International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 6, No. 4, p. 100-111, 2015

RESEARCH PAPER OPEN ACCESS

Comparative influences of manures and NPK fertilizer on growth performance, root yield and reserpine content of *Rauvolfia* spp

Rachna Kumari, Maheshwar, P. Trivedi, Birendra Prasad

Microbial and Molecular Genetics Lab, Depatment of Botany, Patna University, Patna-800005, India

Article published on April 14, 2015

Key words: *Rauvolfia*, Reserpine, Manures, NPK fertilizer, Growth performance, Root yield.

Abstract

The study revealed the growth performance, root yield and reserpine content in the root part of *R. serpentina* and *R. tetraphylla* under four different treatment regimes (T1,T2, T3, T4). Three types of cutting like root, root-stem junction and stem of 2 to 2.5 years old plants of both the species were used. It was concluded that root formation percentage from cuttings of root, root-stem junction and stem under different treatment conditions in *R. serpentina* were varied from 33.33–85.00 %, 31.67– 50.00% and 20.00–33.33 %, respectively. A similar trend was observed in in *R. tetraphylla*. Vermicompost (T1) showed comparatively maximum root biomass increased percentage followed by Cowpat (T2) in *R. serpentina* though it was more or less similar in *R. tetraphylla*. The application of T1, T2, T3 and T4 compositions significantly (P=0.01) increased root mean dry weight in all plant cutting types of both the species against control and the trend was root-stem junction cutting > root cutting > stem cutting. Application of NPK fertilizer decreased the soil pH from 6.73 (T5) to 6.6 (T3 and T4). In T1 and T2 pots the available soil nitrogen significantly increased by 0.0079 % and 0.0072 %, respectively. NAA at 50 ppm had highest impact on root formation followed by IAA and IBA then at 5 ppm of 2,4–D in *R. tetraphylla*. Quantification of reserpine in root of treated plants by HPTLC revealed significant variation.

Introduction

Rauvolfia serpentina (L.) Benth ex Kurz (Apocynaceae) is a well known medicinal plant and source of principal bioactive indole alkaloid reserpine, heavily localized in their root region. *Rauvolfia tetraphylla* genotype is often used as a substitute of *R. serpentina*. The Plant is used in the treatment of hypertension and used as a sedative or tranquilizing agent. In natural habitat *Rauvolfia* species ensure the availability of vegetable mould rich in organic substances. Available organic carbon in soil ranged from 0.95 % to 1.17 %, Phosphorus being 22 Kg to 25 Kg/ha and Potassium from 90 Kg to 200 Kg/ha (Varadranjan, 1963). *Rauvolfia* species need a proper and balanced dose of nitrogen, phosphorous, potassium including carbon sources and others for growth and development often reflect their survival strategy in the community.

Rauvolfia species are threatened in India due to indiscriminate collection and over exploitation of natural resources for commercial purposes to meet the requirements of the pharmaceutical industry. For the fulfillment of the present and future demand these plants need to be cultivated in the already available system from plant cuttings scientifically at commercial scale and it will be of great importance for conservation by optimizing field growth conditions for maximum recovery of phytochemical (reserpine) against chemical synthesis of reserpine. Earlier little success was achieved to develop root propagules from cuttings of *R. serpentina* (Badhwar *et al.*,1956). Partial success was achieved in *R. canescence* cultivation from stem cuttings (Chandra, 1956). Many workers studied the complementary effect of cowdung, vermicompost, other plant waste organic matter and NPK fertilizer on plant growth and yield of several species (Alamgir and Ahamed 2005, Asawalam and Onwudike 2011, Ayeni, 2010). Though the nutrient status of manures like vermicompost and bioprocessed cowpat and other plants and animal wastes are also a good source of nitrogen, phosphous, potassium and many other components like Ca, Mg and Na have their individual effects on soil enrichment and nutrient uptake by plants (Benckiser and Simarmata,1994). As comparative effect of cocoa husk, ash manure and NPK fertilizer on the soil and root nutrient content and growth of kola seedlings was observed (Ajayi *et al.*,2007). Studies were also made on the comparative effects of NPK fertilizer, cowpea pod husk and some tree crops wastes on soil, leaf chemical properties and growth performance of cocoa (*Theobroma cacao* L.) (Adejobi *et al.*, 2014). Inspite of above fragmentary information, no works are available on the comparative effect of organic manure and inorganic fertilizer NPK on plant growth of *Rauvolfia* species which necessitated us to work on these lines.

Materials and methods

Rauvolfia species and growth conditions

The soil was collected from Ganga ghat of Patna which is sandy loam in nature passed through 2 mm sieve and filled in earthen pot of 30 cm height and 20 cm diameter at the rate of 5 Kg per pot. The soil was prepared two days before pot filling and plantation. The soil was mixed separately with vermicompost of cowdung, bioprocessed cowpat and different doses of NPK fertilizer, in the form of urea, superphosphate and muriate of Potash. The treatment consisted @ 250 g/Kg. vermicompost (T1), 250 g/kg. Cowpat (T2) and @ 250 mg/Kg 3N+P+K (T3), 250 mg/Kg N+P+K (T4) against control (T5). Plants of *Rauvolfia serpentina* and *R. tetraphyalla* were collected from cultivated land of Falka of Katihar district and plain land of Rangbhoomi Maidan of Purnea district respectively. The age of the collected sample was 2.0–2.5 years. Three types of plant cuttings of length 20 cm each in the form of root, root-stem junction of 0.8–1 cm diameter and stem cutting of 0.4-0.8 cm diameter of both the species were used. The cuttings were planted between 20–28 February, 2012 in green house of Department of Botany, Patna University and harvested in the month of February, 2013. The basal end of cuttings was inserted into the soil at 45° angle upto 5 cm beneath the soil and two cuttings in each pot and total of 30 cuttings for each treatment were arranged in replication of three.

Survivality problems of Rauvolfia tetraphylla stem *and their treatments with growth regulators*

In another set of experiment for *R. tetraphylla* stem, IAA, NAA, IBA and 2, 4–D at 5 ppm, 50 ppm and 150 ppm were used to overcome the survivality problems as well as the poor performance of rooting. In different concentration of hormone solution 5 cm length of the basal side of cuttings were dipped for 48 hours at 20 ^oC temperature and then planted (Alamgir and Ahamed, 2005). Plants were grown upto one year, providing weeding and irrigation as per requirement. Further, they were harvested and performance of species was judged with respect to mean of plant height, number of leaves, number of shoot branches and shoot sub-branches, root length, number of strong root branches, rooting percentage, fresh and dry weight of root per plant and relative root biomass increased percentage.

