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Effect of plant density and organic manures on growth, root yield and reserpine content of Sarpagandha (*Rauvolfia serpentina* (L), Benth. ex Kurz)

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Abstract
Sarpagandha is one of the most commercially viable indigenous medicinal plants. The root yield and alkaloid content of the plant has been reported to increase under Good Management Practices (GMP). However, the crop has not received the kind of attention that it deserves. Hence, two field experiments were conducted under AICRP on Medicinal & Aromatic Plants and Betelvine, Horticulture Research Station, OUAT, Bhubaneswar during 2013, 2014 and 2015 to study the effect of plant density (90 × 90 cm, 60 × 60 cm, 45 × 45 cm and 30 × 30 cm) and organic manures (FYM @ 20 t/ha, Mustard cake @ 2 t/ha, FYM @ 10 t/ha + Mustard cake @ 1 t/ha and control: without application of organic and inorganic nutrients) on the performance of sarpagandha. The sarpagandha planted at a spacing of 45 x 45 cm obtained maximum growth and fresh and dry root yield. Combined application of FYM @ 10 t/ha + Mustard cake @ 1 t/ha recorded higher dry root yield. However, application of FYM @ 20 t/ha exhibited the highest B:C ratio of 2.01 followed by T_2: FYM @ 10 t/ha +M. Cake@ 1t/ha (1.95). The reserpine content of sarpagandha was not influenced by the different plant spacing and source of organic manures. Hence, it could be recommended that sarpagandha needs to be grown on commercial scale at a spacing of 45 x 45 cm and manured with FYM @ 20 t/ha.

Keywords: Plant density, organic manures, growth, root yield, alkaloid, soil health, sarpagandha

Introduction
Sarpagandha (*Rauvolfia serpentina* (L), Benth. ex kurz) belongs to the family-Apocynaceae is one of the most commercially viable indigenous medicinal plants. The roots of plants are mainly used for medicinal purposes. In India, the root of sarpagandha has a 400 year history of use in treatment of snake-bite, insect stings, nervous disorders, mania and epilepsy, intractable skin disorders, such as psoriasis, excessive sweating and itching, gynaecological ointments for menopause, toxic goitre and in conditions such as angina pectoris and to promote uterine contraction in child birth. The importance of the drug and alkaloids obtained from it has been recognized by the allopathic system in the treatment of hypertension and as a sedative or tranquillizing agent (Mittal *et al*., 2012) [21]. About 80 alkaloids are isolated from *Rauvolfia* species. The most important among these are reserpine, serpentine and ajmalicine etc. (Deshmukh *et al*., 2012) [9]. The total alkaloid content varies from 1.7-3% of the dried roots depending upon varieties and cultivation practices.

The sarpagandha has enormous importance in the health care system. But after reports of its therapeutic properties, natural reserves of sarpagandha have been declining due to over exploitation by the local and tribal people. This has led to listing of this species as "Endangered" by the International Union for Conservation of Nature and Natural Resources (IUCN) (Jain *et al*, 2003) [14]. In India, Government of India has prohibited the collection of plants growing in wild in forests and its export since 1969. Hence, large scale commercial cultivation needs to be promoted. Development of agro-technology is mainly focused on the low productive and high cost rare in a large scale for endangered medicinal plant species (Bordeker, 2002) [4].

For the fulfillment of the present and future demand, this plant needs to be cultivated scientifically at a commercial scale. The plant is vegetatively propagated by root cutting because of poor seed viability and low germination percentage that may be due to the presence of cinnamic acid and its derivatives in the seed (Mitra, 1976) [20].

Propagation from seed is unreliable due to poor germination and death of many young seedlings under natural conditions. Nayar (1955) [22] observed 15-20% seed germination, seed germination, but only 10-13% plant developed from germinated seeds of *Rauvolfia serpentina*.

It is well known that the species has enormous importance. But in order to increase its production, productivity and quality of the produce, the ideal agro-techniques have to be standardized. It is a mandate for the medicinal plants to be cultivated by organic means considering their therapeutic values. The qualities of the medicinal plants are highly influenced by the organic management practices. The perusals of literatures revealed that no research work has been undertaken for standardization of organic management practices for *R serpentina*. Therefore, the present investigations have been carried out with an objective to standardize the plant density and organic manures in sarpagandha for higher production and quality.

