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Influence of Nitrogen, Phosphorus and Potassium Fertilizer on Biochemical Contents of *Asparagus racemosus* (Willd.) Root Tubers

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Abstract: This research attempts to investigate the influence of N, P and K on chlorophyll, carbohydrate, proteins and sapogenin contents of *Asparagus racemosus* (Willd.). The treatment consisted different concentrations of nitrogen (N 20, N 40, N 80 and N 160 mg kg^{-1}), phosphorus (P 20, P 40, P 80 and P 160 mg kg^{-1}) and potassium (K 40, K 80 and K 160 mg kg^{-1}) in the form of Urea (46%, H_2NCONH_2), superphosphate (16%, P_2O_5) and muriate of potash (60% K_2O), respectively. A significant increase in the chlorophyll content was recorded with all the applications of N, P and K. Root protein and carbohydrate contents were found linearly increase with K treatment while a slight decline was found with the higher dosage of N. Root sapogenin content was 1.66, 1.87 and 1.75 folds higher than the control with N, P and K, respectively. Application of Phosphorus was found to be best for growth and biochemical contents of root tuber.

Key words: *Asparagus racemosus*, Sapogenin, protein, carbohydrate and chlorophyll

INTRODUCTION

Agricultural production can be associated with the extent to which farmer are able to effectively and suitably combine the production factors. One of the most critical aspects of optimizing crop growth is plant nutrition (USDA, 2002; Aiyelaegbe *et al*., 2005). Medicinal plants are the nature's gift to human being to make disease free healthy life. It plays a vital role to preserve our health. The genus, Asparagus consists of herbs, shrubs and vines that are widespread all over the World and represents highly valuable plant species having therapeutical and nutraceutical importance in addition to being consumed as food (Shasany *et al*., 2003). *Asparagus racemosus* (locally known as Shatavari) is one of the important medicinal plants extensively used by the traditional practioners in India for its medicinal value. The leaves and the tuberous roots of *Asparagus* are medically important to cure minor to severe disease. *Asparagus racemosus* is distributed throughout tropical and sub-tropical parts of India up to an altitude of 1500 m (Velavan *et al*., 2007). The healing qualities of Shatavari are useful to a wide array of ailments. Being a rasayana or rejuvenating herb, its restorative action is beneficial in woman's complaints. *Asparagus racemosus* is mainly known for its phytoestrogenic properties. With an increasing realization that hormone replacement therapy with synthetic oestrogens is neither as safe nor as effective as previously envisaged, the interest in plant-derived oestrogens has increased tremendously making *Asparagus racemosus* particularly important (Bopana and Saxena, 2007). Roots of *A. racemosus* were found to possess antioxidant and anti-ADH activity (Kamat *et al*., 2000; Wiboonpun *et al*., 2004), antitumour and anticancer activity (Senna *et al*., 1993; Shao *et al*., 1996; Diwanay *et al*., 2004), anti-ulcerogenic activity (Datta *et al*., 2002), anti-inflammatory activity (Mandal *et al*., 1998) and antimicrobial activity (Mandal *et al*., 2000).

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Most of the herbal medicine available is derived from the roots of the plant. The commercial success largely depends on quality and yield of root, which is the product of commerce. Among factors responsible for the low yield is low soil fertility, as most tropical soils are deficient in essential nutrients particularly N and P (Jones and Wild, 1975). Poor availability of nutrients in soluble form in the arid and semi-arid soils is the most important limiting factor as compared to that of moist areas. It has been suggested that organic manure should be used in place of chemical fertilizer to avoid long term negative effects of chemical fertilizers on the soil (Parr *et al*., 1990). However, organic manure is usually required in large quantity to sustain crop production and may not be available to the small-scale farmers (Nyathi and Campbell, 1995), hence the need for inorganic fertilizer. The positive effect of the application of inorganic nutrients on the crop yield and yield improvement has been reported by Carasky and Iwuafor (1999). N, P and K are among the common major nutrients, which are essential for the growth and development of all the plant species. There are various ways for improving yield and quality of Asparagus but the best way to improve yield and quality is to apply appropriate amount of fertilizers and to select high yielding varieties. Buries (1959) stated that nitrogen has stimulating effect on root activity and rooting pattern of the crop. It has also been reported available nitrogenous compounds allowed seedling to make a good start. Phosphorus is a major component of important metabolic structure involved in energy utilization and storage mechanism. This is also essential for carbon metabolism which increases the biomass production, its partitioning and ultimately the yield of crop plants (Blevins, 1994). Application of Potassium in different forms has also found to influence plant yield and its chlorophyll contents (Chapagain and Wiesman, 2004).

