 20, 40 and 60 kg/ha in the form of urea) and 1 level of FYM (10 t/ha). The entire doses of nitrogen were applied at 30 days after transplanting and the FYM was incorporated in the field before ploughing. The treatments were replicated thrice in randomized block design. The general characteristics of the soil were: pH (1:2 soil: water 7.9, EC 0.52 dSm^{-1}, organic carbon 4.1 g kg h^{-1}). The soil was clayey in texture and classified as Typic Haplustert. The crop was raised by following the standard agconomic practices.

The content of pigments (chlorophyll and carotene), total phenols, alkaloids and activity of polyphenol oxidase (PPO) were estimated in the leaves at pre-flowering and post-flowering stages. The pigment contents in fresh leaves were analysed by adopting the non-macerating procedure described by Hiscox and Israelstam (1979).

* Corresponding author

Total phenol content in leaves was determined as per procedure given by Yubedee and Arinze (1994). The activity of polyphenol oxidase (PPO) was assayed following the procedures given by Chance and Maehly (1955). The observations on photosynthesis and respiration were taken with IRGA (CID-510) at both pre and post-flowering stage.

The crop was harvested at physiological maturity (approx. 140 days after transplanting) and the roots were collected and sun-dried. The grading of the root was done based on its length. Later, the root was powdered and the alkaloid content was determined by acid titration (Mishra 1998) and the data was analyzed statistically (Gomez and Gomez 1984).

The effect of different levels of nitrogen and FYM on pigment content is given in Table 1. Significant differences were observed in the pigment contents at both pre-flowering and post-flowering stages. The content of chlorophyll *a*, chlorophyll *b* and total chlorophyll was higher in the pre-flowering stage. However, the total carotene content increased in the post-flowering stage. Among the different levels of nitrogen N_{20} recorded the highest chl a in the pre-flowering stage, whereas, N_{60} and FYM recorded the highest chlorophyll content during post-flowering stage. N_{40} and N_{60} at pre-flowering stage and N_{60} and FYM at the post-flowering recorded the highest carotene content. At lower nutrient levels the pigment content was maintained. At later stages, the chlorophyll content declined significantly.

Table 1. Pigment content (mg g^{-1} fw) photosynthesis (µmol $m^{-2}s^{-1}$) and respiration (µmol $m^{-2}s^{-1}$) rates in the leaves of ashwagandha at pre-flowering and post-flowering stages as affected by different levels of nitrogen and FYM.

Treatment	Chlorophyll a	Chlorophyll b	Carotene	Total Chl	Photosynthetic rate	Respiration
			Pre-flowering stage			
N_0	0.56	0.26	0.19	0.83	12.47	6.18
N_{20}	0.63	0.26	0.19	0.89	15.73	6.22
N_{40}	0.62	0.26	0.21	0.90	15.54	6.33
N_{60}	0.62	0.26	0.21	0.91	16.36	6.91
$FYM_{10t\ ha\text{-}1}$	0.59	0.23	0.19	0.82	18.69	5.64
CD at 5%	0.03	0.01	0.01	0.03	1.66	0.54
Average	0.60	0.25	0.20	0.87	15.75	6.26
			Post-flowering stage			
N_0	0.36	0.19	0.23	0.56	8.67	6.28
N_{20}	0.39	0.19	0.23	0.59	9.62	7.66
N_{40}	0.39	0.21	0.26	0.60	10.45	7.50
N_{60}	0.42	0.21	0.28	0.63	12.57	8.55
$FYM_{10t\ ha\text{-}1}$	0.42	0.19	0.27	0.62	10.14	8.77
CD at 5%	0.02	0.01	0.02	0.02	1.75	0.74
Average	0.40	0.20	0.25	0.60	10.29	7.75

However, carotene increased, may be due to senescence effects (Rosenthal and Camm 1996, Suzuki and Shioi 2004).

There were significant changes in physiological parameters as shown in the Table 2. The photosynthetic rate increased with the increased levels of nitrogen at both the pre-flowering and post-flowering stages. Higher rate of photosynthesis was observed at pre-flowering stage compared to post-flowering stage. FYM recorded higher photosynthesis over control. Rate of respiration also increased with the increase in the level of nitrogen. However, the higher rate of respiration was observed in the post-flowering stage compared to pre-flowering stage (Table 2). The photosynthetic rate in N_{60} treatment was higher by 34% over control, but at the same time the rate of respiration was also higher by 11%, indicating balance in the favour of photosynthesis. Higher balance in favour of photosynthesis normally results in higher total biomass productivity, provided other metabolic functions occur at higher rate. The rate of respiration was more in stress (N_0) as compared to higher levels of nitrogen. This whole process of respiration vs photosynthesis in relation to carbon balance has also been discussed by Amthor (1994).