Assessment of chemical properties of soil

Soil samples of pots before planting and after harvest of plants were sieved by 1mm pore size sieve. Soil pH was determined in water (1:1) using a combined electrode pH meter (Thomas, 1996). Available nitrogen was determined by the procedure involves distilling the soil with alkaline potassium permanganate solution to liberate ammonia (Subbiah and Asija, 1956). Available phosphorus was extracted by molybdenum blue method (Kuo,1996). Potassium was determined by flame photometer method using ammonium acetate reagent and organic carbon was determined by chromic acid wet oxidation method of Nelson and Sommers (1996). All assessment repeated three times for producing significant result.

Assessment of reserpine content

Roots were dried in the oven below 60 ^oC until constant weight was attained. Further phytochemical screening of all root samples was done by Dragendorff's reagent.

Assessment of reserpine content in samples of root by TLC and HPTLC was also done (Panwar *et al*., 2011, Sangram *et al*.,2010, Sarika *et al*., 2012). The results were produced using three replications for each set of experiment.

Qualitative analysis by TLC

The qualitative estimation of resperpine was done by thin layer chromatography technique on preparative silica gel 60. Solvent system used for alkaloid estimation was chloroform : methanol (97:3). Bands were visualized by spraying Dragendorff's reagent uniformly over the plates. Further plates were observed under UV- transilluminator. Identification was done on the basis of colour of bands and their Rf values under UV light (Sarika *et al.,* 2012).

Quantitative Analysis by HPTLC

The content of reserpine in different samples was evaluated by HPTLC analysis. The HPTLC profile of methanolic crude alkaloid extract (MCAE) of treated root samples of both the species along with reserpine standard was observed in the mobile phase of Toluene : Ethylacetate : diethylamine (7:2:1) in which Rf value for reserpine was found to be 0.49 ± 0.02.

Methanolic extract of each sample after filtration was dried in vacuum desiccator and amount measured called as MCAE. The percentage of these extractive value which is present in the crude powdered sample referred as an effective value percentage (EVP).

Statistical analysis

The mean dry wt. of root due to effect of treatments on cutting types of both the species were analysed by ANOVA. Coefficient of Correlation between root length and root mean dry weight, root mean dry weight and root biomass increase percentage and among plant height and root mean dry weight was also worked out in both the species.

Results

The growth parameters of plant cuttings of *R. serpentina* and *R. tetraphylla* in response to different treatments for nutrient uptake of plant types presented in Table 1a and 1b. The cuttings from root-stem junction revealed the highest root dry weight (8.25 ± 2.98 g/plant), root biomass increased percentage (589.28%) and plant height (53.00 ± 1.00

cm) in T1 condition followed by T2 condition i.e. 7.20 ± 0.72 g/plant (dry wt. of root), 514.57 (root biomass increased percentage) and 48.33 ± 0.96 cm (plant height) in *R. serpentina*. *R. tetraphylla* did not show much significant difference among morphological parameters studied like mean root dry weight (10.02 ± 1.68 and 9.98 ± 0.25 g/plant), root biomass increased percentage (447.32% and 445.54%) and plant height/plant (82.33 ± 6.50 and 81.67 ± 3.60 cm) in T1 and T2 conditions respectively related to other treatment conditions followed in descending order by T3 > T4 (Fig. 1). The growth performance by means of root mean dry weight of all plant cutting types of both the species under treatment conditions were shown by ANOVA in Table 2. In T1 condition root junction cutting of *R. serpentina* and *R. tetraphylla* showed much branched and heavier root i.e. 10 to 15 in former and 9 to 10 in later. Coefficient of Correlation between root length and root mean dry weight, root mean dry weight and root biomass increase percentage and among plant height and root mean dry weight were significant (p=0.01) in both the species and positive considering root stem junction cutting as a factor (Table 3). Root formation percentage in this plant type was varied from 31.67 to 50.00 % and 60 to 80 % respectively in *R. serpentina* and *R. tetraphylla*. Maximum root propagule development in *R. serpentina* from root-stem junction was also discussed (Alamgir and Ahamed, 2005).

Table 1 a. Growth performance of *Rauvolfiaserpentina* plant cutting type in response to treatments.

Root Sample	Mean plant height (cm/plant)	Mean No of shoot branch/plant	Mean No of shoot sub branch/ plant	Mean No of leaf/plant	Mean No of strong root branches/ plant	Rooting (%)	Mean Root length (cm/plant)	Mean fresh wt. of root (g/plant)	Mean dry wt. of root (g/plant)	Moisture content of root (%)	Root biomass increase (%)
					Root Cutting						
T1	48.67±5.50	1.3±0.5	2.0±0.0	26.00±2.00	1.33±0.58	83.33±1.52	24.00±1.00	8.87±1.10	3.79±0.63	57.27	293.79
T2	46.67±7.02	1.6±0.5	2.0±0.0	25.67±3.05	1.67±0.58	85.00±2.51	24.0±2.00	9.77±4.10	4.00±0.84	59.06	310.08
T3	31.33±5.73	1.3±0.5	1.3±0.5	21.67±2.08	1.33±0.58	33.33±4.27	23.0±1.73	7.31±4.36	2.99±0.64	59.1	231.78
T4	25.33±2.02	1.0±0.0	1.3±0.5	20.33±1.52	1.00±00	33.33±3.21	18.67±1.52	4.97±1.80	1.56±0.89	68.61	120.93
T5	23.67±0.81	1.0±0.0	1.0±0.0	12.00±3.00	1.0±00	50.00±5.73	11.5±1.25	2.69±1.31	1.29±0.61	52.00	-
					Root stem junction cutting						
T1	53.00±1.00	1.6±0.5	2.0±0.0	31.00±3.60	12.33±2.52	50.00±0.57	25.33±1.52	26.53±5.50	8.25±0.2.98	68.9	589.28
T2	48.33±0.96	1.6±0.5	2.0±0.0	32.50±3.05	11.00±1.00	50.00±0.57	24.67±3.21	20.89±3.51	7.20±0.72	65.46	514.57
T3	36.33±3.51	1.3±0.5	2.0±0.0	24.33±3.05	3.67±0.58	33.33±3.04	23.00±1.52	11.06±1.80	4.52±1.79	68.17	251.42
T4	24.00±1.01	1.0±0.0	1.3±0.0	15.00±2.00	2.67±1.15	31.67±1.00	18.67±2.01	6.70±1.01	2.30±0.62	65.67	164.28
T5	21.20±2.83	1.0±0.0	1.0±0.0	12.67±0.57	1.67±0.58	27.00±0.57	9.33±0.65	3.51±0.92	1.40±0.33	60.11	-
					Stem cutting						
T1	31.33±3.04	1.0±0.5	1.3±0.0	23.33±3.05	1.00±00	33.33±0.57	18.67±0.57	6.87±2.00	2.86±0.32	58.37	232.52
T2	31.33±2.06	1.0±0.5	1.3±0.0	24.33±2.88	1.00±00	33.33±0.57	18.67±1.05	6.71±1.01	2.80±0.32	59.76	227.64
T3		-	-	-	-	-	-	-	-	-	-
T4		-	-	-	-	-	-	-	-	-	-
T5	20.00±0.90	1.0±0.0	1.0±0.0	8.33±0.57	1.00±00	20.00±1.00	8.3±0.57	2.49±0.57	1.23±0.22	50.6	-