Balanced fertilizer nutrients can also play a vital role in sustaining high yield of medicinal plants as well as maintaining fertility status of soils on long-term basis. The present investigation was undertaken with nitrogen, phosphorus and potassium to investigate their effects on growth and biochemical contents of root tubers of *Asparagus racemosus* Willd.

MATERIALS AND METHODS

The study was conducted at the Medicinal Plant Nursery, Department of Botany, University of Rajasthan, Jaipur during August, 2004 to January, 2005. Three month-old healthy and uniform sized seedlings of *Asparagus racemosus* Willd. were taken for the present study. Ten replicates were taken for each set of experiment and were conducted in 30 cm earthen pots filled with approximately 4 kg soil (soil: silt: clay, 1:1:1). Plants were irrigated to 50-60% of the field capacity. Different concentrations of nitrogen (N 20, N 40, N 80 and N 160 mg kg^{-1}), phosphorus (P 20, P 40, P 80 and P 160 mg kg^{-1}) and potassium (K 40, K 80 and K 160 mg kg^{-1}) were employed in the form of Urea (46%, H_2NCONH_2), superphosphate (16%, P_2O_5) and muriate of potash (60% K_2O), respectively. The amount of N, P and K was calculated in these compounds on the basis of available nitrogen, phosphorus and potassium, respectively. Nutrients were properly mixed with 4 kg of soil on the blotter paper and then transferred to the 30 cm earthen pots. Plants were harvested in general by uprooting. Fresh weight of aboveground and underground parts was taken just after harvest. The length of shoot and root tubers was also measured. Dry weight was determined after drying the plants in oven at 60±2°C, till the weight became constant. The dried samples were ground by grinding machine and stored in paper bags for biochemical analysis. Chlorophyll content in fresh leaves was estimated following the method of Arnon (1949) using 80% acetone and absorbance was read at 663 and 645 nm. Carbohydrate contents were estimated following Roe (1955) method using Anthrone reagent. Protein contents were estimated following Bradford (1976) and Sapogenin contents were analyzed following method described by Bhagat and Jadeja (2003) using Liberman-Burchard reagent. Yield-contributing parameters including height, fresh and dry weight of both aboveground and underground parts of the plant was also recorded. The data is represented as Mean±SEM (Standard error of mean) of five replicates.

RESULTS AND DISCUSSION

Different forms of inorganic fertilizer in different dosages had a significant effect on all the tested biochemical contents of *Asparagus racemosus* (Willd.). Yield-contributing parameters were also significantly influenced.

Effect of Nitrogen

N favours maximum chlorophyll biosynthesis. Highest concentration of N (160 mg kg^{-1}) increased the chlorophyll content two fold (3.119 mg g^{-1}) over the control plants. All other biochemical contents were also increased significantly with the N treatment compared to control plants (Table 1). A gradual increase in carbohydrate (69.35 mg g^{-1}), protein (10.83 mg g^{-1}) and sapogenin (0.313 mg g^{-1}) contents was recorded with the increasing concentration of N upto 80 mg kg^{-1}, which also favoring by maximum biomass accumulation 29.25 and 12.44% in both aboveground and underground parts, respectively. Application of 80 mg kg^{-1} N was found more effective for development of root tuber of *Asparagus* as observed in present study is in the relation with the findings of Hossain *et al*. (2006) and Krarup *et al*. (2002), that 50 kg N ha^{-1} was enough rather than applying higher concentration of nitrogen such as 100, 150 and 200 kg N ha^{-1}. This is due to the fact that higher concentrations of N ion in the soil limit the uptake of other essential macro and micronutrients by the plant. Paschold *et al*. (1999) reported that excessive N supply can result in less vigorous spears of *Asparagus* while N deficiency reduces quality. A slight decline in shoot length was recorded with 20 mg kg^{-1} concentration of N, but no inhibitory effect on shoot biomass was observed. Influence of N on photosynthetic capacity of plants may be due to the content of activated RUBISCO (Jia and Gray, 2004). The results are in agreement with the findings of Hussain *et al*. (2006) and Nicola (2000) that increasing concentration of nitrogen beyond optimum level did not increase root number and root weight. Nitrogen is the most important inorganic nutrient in plants and major constituents of proteins, nucleic acids, many cofactors and secondary metabolites (Marschner, 1995). NO_3^- addition also modifies resource allocation, growth and development by modulating root-shoot allocation (Scheible *et al*., 1997; Stitt and Krapp, 1999) and promoting flowering and tuber initiation (Marschner, 1995).