Table 2. Biochemical constituents of root and activities of polyphenol oxidase (PPO) in the leaves of ashwagandha as affected by different levels of nitrogen and FYM at pre-flowering and post-flowering stage.

Treatment	Phenol content (µg/g DW)	Ortho-dihydric phenol (µg/g DW)	PPO (Unit/g fw)
Pre-flowering stage			
N_0	0.39	0.005	47.40
N_{20}	0.39	0.006	46.70
N_{40}	0.34	0.005	52.23
N_{60}	0.33	0.003	55.90
FYM_{10t} ha^{-1}	0.38	0.007	54.70
Average	0.37	0.005	51.39
CD at 5%	0.01	0.0004	1.24
Post-flowering stage			
N_0	0.45	0.007	59.67
N_{20}	0.48	0.009	56.53
N_{40}	0.49	0.006	51.37
N_{60}	0.47	0.006	51.40
FYM_{10t} ha^{-1}	0.55	0.007	58.67
Average	0.49	0.007	55.53
CD at 5%	0.02	0.0004	1.32

Changes in biochemical parameters as affected by different levels of nitrogen and FYM is presented in Table 3. Higher total phenol content was recorded in post-flowering stage compared to the pre-flowering stage. In the pre-flowering stage the total phenol content decreased with the increasing nitrogen. In the post-flowering stage, the treatment of FYM recorded the highest total phenol content (Table 3). The effect of the nitrogen and FYM on PPO showed an inverse relationship with nitrogen levels. Higher phenol content and higher PPO activity under stress conditions was also observed by Mayer and Harel (1979). Ashwagandha requires low input of nitrogen. The higher phenol content helps the plant to tolerate very harsh growing conditions. The ortho-dihydric phenol is an important compound, which imparts disease resistance to the plants. The content of o-DHP also decreased with the increase in the level of nitrogen (Table 3). The metabolic differences with growth stages were also reported by Szecskó1 *et al*. (2002).

The yield and quality of roots were found to be affected with the application of nitrogen and FYM (Table 4). Based on the thickness of the root, Ashwagandha has been classified into five grades ie A, B, C, D and E. The valuable grade B, C and D have been found in greater proportion across all the nitrogen levels and FYM. Similar trend has been observed from absolute yield point of view as the root grades were derived from the yield. The fine powder of ashwagandha root is correlated with size and starch content. The higher powder content has been found from N_{20} to N_{60}, with slight reduction in N_0 and FYM. Grade wise, it shows continuous decline from A to E, because of higher fibrous content formation as root got matured. The quality of root, the main economic part is dependent upon the alkaloid content in root, which was

Table 3. Root yield and quality of ashwagandha as affected by different levels of nitrogen and FYM at harvest.

Root quality	Root grade	Nitrogen levels (kg/ha)				
		N_0	N_{20}	N_{40}	N_{60}	FYM 10t ha^{-1}
Dry root yield (g/m^2)	A	58.9	61.6	43.5	41.5	58.0
	B	101.1	101.5	85.0	74.1	84.3
	C	72.1	83.4	82.0	72.1	80.6
	D	63.9	68.8	81.4	80.6	68.9
	E	43.5	47.1	61.6	66.1	50.8
Average		67.9	72.5	70.7	66.9	68.5
Root grade* (%)	A	19	17	12	12	16
	B	29	28	24	21	26
	C	21	23	24	21	25
	D	19	19	23	25	19
	E	12	13	17	21	14
Average (%)		100	100	100	100	100
Fine powder (%)	A	86	87	97	96	92
	B	72	97	84	81	70
	C	74	76	73	71	71
	D	68	67	64	66	6
	E	58	56	58	56	59
Average		72	77	75	74	72
Alkaloid content (%)	A	0.67	0.68	0.68	0.65	0.67
	B	0.73	0.71	0.68	0.68	0.69
	C	0.67	0.68	0.68	0.60	0.62
	D	0.58	0.60	0.60	0.53	0.55
	E	0.55	0.55	0.57	0.53	0.53
Average		0.64	0.64	0.62	0.60	0.64