- Not detected; Value (mean±SE, n=9).

Plant cuttings grown in soil already prepared using T3 and T4 composition sprouted within 3 to 4 days after plantation. But consequently struck poor rooting percentage, root biomass increased percentage as well as plant height in all plant cutting types of both the species in comparison to T1 and T2 conditions.

To increase the rooting potential and phytochemicals present in them only stem cuttings of *R. tetraphylla* were tried with auxins like NAA, IAA, IBA, 2, 4–D and effect was worked out (Tables 4 and Fig. 2). Lower and moderate concentrations of NAA and IAA, (5 ppm and 50 ppm) stimulated root formation, root length, width as well as the secondary root formation while 150 ppm concentration showed inhibitory effects on root growth. IBA at 50 ppm produced 85% rooting while higher concentration (150 ppm) decreased root formation. 66.7% rooting was achieved at lowest concentration (5 ppm) of 2,4–D and with further increased concentration (50 ppm) root formation declined and finally inhibited at 150 ppm. Root mean dry weight (g/plant) was found to be in order of NAA>IAA>IBA>2,4–D (8.20>8.09>7.98>3.20) significant at p=0.001.

Table 1 b. Growth performance of *R. tetraphylla* plant cutting type in response to treatments.

Root Sample	Mean plant height (cm/plant)	Mean No of shoot branch/plant	Mean No of shootsub branch/ plant	Mean No of leaf/plant	Mean No of strong root branches/ plant	Rooting (%)	Mean Root length (cm/plant)	Mean fresh wt. of root (g/plant)	Mean dry wt. of root (g/plant)	Moisture content root (%)	Root biomass of increase (%)
						Root Cutting					
T1	78.33±7.40	4.3±1.5	10.6±3.0	176.33±7.00	2.0±1.00	100.00±1.52	37.67±1.52	12.53±1.31	4.66±1.31	62.81	201.73
T2	76.67±5.50	4.0±2.0	8.6±2.6	147.33±7.02	2.0±1.00	100.00±1.52	34.33±2.64	11.86±1.52	4.14±1.33	65.09	179.22
T3	76.33±5.72	3.6±0.5	7.0±0.5	137.33±5.50	2.0±1.00	82.00±2.08	37.00±2.64	8.87±2.62	3.81±0.30	57.05	164.94
T4	57.33±3.04	2.6±0.5	4.6±0.5	86.67±5.50	2.0±1.00	80.76±2.00	20.67±1.52	7.52±1.80	2.79±0.57	62.89	120.78
T5	44.67±3.51	1.3±0.5	3.6±0.5	83.67±4.16	1.33±0.58	88.90±1.52	16.67±1.94	6.17±1.52	2.31±0.33	62.56	-
						Root stem junction cutting					
T1	82.33±6.50	4.3±2.0	11.6±4.3	189.00±5.00	10.33±0.58	80.00±1.52	27.33±1.52	25.52±3.78	10.02±1.68	60.74	447.32
T2	81.67±3.60	4.3±1.0	9.3±2.5	196.00±5.00	9.00±1.00	80.00±1.52	26.33±2.00	25.21±3.51	9.98±0.25	60.41	445.54
T3	62.67±3.51	4.3±0.5	9.3±5.7	191.00±4.16	3.00±1.00	69.67±5.00	23.33±1.52	16.00±1.52	5.72±1.48	64.25	255.36
T4	51.00±2.00	2.6±0.5	3.6±0.5	92.67±4.16	2.67±1.15	60.00±2.00	21.00±1.80	7.88±1.80	2.85±0.18	63.83	127.23
T5	45.00±5.68	1.6±0.5	3.6±0.5	83.67±11.84	2.67±0.58	33.33±1.00	15.00±0.76	6.15±4.36	2.24±0.50	63.58	-
						Stem cutting					
T1	81.67±3.51	3.3±1.0	9.3±2.0	159.67±10.59	2.67±1.54	73.35±1.52	51.67±3.78	17.62±2.00	7.76±1.35	55.96	338.00
T2	78.67±3.51	2.6±0.5	9.3±2.0	181.91±10.59	2.67±1.54	72.24±1.52	48.67±2.84	17.01±1.00	6.96±2.21	59.08	323.00
T3	65.33±5.68	2.3±0.5	9.3±2.5	148.33±7.02	2.00±1.00	33.33±12.28	37.33±3.51	11.38±2.00	3.94±0.96	65.37	167.00
T4	48.68±5.68	2.0±0.0	4.0±2.5	96.00±7.00	2.00±1.00	33.33±12.28	25.00±3.00	5.99±1.00	2.15±0.90	64.11	106.00
T5	44.67±5.03	1.3±0.5	3.0±2.0	80.67±10.59	1.00±0.00	50.00±1.00	12.00±2.64	5.87±1.00	2.00±0.84	65.93	-
	-										

Not detected; Value (mean±SE, n=9.