Materials and Methods

Two field experiments were conducted under AICRP on Medicinal & Aromatic Plants and Betelvine at Horticulture Research Station, OUAT, Bhubaneswar during 2013, 2014 and 2015 to study the effect of plant density ($T_{1:}$ 30 × 30 cm, $T_{2:}$ 45 × 45 cm, $T_{3:}$ 60 × 60 cm and $T_{4:}$ 90 × 90 cm) and organic manures ($T_{1:}$ FYM @ 20 t/ha, $T_{2:}$ Mustard cake @ 2 t/ha, $T_{3:}$ FYM @ 10 t/ha + Mustard cake @ 1 t/ha and $T_{4:}$ control: without application of organic and inorganic nutrients) on the performance of Sarpagandha. The recommended dose of Sarpagandha is 100:45:45 kg N: P: K/ha. The organic manures were applied to supply 100% of recommended dose of nitrogen.

Each experiment was laid out as per Randomized Block Design consisting of four treatments with six replications. The observations were recorded after 18 months of crop growth. The physico-chemical characters of the soil of the experimental field were studied before undertaking the experiment is presented as below:

Sl. No.	Characters	Content
1	pH	5.10
2.	E.C.(ds/m)	0.027
2	Organic carbon (%)	0.38
3	Available nitrogen (kg ha^{-1})	215
4	Available phosphorus(kg ha^{-1})	45
5	Available potassium (kg ha^{-1})	58

The electrical conductivity of the soil was determined by using a wheat stone bridge electrical conductivity meter (Jackson, 1973) [13]. Organic carbon content and pH of soil were estimated by Walkey and Black method (Jackson, 1973) [13] and Systronics pH meter (Jackson, 1973) [13], respectively. Available nitrogen, phosphorus and potassium content of soil was estimated by alkaline permanganate method (Subbiah and Asija, 1956), Bray's extractant spectrophotometry method (Jackson, 1973) [13] and ammonium acetate extractant flame photometry method (Jackson, 1973) [13], respectively. The data recorded on various characteristics of plant and soil parameters were subjected to Fisher's method of analysis of variance and interpretation of data was taken up as per Sukhatme and Amble (1995) [33].

Results and Discussion

Effect of plant density on growth, root yield and reserpine content of sarpagandha

Vegetative growth

The perusal of data in Table 1 revealed that the growth parameters of sarpagandha were significantly influenced by the planting density. The maximum plant height was recorded with T_3 (60 x 60 cm) which was statistically at par with T_1(30 x 30 cm) and T_2(45 x 45 cm). The minimum plant height was observed with T_4(90 x 90 cm). The number of primary branches, number of leaves per plant and root length were recorded maximum with T_1(30 x 30 cm) closely followed by T_2(45 x 45 cm). The results revealed that the growth of the plant decreases with increasing of plant spacing. The sarpagandha is a shade loving plant and plants grown at closer spacing levels could provide congenial environment owing to desired sunlight interception by plants for better plant growth. Higher plant height was due to more linear growth of plants as a result of higher density of planting per unit area. The results of the present study are in agreement with those of Ramachandran and Muthuswami (1982) [27] in turmeric. However, the higher growth components such as number of primary branches, number of leaves per plant and root length were observed at wider spacing than closer spacing by Jayalakshmi (2003) [15] in coleus., Rajashekaran *et al*. (1982) [27] in periwinkle, Singh *et al*. (1994) [30] in *Diascorea alata*, Sathish (2000) [28] in *Gloriosa superba*, Sharma and Singh (1981) [21] in carrot, Maheshwarappa *et al.* (2000) [18] in galangal, Bharathi *et al*. (2004) [3] in Chinese potato and Dineshkumar *et al*. (2005) [10] in senna (*Cassia angustifolia*). The diameter of tuber root of sarpagandha was not influenced by the planting density.