*[Root grade based on thickness in mm: A = <8; B = >8-10; C = >10-12; D = >12-13; E = >13]

found to be better at low nitrogen level. The alkaloid content of Ashwagandha has been found to be higher at lower nitrogen levels and found to be highest at N_0 (0.73%) and N_{20} (0.71%) in grade B roots. The higher the grade size, the lesser is the alkaloid content, with lesser powder content, which is due to higher fibre content. The relationship has been shown very well between secondary metabolites and growth/nutrient levels (Almeida-Cortez *et al* 1999, Almeida-Cortez and Shipley 2002). In summary, the experiment suggest that at low input, the quality of Aswagandha was better due to increase in alkaloid content.

REFERENCES

Almeida-Cortez, J.S. and Shipley, B. (2002). No significant relationship exists between seedling relative growth rate under nutrient limitation and potential tissue toxicity. *Funct. Eco.* **16:** 122-127.

Almeida-Cortez, J.S., Shipley, B. and Arnason, J.T. (1999). Do plant species with high relative growth rates have poorer chemical defenses? *Funct. Eco.* **13:** 819-827.

Amthor J.S. (1994). Plant respiratory responses to the environment and their effects on the carbon balance. In: Wilkinson RE, (ed.), Plant-Environment Interactions, pp. 501-554. Decker, New York.

Chance, B. and Maehly, A.C. (1955). Assay of catalases and peroxidases. *Methods Enzymol.* **2:** 764-775.

Garcia-Mateos, R., Lucas, B., Zendejas, M., Soto-Hetnandez, M., Martinez, M., Sotelo, A. (1996). Variation of total nitrogen, non-protein nitrogen content, and types of alkaloids at different stages of development in *Erythrina americana* seeds. *J Agril. Food Chem. (USA).* **44:** 2987-2991.

Gomez, K.A. and Gomez, A. (1984). Statistical Procedures for Agricultural Research (2nd Ed.) John Wiley & Sons, Inc. New York.

Hageman, R.H. and Hucklesby, D.P. (1971). Nitrate reductase from higher plants. In: A. San Pietro (ed.), Methods in Enzymology, Vol 23A, pp. 491-503. Academic Press, London pp. 491-503.

Hiscox, T.D. and Israelstam, G.F. (1979). A method for the extraction of chlorophyll from leaf tissues without maceration. *Can. J. Bot.* **57:** 1332-1334.

Maheshwari, S.K., Sharma, R.K. and Gangrade, S.K. (2000). Response of aswagandha (*Withania somnifera*) to organic manures and fertilizers in a shallow black soil under rainfed condition. *Indian J. Agron.* **45:** 214-216.

Mayer A. M., Harel, E. (1979). Polyphenol oxidases in plants. *Phytochem.* **18:** 193-215.

Mishra, S. N. (1998). Development of new calorimetric technique for evaluation of total alkaloid in root powder. Pro. of All India Co-ordinated Research Project on Medicinal & Aromatic Plants. 12th Workshop. 1998, Publisher, ???

Nigam, K.B. and Kandalkar, V.S. (1995). Aswagandha. In: K.L. Chadha and Rajendra Gupta (eds.), Advances in Horticulture, Vol 11, pp. 337-344, Malhotra Publishing House, New Delhi.

Rosenthal, S.I. and Camm, E.L. (1996). Photosynthetic decline and pigment loss during autumn foliar senescence in western larch (*Larix occidentalis*). *Tree Physiol.* **17:** 767–775.

Suzuki, Y. and Shioi, Y. (2004). Changes in chlorophyll and carotenoid contents in radish (*Raphanus sativus*) cotyledons show different time courses during senescence. *Physiol. Plant.* **122:** 1399-3054.

Szecskó1, V., Hrotkó1, K. and Stefanovits-Bányai, É. (2002). Seasonal variability in phenol content, peroxidase and polyphenoloxidase enzyme activity during the dormant season in plum rootstocks, *Acta Biolgica Szegediensis* **46:** 211-212.

Yubedee, A.G. and Arinze, A.E. (1994). Discriminatory adsorption of polygalacturonases of *Fusarium moniliforme* on plant tissues and their effect on the host range of the fungal isolates. *Inter. J. Trop. Plant Diseases.***12:** 67-74.