Effect of Phosphorus

Super phosphate, as the source of P showed significant promotory effect on plant growth and its biomass over the control plants. Chlorophyll concentration was significantly increased with all the concentrations of P and found maximum (2.905 mg g^{-1}) in plants given 160 mg kg^{-1} P. A linear increase was recorded in the carbohydrate and protein contents with the increasing concentration of P. Maximum carbohydrate (70.17 mg g^{-1}) and protein (10.69 mg g^{-1}) contents were recorded at 160 mg kg^{-1} P and sapogenin contents were also found to be higher (0.352 mg g^{-1}) at the same concentration. The results are in agreement with Xue *et al*. (1992) that higher dose of phosphorus favored the high yield of asparagus. Phosphorus is most important nutrient element for improving photosynthetic rates, which depends on several factors including nutrient supply (Bisht and Chandel, 1991). Height of plant was also influenced positively with P application, supported by its biomass (Table 2). P supply can modulate the content of activated RUBISCO either directly or indirectly (Usuda and Shimogawara, 1991; Rao and Terry, 1995; Pieters *et al*., 2001) and influencing photosynthetic activity of plant.

Effect of Potassium

Although Rajasthan soil are not significantly deficient in Potassium but the addition of K as muriate of potash upto the level of 160 mg kg^{-1} increased chlorophyll concentration (2.604 mg g^{-1}), which resulted in increased sapogenin contents (0.329 mg g^{-1}) in root tubers over the control plants (0.188 mg g^{-1}) (Table 3). However, maximum chlorophyll a:b ratio was recorded in control

Table 1: Effect of different doses of nitrogen fertilizer on the leaf chlorophyll, root protein, carbohydrate, sapogenin, plant height and percentage dry weight of *Asparagus racemosus*

Nitrogen	Chlorophyll	Carbohydrates	Proteins	Sapogenins	Shoot length	Root length	Dry weight		
(mg kg^{-1})	----------------------------(mg g^{-1})-------------------------				(cm)	(cm)	AG	UG (%)	AG:UG
Control	1.823±0.038	53.86±0.274	7.23±0.101	0.188±0.008	25.46±1.348	3.660±0.291	25.59±0.302	10.16±0.017	2.518±0.026
N 20	2.810±0.020	59.57±0.808	9.51±0.385	0.225±0.016	22.16±1.166	4.533±0.202	26.81±0.479	11.67±0.351	2.303±0.107
N 40	3.001±0.030	63.74±0.613	9.49±0.48	0.274±0.023	38.83±2.166	6.766±0.145	28.43±0.266	12.02±0.150	2.364±0.026
N 80	3.038±0.012	69.35±0.616	10.83±0.293	0.313±0.07	50.76±0.868	9.733±0.409	29.25±0.590	12.44±0.227	2.353±0.074
N 160	3.119±0.025	66.50±0.482	10.29±0.276	0.294±0.056	44.56±0.993	6.866±0.233	29.05±0.347	11.32±0.695	2.582±0.145
F_{cal}	388.4849	240.4816	210.2931	22.5708	68.0114	75.8595	14.3856	5.504	1.8027
F_{crit}	3.47805	3.47805	3.47805	3.47805	3.47805	3.47805	3.47805	3.47805	3.47805
Level of significance	**	**	**	**	**	**	**	**	*

**Significant at 5% level of probability, *Significant at 1% level of probability

Table 2: Effect of different doses of phosphorus fertilizer on the leaf chlorophyll, root protein, carbohydrate, sapogenin, plant height and percentage dry weight of *Asparagus racemosus*

Phosphorus	Chlorophyll	Carbohydrates	Proteins	Sapogenins	Shoot length	Root length	Dry weight		
(mg kg^{-1})	----------------------------(mg g^{-1})-------------------------				(cm)	(cm)	AG	UG (%)	AG:UG
Control	1.823±0.038	53.86±0.274	7.23±0.101	0.188±0.008	25.46±1.348	3.660±0.291	25.59±0.302	10.16±0.017	2.518±0.026
P 20	2.625±0.323	61.05±0.51	8.40±0.194	0.229±0.033	37.16±0.440	5.766±0.43	30.32±0.117	11.09±0.457	2.702±0.072
P 40	2.828±0.020	67.22±0.306	9.42±0.435	0.269±0.024	46.83±1.589	6.500±0.288	32.07±0.355	12.44±0.268	2.580±0.050
P 80	2.905±0.039	69.45±0.366	10.07±0.221	0.278±0.025	48.43±2.796	7.066±0.233	32.47±0.242	12.77±0.127	2.452±0.038
P 160	2.734±0.038	70.17±0.186	10.69±0.201	0.352±0.031	52.76±1.299	8.100±0.208	32.75±0.165	13.36±0.184	2.546±0.044
F_{cal}	159.5164	329.6501	269.1944	21.7237	48.5174	30.54267	139.0827	27.7089	3.5856
F_{crit}	3.47805	3.47805	3.47805	3.47805	3.47805	3.47805	3.47805	3.47805	3.47805
Level of significance	**	**	**	**	**	**	**	**	*