Table 1: Effect of spacing on growth and yield of sarpagandha

Treatment	Plant height (cm)	No. of primary branches /plant	No. of leaves/ plant	Root Length (cm)	Root Diameter (cm)	Fresh root yield (q/ha)	Dry root Yield (q/ha)	Reserpine content (%)
T_1: 30 x 30 cm	64.49	3.99	72.85	25.78	0.64	16.07	7.01	0.054
T_2: 45 x 45 cm	64.93	3.96	70.59	26.38	0.68	17.80	7.66	0.060
T_3: 60 x 60 cm	64.99	3.48	57.35	23.55	0.63	15.00	6.74	0.059
T_4: 90 x 90 cm	49.54	3.00	42.24	23.20	0.64	11.59	5.37	0.056
S.E. (d)	2.26	0.15	4.01	1.05	0.04	0.68	0.29	0.074
CD at 5%	4.90	0.32	8.71	2.29	NS	1.47	0.62	NS

Fresh and dry root yield and reserpine content

The fresh and dry root yield was significantly influenced by the different levels of plant density. The maximum fresh and dry root yield was recorded with T_2 (45 x 45 cm) and the minimum value was recorded with T_4 (90 x 90 cm). The results revealed that with decrease in spacing and consequent increase in plant population in sarpagandha, the fresh and dry tuber yield increased from 11.59 q/ha to 17.80 q/ha and 5.37 q/ha to 7.66 q/ha, respectively. The spacing of 45 cm x 45 cm recorded significantly higher dry and fresh tuber yield than the other spacing levels. The spacing of 30 cm x 30 cm recorded next higher yield. The closer spacing was found better for getting higher yield per unit area though yield per plant was higher at wider spacing. In Sarpagandha, tuber roots (primary roots) are the economic part of the plant. The agronomic manipulations and practices aimed at improving the yield of tubers through optimising source-sink ratio are of more practical significance. Optimum spacing provided to each plant helps to utilize growth resources optimally resulting in better yields. Veeraraghavathatham *et al.* (1985) reported that tuber yield of coleus per unit area increased with increase in population registering the highest at closer spacing. The findings of the present investigation are in line with those of Jayalakshmi (2003) [15], Patil and Hulamani (1999) [24] and Chandrasekhar *et al.* (2007) [6] in coleus. The reserpine content of sarpagandha was not influenced by the different plant spacing. Jayalakshmi (2003) [15] reported that various spacing levels did not influence total alkaloid content significantly.

Effect of organic manures on growth, yield and reserpine content of sarpagandha

Vegetative growth

The results presented in Table 2 revealed that the growth parameters of sarpagandha like plant height, number of primary branches per plant, diameter of branches, number of leaves per plant and LAI were significantly influenced by the organic manures. The maximum plant height (62.90 cm), number of primary branches per plant (3.79), diameter of branches (0.64 cm), number of leaves per plant (72.97), LAI(3.71) were observed in the treatment T_3 (FYM @ 10 t/ha + M. cake @ 1 t/ha). All these parameters were found minimum in the control plots receiving no manures and fertilizers. The increase in growth parameters of the plant might be attributed to physical improvement of soil by the application of manure which facilitated the increased availability of nutrients from the soil pool to the plant. Smitha *et al.*, (2010) [31] reported the better plant growth in Makoi due to increased availability of soil nitrogen and phosphorus to the plant with the application of manures. Similar finding on increase in vegetative growth of plant and LAI due to manuring was reported by Rahman *et al.*, (2014) [26] in Tulsi and Pudina and Olowoake (2014) [23] in Amaranthus. The present findings are in line of the results reported by Kumar and Topal (2015) [16] in Kalmegh. However,

Table 2: Effect of organic manures on vegetative growth of sarpagandha

Treatment	Plant Height (cm)	No. of primary branches/ plant	Diameter of branches (cm)	No. of leaves/plant	LAI
T_1: FYM@ 20 t/ha	62.19	3.56	0.61	61.37	3.15
T_2: M. Cake@ 2 t/ha	57.13	3.30	0.56	58.01	2.94
T_3: FYM @ 10 t/ha + M. cake @ 1 t/ha	62.90	3.79	0.64	72.97	3.71
T_4: Control	49.66	2.87	0.53	46.94	2.21
S.E(d)	0.89	0.12	0.03	2.02	0.26
CD at 5%	1.87	0.26	0.07	4.24	0.56

Table 3: Effect of organic manures on root growth, root and seed yield of sarpagandha

Treatment	Root Length (cm)	Root Diameter (cm)	Fresh root yield (q/ha)	Dry root yield (q/ha)	Seed yield (kg/ha)	Reserpine content (%)
T_1: FYM@ 20 t/ha	22.95	0.50	17.90	6.20	107.52	0.058
T_2: M. Cake@ 2 t/ha	21.37	0.46	16.55	5.54	98.57	0.057
T_3: FYM @ 10 t/ha + M. cake @ 1 t/ha	24.73	0.55	20.09	6.33	120.24	0.059
T_4: Control(no manure)	20.45	0.48	10.05	4.08	75.28	0.050
S.E(d)	0.59	0.08	0.52	0.22	4.77	0.083
CD at 5%	1.25	NS	1.09	0.46	10.03	NS