Significant differences in soil chemical properties such as soil pH, available nitrogen, phosphorus, potassium and organic carbon were evident. Application of NPK fertilizer decreased the soil pH in water from 6.67 (T5) to 6.6 (T3 and T4), whereas combination of vermicomposts increased the soil pH from 6.67 (T5) to 6.73 (T1). In T1 and T2 soil nitrogen significantly increased by 0.0079 % and 0.0072 %, respectively. Application of NPK showed comparatively less available soil nitrogen in T3 (0.0067 %) and T4 (0.0070 %) (Table 5).

Table 3. Coefficient of Correlation between root length and root mean dry weight using cutting type as well as treatment as a factor.

Plant species	factor	Root length and root mean dry weight	Plant cutting types	factor	Root length and root mean dry weight: *R. serpentina*	Root length and root mean dry weight: *R. tetraphylla*
R. serpentina	R-C	0.893**	R-C	T1	0.968**	0.975**
	R-S-J-C	0.869**		T2	0.999**	0.953**
	S-C	0.726*		T3	0.891**	0.958**
R. tetraphylla	R-C	0.955**		T4	0.871**	0.920**
	R-S-J-C	0.931**		T5	0..998**	0.992**
	S-C	0.964**				
			R-S-J-C	T1	0.996**	0.801**
				T2	0.840**	0.710*
				T3	0.976**	0.971**
				T4	0.838**	0.903**
				T5	0.936**	0.820**
			S-C	T1	0923**	0.966**
				T2	0.971**	0.932**
				T3	_	0.986**
				T4	_	0.994**
				T5	0.882**	0.999**

R-C = Root Cutting, R-S-J-C=Root Stem Junction Cutting, S-C=Stem Cutting

** = Significant at P=0.01, * = Significant at P=0.05.

The result of phytochemical screening by Dragendorff's reagent was positive in all root samples of both the species. It produced orange brown precipitate. When methanolic crude alkaloid extract of root samples of *R. serpentina* and *R. tetraphylla* were subjected to the solvent system chloroform : methanol (97:3) both the species showed fluorescent green and blue band in UV light. In the fingerprinting chromatograms of TLC the bands of T1 and T2 treated roots of *R. serpentina* were more prominently seen as compared to the T3 and T4 treated roots (Fig. 3). *R. tetraphylla* root samples under these treatments exhibited poor fluorescence as compared to *R. serpentina*. The Rf values were shown to be very close to the standard Rf value 0.96 which indicated the presence of alkaloid reserpine.

Table 4. Growth performance of*Rauvolfiatetraphylla* stem cutting in response to growth hormones.

Root Sample	Concentration of GR (ppm)	Mean plant height (cm/plant)	Mean No of branch/plant	Mean No of sub branch/ plant	Mean No of leaf/plant	Mean No of strong root branches/ plant	Rooting (%)	Mean Root length (cm/plant)	Mean fresh wt. of root (g/plant)	Mean dry wt. of root (g/plant)	Moisture content of root (%)	Root biomass increase (%)
Control	-	44.67±5.03	1.3±0.5	3.0±2.0	80.67±10.5	1.0±0.0	50.00±1.0	12.00±2.64	5.87±1.00	2.00±0.84	65.93	-
IAA	5	65.00±0.01	3.4±0.02	10.0±0.05	169.00±1.0	4.8±1.0	33.33±1.0	29.33±0.10	16.33±0.10	5.60±0.05	65.70	280.00
	50	81.33±0.05	4.0±0.05	10.3±0.05	179.00±1.0	7.5±1.0	85.35±1.0	48.33±0.05	24.30±0.05	8.09±0.02	66.70	404.00
	150	38.00±1.00	3.1±0.05	7.1±1.00	154.00±1.0	3.7±0.5	16.64±1.0	22.45±0.05	10.45±0.10	3.99±0.02	61.82	199.50
NAA	5	73.33±0.05	3.86±0.1	10.3±0.05	178.60±1.5	7.6±0.5	33.33±1.0	28.16±0.05	19.10±0.05	6.33±0.05	66.86	316.50
	50	81.33±0.05	4.0±0.0	10.3±0.05	182.00±1.5	8.3±1.0	100.00±1.0	43.00±0.05	25.60±0.05	8.20±0.05	67.96	410.00
	150	33.33±1.00	3.1±0.1	6.7±0.05	146.30±1.0	4.0±0.5	20.74±1.0	27.67±0.05	12.98±0.10	4.12±0.02	68.26	206.00
IBA	5	54.33±0.01	2.6±0.05	9.3±0.05	170.00±1.0	5.8±1.0	64.00±1.0	30.10±0.01	16.00±0.05	5.46±0.05	65.86	273.00
	50	64.00±0.05	2.6±0.05	9.3±0.05	175.67±1.5	7.0±1.0	80.00±1.0	39.00±0.10	19.99±0.05	7.98±0.02	60.08	399.00
	150	50.00±0.01	2.0±0.01	8.0±0,05	155.33±1.0	5.1±0.5	50.00±1.0	27.67±0.01	12.90±0.05	4.00±0.02	68.99	200.00
2, 4-D	1	48.33±0.10	1.1±0.05	2.6±0.05	40.00±1.5	2.6±0.0	64.70±1.5	6.80±0.05	6.67±0.05	2.48±0.02	62.82	124.00
	5	48.67±0.01	1.3±0.05	3.0±0.05	47.67±1.0	3.0±0.1	66.70±1.5	11.67±0.02	9.42±0.05	3.20±0.01	66.03	160.00
	50	35.16±0.10	1.1±0.0	2.0±0.0	29.67±1.0	2.1±0.0	33.50±1.5	5.00±0.02	5.67±0.02	2.00±0.0	64.73	100.00
	150	20.00±0.0	-	-	22.67±1.0	-	-	-	-	-	-	-
ANOVA (50ppm) treatment		233.62 *	10.68*	11.35*	10244.9*	98.11*	2378.2*	46.61*	104.92*	89.85*	133.83*	15171.7*

- Not detected; Value (mean±SE, n=9), * Significant at 1% level.