**Significant at 5% level of probability, *Significant at 1% level of probability

Table 3: Effect of different doses of potassium fertilizer on the leaf chlorophyll, root protein, carbohydrate, sapogenin, plant height and percentage dry weight of *Asparagus racemosus*

Phosphorus	Chlorophyll	Carbohydrates	Proteins	Sapogenins	Shoot length	Root length		Dry weight	
(mg kg^{-1})	----------------------------(mg g^{-1})-------------------------				(cm)	(cm)	AG	UG (%)	AG:UG
Control	1.823±0.038	53.86±0.274	7.23±0.101	0.188±0.008	25.46±1.348	3.66±0.291	25.590±0.302	10.16±0.017	2.518±0.026
K 40	2.227±0.038	59.82±0.567	8.84±0.784	0.259±0.032	29.16±1.641	5.83±0.440	27.129±0.179	12.28±0.145	2.068±0.149
K 80	2.073±0.040	63.61±0.441	9.71±0.616	0.248±0.008	30.83±1.166	8.20±0.378	27.990±0.184	12.80±0.318	2.307±0.158
K 160	2.604±0.040	66.81±0.596	10.37±0.61	0.329±0.028	56.76±1.105	8.70±0.435	28.516±0.223	13.07±0.225	2.182±0.045
F_{cal}	67.14838	207.5674	237.6161	18.0188	115.5485	35.2561	31.4497	40.1225	2.9665
F_{crit}	4.06618	3.47805	3.47805	3.47805	4.06618	4.06618	4.06618	4.06618	4.06618
Level of significance	**	**	**	**	**	**	*	*	*

**Significant at 5% level of probability, *Significant at 1% level of probability

plants. It has been studied that potassium in different forms influenced the plant yield and its chlorophyll contents (Chapagain and Wiesman, 2004) also. Carbohydrate (66.81 mg g^{-1}) and protein (10.37 mg g^{-1}) contents were also found maximum at 160 mg kg^{-1} K.

The endogenous level of plant growth regulators also affects all the uptake and utilization of minerals. The active principles or precursors are synthesized in the leaves, translocated, biosynthesised and stored in root tubers. A large proportion of leaf photosynthates are required for the growth and development of tuberous root. The rate and amount of photosynthate produced by the leaves and the proportion of photosynthate that is translocated greatly influence size, yield, development and growth of tuber as well as secondary metabolite accumulation. This transport and partitioning of leaf assimilate to the sink tuber is one of the important factors controlling productivity. However from the present study, we concluded that treatment with N P K fertilizer could improve the biochemical status of the plant. Chlorophyll content was found to be improved best with the application of nitrogen at its highest tested concentration and medicinally important saponins are improved with Phosphorus application. Steroidal saponins are a diverse class of secondary metabolites that are structurally constructed of aglycones (Sapogenins) and sugars (glucose/rhamnose).

Asparagus racemosus contains several steroidal saponin glycosides i.e. Shatavarin I-IV. I, II and IV are derived from a common aglycone moiety, Sarsapogenin. Recently, Patricia *et al.* (2008) reported five new steroidal saponins, Shatavarin VI-X from the roots. An appropriate hydrolysis of saponin yield sugars and aglycone moiety sarsapogenin. A correlation between sugar and sapogenin contents was observed in the present study.

Applications of nitrogen, phosphorus and potassium fertilizer are closely related with the yield of *Asparagus racemosus*. Highest root growth in terms of percentage dry weight was observed in plants treated with P, which was further supported by its root sapogenin content. Increase in root growth in all the tested plants is closely associated with biochemical attributes. Compared to control plants root sapogenin content was 1.66, 1.87 and 1.75 folds higher with N, P and K, respectively. Positive correlation between root growth and these parameters could possibly be used for production of high quality plants, as root tubers are the commercial product of interest. Also the estimation of these biochemical attributes under different treatments is informative for finding the best fertilizer nutrition required for the production of its active ingredient. Proteins, carbohydrates, chlorophyll being the direct gene product reflect the genomic composition of cultivars accurately and therefore ideal for finding distinctness. The study therefore has provided NPK fertilization requirement for best growth of such a potential plant commonly used in herbal medicine. The result of this study also showed that there might be differential genotype response to different fertilizer applications, hence the need to determine the fertilizer requirement of the individual genotypes before applying to field production.

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