Root growth, root and seed yield and reserpine content

The data relating to root length and root diameter are presented in Table 3. The results indicated that the root length of plant was significantly influenced by the organic nutrition at 18 month after planting. The maximum root length of sarpagandha was recorded in T_3: FYM @ 10 t/ha + M. cake @ 1 t/ha (24.73 cm) and the minimum root length (19.80 cm) was noticed in T_4: Control (no manure). The root diameter of sarpagandha recorded at 18 month after planting was not influenced by the application of manures, which ranged from 0.48 cm (T_4: Control) to 0.55 cm (T_3: FYM @ 10 t/ha + M. cake @ 1 t/ha). Application of organic manures provide balanced nutrition in addition with enhancing water holding capacity and improving physical, chemical and biological properties of soil. According to Zhang *et al.*, (2014) [35], the application of manures increases rhizosphere soil carbon mineralization and stimulates root growth in cucumber crop. The present finding agrees well to the results obtained by Singh *et al.*, (2015) who reported higher root growth in Satavari by the application of organic manures.

The effect of organic manures on fresh and dry root yield and seed yield of sarpagandha was studied under the experiment which revealed that fresh and dry weight of root and seed yield of sarpagandha were significantly influenced by the organic manures. The fresh and dry root yield at 18 month after planting of sarpagandha was obtained significantly maximum in T_3: FYM @ 10 t/ha + M. cake @ 1 t/ha (20.09 q/ha and 6.33 q/ha) and the minimum fresh and dry root yield was recorded with T_4: Control (10.05 q/ha and 4.08 q/ha). The dry root yield recorded in T_3: FYM @ 10 t/ha + M. cake @ 1 t/ha was found statistically at par with T_1: FYM@ 20 t/ha. Organic manures provide balanced nutrition and improve physical, chemical and biological properties of soil with balanced C/N ratio might have increased the synthesis of carbohydrates which ultimately promoted higher yield (Smitha *et al.*, 2010) [31]. Similar finding on the influence of organic manures on increase in dry root yield of sarpagandha has been reported by Ghosh *et al.*, (2011) [12] and Kumari *et al.*, (2015) [17]. In the present study sarpagandha responds well to the organic manures in respect of increase in dry root yield which is in line of the results obtained by Mbatha *et al.*, (2014) [19] in Parsley. Celick *et al.*, (2004) [5] reported that compost and manure treatments increased available water content of soil which facilitates better root growth.

The combined application of FYM @ 10 t/ha + M. cake @ 1 t/ha increased the seed yield to the tune of 120.24 kg/ha recorded within 18 month of planting followed by T_1: FYM@ 20 t/ha. The control plots recorded the minimum seed yield of 75.28 kg/ha. The increased seed yield due to combined application of FYM @ 10 t/ha + M. cake @ 1 t/ha might be attributed to increased supply of different nutrient elements to the plants. Besides it provides beneficial microbes like nitrogen fixing bacteria, mycorrhizae and growth promoting substances for betterment of crop as reported by Barik *et al.*, (2006) [1] in different field crops. This corroborates the finding of Deivasigamani and Thanunathan (2011) [8]. The results indicated that reserpine content of sarpagandha was not influenced by the different sources of organic nutrition. This might be due to the fact that quality of the plant is governed by the genetic potential of the plant and hence it is not influenced by the treatment of different organic manures. This result is in line of the results obtained by Patne (2003) [25] who reported that fertilizer levels did not influence the forskolin content but increased forskolin yield was due to higher tuber yield.