The EVP differed significantly among different root part of T1, T2, T3, T4, T5 and wild root of both the species. The EVP was higher in T1 than T2 and then T3, T4 and T5 respectively. The EVP of wild root was also measured and it was comparatively lower to T1, more or less equivalent to T2 and higher to T3, T4, T5 in *R. serpentina* while wild root of *R. tetraphylla* showed maximum EVP. Forty microliter of volume of sample concentration 10 mg/ml were applied for quantification of crude extract in methanol. Percentage of reserpine was observed using three replications for each set of experiment (Fig. 4). The sequence of different treatment conditions for the increase of alkaloid percentage of reserpine was found in descending order by T1, T2, wild root, T3, T4 and T5 in *R. serpentina* whereas in *R. tetraphylla* the trend was observed in order of wild root>T1>T2>T3>T4>T5 (Table 5).

Table 5. Effect of vermicompost, cowdung and NPK fertilizer on the chemical properties of soil in pot cultureof *Rauvolfia* species.

Period of sampling	Treatments	pH (H_2O)	N (%)	P (%)	K (%)	OC (%)
Before Planting	Soil Samples	6.67	0.0073±0.0010	0.0008±0.000	0.008±0.001	0.052±0.010
	T1	6.73	0.0079±0.0014	0.0054±0.0016	0.080±0.005	0.196±0.010
	T2	6.68	0.0072±0.0010	0.0034±0.0010	0.096±0.005	0.180±0.010
After Harvesting	T3	6.60	0.0067±0.0010	0.0030±0.0005	0.020±0.010	0.140±0.020
	T4	6.60	0.0070±0.0012	0.0026±0.0005	0.018±0.005	0.165±0.010
	T5	6.67	0.0063±0.000	0.0015±0.0001	0.013±0.005	0.137±0.005

Value (mean±SE, n=3).

Discussion

Organic manures not only increase available N, P, exchangeable K but also provide carbon and other constituents like Na, Mg, Ca that affect soil humus content, biological activity and soil physical structure (Ayeni, 2011, Pattnaik and Reddy, 2011). The use of vermicompost has been recognized generally as an effective means for improving soil aggregation, structure, aeration and fertility and it contains most of the nutrients in plant-available form such as nitrates, phosphates, exchangeable calcium and soluble potassium; increases beneficial microbial population, diversity and activity; and improves the soil moisture-holding capacity. It finally enriches valuable vitamins, enzymes and hormones (Aggelides and Londra1999, Albiach *et al*.,2000, Arancon *et al*., 2006, Azarmi *et al*.,2008, Bhasker 1992, Marinari *et al*.,2000, Mascolo *et al*.,1999, Orozeo *et al*.,1996, Prabha *et al*.,2007, Sailaja and Kumari 2002, Sainz *et al*.,1998, Suther 2009 , Tomati and Galli,1995).

Table 6. Effective value percentage and Reserpine percentage in root samples of *R. serpentina* and *R. tetraphylla*.

Sample	Age of the Plant (Year)		Effective Value (%)		Reserpine (%)	
	R. serpentina	*R. tertraphylla*	*R. serpentina*	*R. tetraphylla*	*R. serpentina*	*R. tertraphylla*
Wild root	-	-	8.52	8.20	0.5849	0.1515
T1	1	1	9.00	7.02	0.7441	0.1423
T2	1	1	8.68	7.00	0.5849	0.1195
T3	1	1	8.00	6.65	0.5091	0.0581
T4	1	1	6.98	6.00	0.1393	0.0139
T5	1	1	4.92	3.80	0.1077	0.0069
NAA	1	1		6.90		0.0678
IAA	1	1		6.65		0.0639
IBA	1	1		6.60		0.0540
2, 4-D	1	1		6.20		0.0508

Vermicompost increases microbial activity in the rhizosphere of plant in comparison to the plants supplemented with inorganic fertilizers or other type of organic fertilizers such as manure (Aira *et al*., 2010). Soil enzyme activity is also significantly increased by the addition of vermicompost at Research Farm of ICAR Research complex of NEH Region, Mizoram during experimental cultivation of *Solanum lycopersicum* L. as compared to equivalent rates of inorganic fertilizers (Azarmi *et al*.,2008). The decreased level of available nitrogen in T3 and T4 could be attributed to high rate of nutrient release in NPK fertilizer which makes N readily available and easily leached. Available phosphorus was also decreased in NPK combination as also reported by Asawalam and Onwudike (2011). Virekanand and Fixen (1990) have reported that the build up of phosphorus through application of vermicompost and cowdung is as a result of mineralization of organic phosphate due to microbial population and the production of organic acids, which make soil P available. Significant increase of potassium in T1 and T2 as compared to T3 and T4 may be attributed to the better buffer capacity of the soil as a result of the high organic matter content of manure (Meelu and Gill, 2001). Compared to control, there was significant increase in organic carbon of soil when vermicompost and cowpat was applied. Application of (3.7 t/ha) cowdung at Umudike (Nigeria) for the cultivation of *Ipomea batatas* significantly increased the soil organic matter more than other treatments like combination of cowdung + NPK and NPK alone (Asawalam and Onwudike, 2011). Application of 1 ton/hac cow dung increases soil organic matter by 0.025 % per annum (Asawalam and Onwudike, 2011).

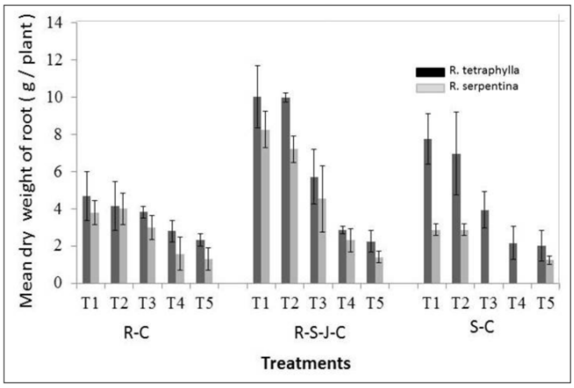

Fig. 1. Mean dry weight of root of three types of plant cutting of *R. serpentina* and *R. tetraphylla* under different treatment condition where RC = Root cutting, R-S-J-C = Root stem junction cutting, S-C= Stem cutting.