Economics of sarpagandha grown under different sources of organic manures

The economics of sarpagandha grown under different sources of organic manures was estimated in the present investigation in order to find out the most remunerative one (Table 4). The results indicated that application of M. Cake@ 2t/ha (T_2) incurred maximum cost of cultivation (Rs.2,30,000/ha) followed by T_3: FYM @ 10 t/ha +M. Cake@ 1t/ha (Rs.2,26,000/ha). The minimum cost of cultivation was estimated in the treatment T_1: FYM@ 20 t/ha. The higher cost of cultivation was mainly due to higher expenditure incurred towards purchase of organic inputs. Although organically production of sarpagandha was found profitable in all cases, the highest net return of Rs.2,13,920/- was obtained with T_3 (FYM @ 10 t/ha +M. Cake@ 1t/ha). The minimum net return (Rs.1, 02, 440/ha) was realized when sarpagandha was grown without application of any manure and fertilizers. So far as the benefit and cost ratio of different treatment are concerned, the treatment T_1 supplied with FYM @ 20 t/ha exhibited the highest B: C ratio of 2.01 followed by T_2: FYM @ 10 t/ha +M. Cake@ 1t/ha (1.95). The lowest B: C ratio of 1.57 was recorded with the treatment T_4: Control(No manure). The higher cost-benefit ratio in the above treatments was attributed to higher biological productivity and less cost of cultivation as compared to other treatment. The higher net return and benefit cost ratio obtained by the organically cultivation of medicinal plant were reported by Gera *et al.*, (2005) [11] in Kalmegh, Buch, Safed musli, Aswagandha and Akarakara and Vanitha and Manimalathi (2013) [34] in Glory lilly.

Table 4: Economics of sarpagandha grown through different sources of organic manures.

Treatment	Root Yield (q/ha)	Seed yield (kg/ha)	Treatment cost (Rs/ha)	Cost of cultivation (Rs/ha)	Gross Return (Rs/ha)	Net Return (Rs/ha)	B: C ratio
T_1: FYM@ 20 t/ha	6.2	107.52	32,000	212000	425760	213760	2.01
T_2: M. Cake@ 2t/ha	5.54	98.57	50,000	230000	381685	151685	1.66
T_3: FYM @ 10 t/ha +M. Cake@ 1t/ha	6.33	120.24	46,000	226000	439920	213920	1.95
T_4: Control(No manure)	4.08	75.28	0	180000	282440	102440	1.57

Sale price: Dry root-Rs.60,000/q, Seed-Rs. 500/kg

Effect of organic manures on physico-chemical parameters of soil

The data recorded on physico-chemical characteristics of soil within 0-30 cm of soil depth after completion of the experiment are presented in Table 3. The results indicated that the physico-chemical characteristics of soil such as pH, electrical conductivity, organic carbon, available N and K were not influenced by the application of organic manures. It was revealed from the table that there was significant variation in the available P of soil within 0-30 cm depth. The maximum available P of 49.5 kg/ha was estimated in T_3: FYM @ 10 t/ha + Mustard cake @ 1 t/ha and the minimum available P (44.9 kg/ha)was estimated in T_4: Control. This might be due to the fact that the application of organic manures affects the soil microbes, which increase the mineralization of manures and hence improve the available P. Choudhary *et al.* (1981) [7] observed an increase in available P content of the soil due to its presence in manures and release from native soil. Basak *et al.* (2013) [2] found significantly high enzyme activity in soil due to application of organic manures which played crucial role in nutrient transformation.

Table 5: Effect of organic manure on physico-chemical characteristics of soil.

Treatment	E.C. (dS/m)	pH	Organic Carbon (%)	Avail. N (Kg/ha)	Avail. P (Kg/ha)	Avail. K (Kg/ha)
T_1: FYM@ 20 t/ha	0.027	5.36	0.42	228.95	46.05	64.70
T_2: M. Cake@ 2 t/ha	0.025	5.30	0.40	225.95	46.35	62.25
T_3: FYM @ 10 t/ha + M. cake @ 1 t/ha	0.02	5.33	0.39	230.95	49.50	66.00
T_4: Control	0.02	5.25	0.38	222.25	44.90	63.10
S.E(d)	0.005	0.13	0.02	4.67	1.22	1.69
CD at 5%	NS	NS	NS	NS	2.57	NS

Conclusion

The sarpagandha planted at a spacing of 45 x 45 cm obtained maximum growth and fresh and dry root yield. Combined application of FYM @ 10 t/ha + Mustard cake @ 1 t/ha recorded higher dry root yield. However, application of FYM @ 20 t/ha exhibited the highest B:C ratio of 2.01 followed by FYM @ 10 t/ha +M. Cake@ 1t/ha (1.95). The alkaloid (reserpine) content of sarpagandha was not influenced by the different plant spacing and source of organic manures. Hence, it can be concluded that sarpagandha can be recommended for commercial cultivation at a spacing of 45 x 45 cm with application of FYM @ 20 t/ha.

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