Stem cuttings of *R. serpentina* dried and did not gain root biomass. Only 12 % success with stem cuttings and 36% with root cuttings was also found earlier in *R. serpentina* (Hedayatullah,1959). The enhanced used of N in the form of triple dose under constant PK (T3) comparatively increased plant height, leaf number, branching, dry weight of root but still showed poor performance than plant treated under T1 and T2. Application of N+P+K fertilizer on stem cuttings of *R. tetraphylla* had also much poor impact regarding their survivality and rooting percentage. This clearly indicates that growth attributes of both the species were more sensitive to nutrient uptake. There are reports that the humic substances extracted from earthworm compost were capable of inducing root growth in maize plants by stimulation of the plasma membrane H^+ ATPase activity (Canellas *et al.*, 2002, Zandonadi, 2006). The induction of lateral root initiation by vermicompost derived humic substances in *Arabidopsis* has been also related to the activation of transcription of some auxin responsive genes (Trevisan *et al.*, 2010). It has been suggested that other vital nutrient present in organic fertilizer required for good growth are absent in NPK fertilizers hence shows limited residual effects on plant (Adejobi *et al.*, 2014, Asawalam and Onwudike 2011, Ajayi *et al.*, 2007, Ayeni, 2010).

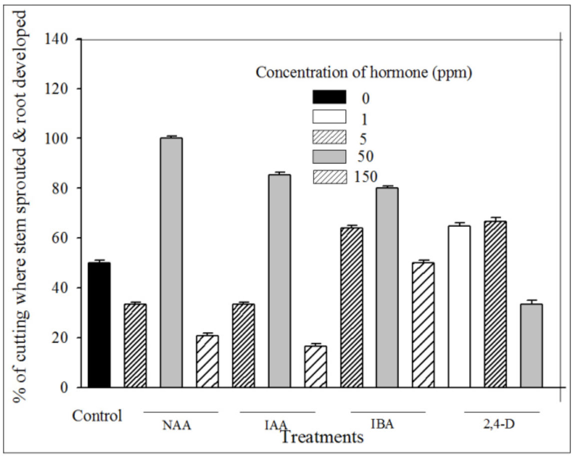

Fig. 2. Effect of concentrations of hormone on rooting percentage of *R. tetraphylla*, values (mean $\pm$ SE, n=9).

The positive effect of different concentrations of IBA, NAA, IBA+NAA and 2,4-D was reported for root propagule development of *R. serpentina* (Alamgir and Ahamed, 2005). Auxins might have played an important role in plant height, root length and rooting percentage (Alamgir and Ahamed 2005, Singh *et al.*, 2010).

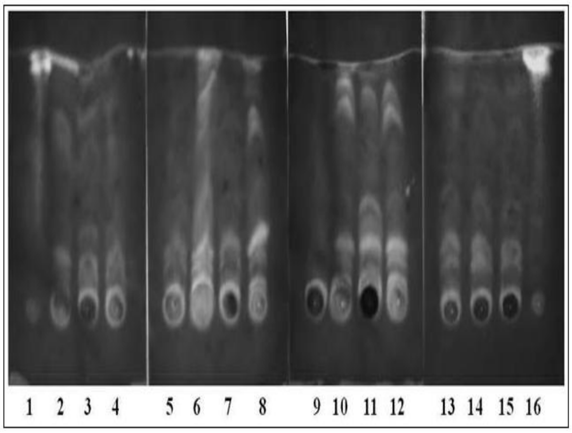

Fig. 3. TLC of methanolic extract of root part of *R.serpentina* and *R.tetraphylla* under different treatment conditions showing presence of reserpine (Track 1:Standard;Track 2: *R.serpentina*(T1);Track 3: *R.tetraphylla*(T1);Track 4*: R.serpentina*(T3);Track 5: *R.tetraphylla*(T3);Track 6*: R.serpentina*(T2);Track 7: *R.tetraphylla*(T2);Track 8: *R.serpentina*(T5);Track 9: *R.tetraphylla*(T5);Track10: *:R.serpentina*(WR);Track 11: *R.tetraphylla*(WR);Track 12: Not of use; Track 13:Not of use;Track 14: *R.tetraphylla*(IAA);Track 15: *R.tetraphylla*(NAA);Track 16: standard).

Alkaloid content was increased more when N level was increased under constant PK level as in T3>T4 condition. Dry mass of *Rauvolfia serpentina* increases at the elevated NPK and N level during combined application of cowdung and NPK and it was accompanied by the total crude alkaloid contents (Alamgir and Ahamed, 2005). The combined application of cow dung and N+P+K fertilizer significantly increased soil nitrogen from 0.09 %–0.16 % at Umudike (Nigeria) since the complementary use of these fertilizers reduces N loss from soil thereby increasing available nitrogen in the soil. In the treatment condition of T1 and T2 where percentage of reserpine was found comparatively greater in comparison to T3 and T4 also indicates the elevated N level could be due to high rate of nutrient release in NPK fertilizer, which makes N readily available and easily leached hence did not significantly increased soil nitrogen in comparison to

T1 and T2 condition (Asawalam and Onwudike 2011, Reijntjes *et al.*,1992). The application of vermicompost may produce a significantly greater increase in the abundance of N-fixers, actinomycetes and spore formers than in soil supplemented with inorganic fertilizers (Kale *et al.*,1992).

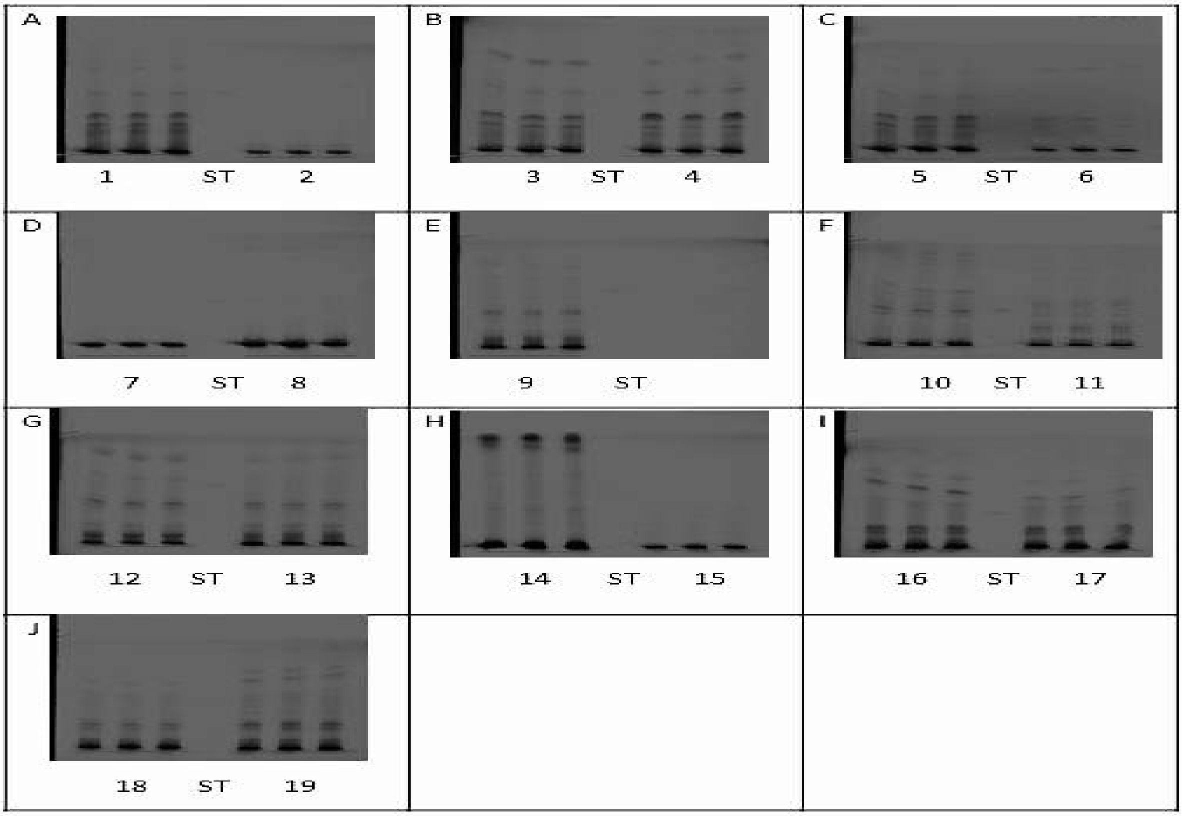

Fig. 4. Fingerprinting chromatogram of root part (collected from one year old plant) of *R.serpentina* and *R. tetraphylla* under different treatment conditions in three sets of replication indicating presence of reserpine(ST-standard). (A) 1: *R serpentina* (WR) ; 2: Not of use, (B) 3*: R serpentine* (T2); 4: *R serpentina* (T1), (C) 5: *R serpentina* (T3); 6: Not of use, (D) 7: *R.tetraphylla* (T4) 8: *R serpentina* (T4), (E) 9: *R serpentina*(T5), (F) 10: Not of use, 11: *R. tetraphylla* (WR), (G) 12-:*R.tetraphylla* (T1); 13: *R.tetraphylla* (T2), (H)14:*R.tetraphylla*(T3) ;15: *R. tetraphylla* (T5), (I) 16: *R.tetraphylla* (IAA); 17: *R.tetraphylla* (NAA), (J) 18:*R.tetraphylla* (IBA);19:*R.tetraphylla* (2,4-D) [T1-Vermicompost, T2-Cowpat, T3-3N+P+K, T4- N+P+K, T5-Control].

Conclusions

The use of both organic manures and chemical fertilizers significantly enhanced *R. serpentina* and *R. tetraphylla* growth parameters, fresh and dry root weight yield and root biomass increased percentage. However, the addition of organic manure like vermicompost and cowpat as nutrient sources showed, promising effect on both species root yield percentage as well as percentage of reserpine comparable to NPK fertilizer. Hence, these manures either separately or with NPK fertilizer are advised for the use of *Rauvolfia* plant cutting establishment but, NPK must be used in combination with manures.

Acknowledgements

We thank Prof. S.N.Sharma, Head, Department of Botany, P.U. for providing laboratory facilities and Director, NIPER Chandigarh for quantitative analysis by HPTLC. This work was supported by Department of Science and Technology (DST) under Women Scientist Scheme-A (WOS-A) Government of India vide Sanction Order No.100/(IFD)/8060/2011-12.

References

Adejobi KB, Akanbi OS, Ugioro O, Adeosun SA, Mohammed I, Neluka BA, Adeniyi DO. 2014. Comparative effects of NPK fertilizer, cowpea pod husk and some tree crops wastes on soil, leaf chemical properties and growth performance of cocoa (*Theobroma cacao* L.). African Journal of Plant Science **8,** 103-107.

Aggelides SM, Londra PA. 1999. Effect of compost produced from town waste and sewage sludge on the physical properties of a loamy and a clay soil. Bioresource Technology **71,** 253–259.

Aira M, Gomez–Brandon M, Lazcano C, Baath EJ. 2010. Bominyuez, Plant genotype strongly modifies the structure and growth of maize rhizosphere microbial communities. Soil Biology and Biochemistry **42,** 2276–2281.

Ajayi CA, Awodun MA, Ojeniyi SO. 2007. Comparative effect of cocoa pod husk ash and NPK fertilizer on soil and nutrient content and growth of Kola seedlings. International Journal of Soil Science **2,** 148–153.

Alamgir ANM, Ahamed M. 2005. Growth and Phytochemical investigation of *Rauvolfia serpentine* Benth. propagule. Bangladesh Journal of Botany **34**, 7–10.

Albiach R, Canet R, Pomares F, Ingelmo F. 2000. Microbial biomass content and enzymatic activities after application of organic amendments to a horticultural soil. Bioresource Technology **75,** 43–48.

Arancon NQ, Edwards CA, Bierman P. 2006. Influences of vermicompost on field strawberries: Part 2. Effects on soil microbiological and chemical properties. Bioresource Technology **97,** 831–840.

Asawalam DO, Onwudike SU. 2011. Complementary use of cowdung and mineral fertilizer: Effect on soil properties, growth, nutrient uptake and yield of sweet potato (*Ipomea batatas*). PAT **7** , 36–48.

Ayeni LS. 2010. Integrated application of cocoa pod ash and NPK fertilizer. Effect on soil and plant nutrient status and maize performance, Field experiment, Journal of American Sciences **6,** 96–102.

Ayeni LS. 2011. Cumulative effect of combined cocoa pod ash, poultry manure, NPK 20:10:10 fertilizer on major cations release for crop production in southwestern Nigeria. International Journal of Agriculture and Soil Science **1,** 248–253.

Azarmi R, Giglou MT, Taleshmikail RD. 2008. Influence of vermicompost on soil chemical and physical properties in tomato field. African Journal of Biotechnology **7,** 2397–2401.

Badhwar RL, Kasira GV, Ramaswami. 1956. *Rauvolfia serpentina*:methods of propagation and their effect on root production. Indian Journal of Pharmacy **18,** 170–175.

Benckiser G, Simarmata T. 1994. Environmental impact of fertilizing soils by using sewage and animal wastes. Fertilizer Research **37,** 1–22.

Bhasker A, Macgregor AN, Kirkman JH. 1992. Influence of soil ingestion by earthworms on the availability of potassium in soil : an incubation experiment. Biology and Fertility of Soils **14,** 300–303.

Canellas LP, Olivares FL, Okorokova AL, Facanha AR. 2002. Humic acids isolated from earthworm compost enhanced root elongation, lateral root emergence, and plasma H^+ – ATPase activity in maize roots. Plant Physiology **130,** 1951–1957.

Chandra V. 1956. Inducing rooting in stem cuttings of *Rauvolfia canescens* L. Science and Culture **22,** 101.

Hedayatullah S. 1959. Culture and propagation of *Rauvolfia serpentina* Benth. in East Pakistan. Pakistan Journal of Science and Industrial Research **2,** 118–122.

Kale RD, Mallesh BC, Kubra B, Bagyaraj DJ. 1992. Influence of vermicompost application on the available macronutrients and selected microbial populations in a paddy field. Soil Biology and Biochemistry **24,** 1317–1320.

Kuo S. 1996. Methods of soil analysis part 3–Chemical Methods,Phosphorus,In : D.L.Sparks (Editor),SSSA Book Series 5, Madison Wisconsin USA 869–920 P.

Marinari S, Masciandaro G, Ceccanti B, Grero S. 2000. Influence of organic and mineral fertilizers on soil biological and physical properties. Bioresource Technology **72,** 9–17.

Mascolo A, Bovalo F, Gionfriddo F, Nardi S. 1999. Earthworm humic matter produces auxin like effects on *Daucus carota* cell growth and nitrogen metabolism. Soil Biol Biochem **31,** 1303–1313.

Meelu OP, Gill HS. 2001. Studies on the substitution of inorganic fertilizer with organic manure and their effect on soil fertility in rice–wheat rotation. Fertilizer Research **3,** 303–314.

Nelson DW, Sommers LE. 1996. Total Carbon,Organic Carbon and Organic Matter,In : D.L.Sparks(Editor),Method of soil analysis part 3–Chemical Methods,SSSA Book Series 5, Madison Wisconsin USA. 961–1010 P.

Orozeo FH, Cegarra J, Trujillo LM, Roig A. 1996.Vermicomposting of coffee pulpusing the earthworm *Eisenia foetida* : effects on C and N contents and the availability of nutrient. Biology and Fertility of Soils **22,** 162–166.

Panwar GS, Guru SK. 2011. Alkaloid profling and estimation of reserpine in *Rauvolfia serpentine* plant by TLC, HPTLC and HPLC. Asian Journal of Plant Science **10,** 393–400.

Pattnaik S, Vikram Reddy N. 2010. Nutrient status of vermicompost of urban green waste processed by three earthworm species. *Eisenia fetida, Eudrillus eugeniae* and *Perioinyx excavatry*. Applied and Environmental Soil Science 1–13.

Prabha KP, Loretta YL, Usha RK. 2007. An experimental study vermin–biowaste composting for agricultural soil improvement. Bioresource Technology **99**, 1672–1681.

Reijntjes C, Haveskort B, Waterbayer A. 1992. Farming for future : An introduction to low external inputs and sustainable Agriculture, Mcmillan. Leuden : ILEIA, Netherlands, 250 P.

Sailaja Kumari MS, Usha Kumari K. 2002. Effect of vermicompost enriched with rock phosphate on the yield and uptake of nutrients in cowpea (*Vigna unguiculata* L.Walp.). Journal of Tropical Agriculture **40**, 27–30.

Sainz MT, Taboada–Castro MT, Vilarino A. 1998. Growth, mineral nutrition and mycorrhizal colonization of red clover and cucumber plants grown in a soil amended with composted urban waste. Plant and Soil **205,** 85–92.

Sangram KP, Pattanayak P, Oraon A, Pravat KP. 2010. Quantitative estimation of reserpine in different parts of *R. serpentina* and *R. tetraphylla* by using HPTLC. Asian Journal of Plant Science and Research **2,** 151–162.

Sarika Deshmukh R, Dhanashree Ashrit S, Bhausaheb Patil A. 2012. Extraction and evaluation of indole alkaloids from *Rauvolfia serpentina* for their antimicrobial and antiproliferative activities. International Journal of Pharmacy and Pharmaceutical Sciences **4,** 329–334.

Singh BK, Pathak KA, Boopathi T, Deka BC. 2010. Vermicompost and NPK fertilizer effects on morpho–physiological traits of plants, yield and quality of tomato fruits (*Solanum lycopersicum* L.). Vegetable Crops Research Bulletin **73,** 77–86.

Subbiah BV, Asija GL. 1956. A rapid procedure for determination of available nitrogen in soils.Current Science **25,** 259–260.

Suther S. 2009. Earthworm communities a bioindicator of arable land management practices: a case study in semi arid region of India. Ecological Indicators **9,** 588–594.

Thomas GW. 1996. Soil pH and soil acidity,In: D.L.Sparks(Editor), Methods of soil analysis part 3– Chemical Methods, SSSA Book Series 5, Madison,Wisconsin, USA, 475–490

Tomati U, Galli E. 1995. Earthworm, soil fertility and plant productivity. Acta Zoologica Fennica **196,** 11–14.

Trevisan S, Pizzeghello D Ruporti B, Francioso O, Sassi A, Palme K, Quaggiotti S, Nardi S. 2010. Humic substances induced lateral root formation and expression of the early auxin responsive IAA 19 gene and DR5 synthetic element in *Arabidopsis*. Plant Biology **12,** 604–614.

Varadranjan PD. 1963. Climatic and soil conditions of *Rauvolfia serpentina* in India. Economic Botany **17,** 111–115.

Vivekananda M, Fixen PE. 1990. Effect of large manure application on soil P intensity,Comun.Soil Plant Analysis **21,** 287-293.

Zandonadi DB, Canellas LP, Facanha AR. 2006. Indolacetic and humic acids induce lateral root development through a concerted plasma lemma and tonoplast H^+ pumps activation. Planta **225,** 1583–1595.