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APPLICATION OF NITROGEN, SULPHUR AND NEEM CAKE IMPROVES BIOMASS AND YIELD OF QUERCETIN IN A MEDICINAL PLANT, *PLUCHEA LANCEOLATA*

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SUMMARY

Field experiments were conducted to study the effect of nitrogen, sulphur and neem cake on biomass and yield of active constituent, quercetin in a medicinal plant, *Pluchea lanceolata.* Application of urea at the rate of 60, 100 and 150 kg ha^{-1} significantly enhanced both, biomass as well as quercetin. The combined effect of sulphur and nitrogen along with neem cake proved better than N (urea) alone. Our results indicate that a judicious and balanced application of inorganic fertilizers, and/or neem cake can be effective in increasing the productivity of medicinal herbs.

***Key words* : Biomass, neem cake, nitrogen, *Pluchea lanceolata,* quercetin, sulphur.**

INTRODUCTION

Owing to their wide distribution and easy availability medicinal plants did not require domestication in the past. Their use and practice registered a decline due to the development of synthetic drugs and antibiotics. But, the reported toxicity and harmful side effects associated with synthetic drugs brought the herbal system of cure back to the forefront (Srivastava and Pande 1998). Consequently, the demand for herbal raw material has increased enormously. Barring a few, majority of medicinal plants are collected from wild sources and negligible systematic effort has been made for developing package of practices for their cultivation, maintenance and optimum extraction of material for making drugs. Because of rapid depletion of medicinal plants in natural populations, large scale plantations are required (Srivastava *et al.* 2002). New technologies in agriculture ushered the "Green revolution" in which use of high yielding varieties, improved agricultural practices and proper fertilizer management have played a significant role. Unfortunately medicinal plants have not received the attention they deserve. Nitrogen is most important among all the major elements required to boost the yield of a number of plants. But , a major part of fertilizer N is lost through leaching or denitrification after its conversion to nitrate (Bronson *et al* 1989, Mader *et al*.2002). These losses account for the low fertilizer N use efficiency by crops (Frye *et al.* 1989). However, Loss of ammonium-N can be reduced by delaying the conversion of ammonium to nitrate through the use of nitrification inhibitors (Serna *et al.* 1996). Nitrification inhibitors thus, not only reduce fertilizer N losses, but also improve its uptake as well as N-use efficiency (Norman *et al.* 1989, Prasad and Power 1995). Nitrification inhibiting properties of neem seed and its cake after oil extraction have been reported by Jat and Pal (2002) and neem cake coated urea has proved superior to prilled urea (Majumdar *et al.* 2000).Neem (*Azadirachta indica*) cake has good nitrification inhibition capacity due to the presence of alkaloids-solanin, meliantrool, azadiractin, nimbin and nimbidin that possess a furan ring system (Jotwani and Srivasatva 1981). Neem

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cake when mixed with soil also reduces alkalinity by producing organic acids. Neem cake contains 3.56% nitrogen, 0.83% phosphorus, 1.67% potassium, 0.77% calcium and 0.75% magnesium.

Pluchea lanceolata, a medicinal plant commonly known as "Rasana' is a small herb found in Punjab, Rajasthan, West Bengal and Uttar Pradesh in India. It is used in the indigenous system of medicine as an antipyretic, analgesic, laxative and nervine tonic (Anonymous 1998). It is also effective in the treatment of dyspepsia, rheumatoid arthritis and bronchitis (Srivasatva *et al.* 1990).The aerial parts of the plant contain the active constituent, quercetin (flavanoid), a radical scavenger (Inderjit *et al.* 1998). Quercetin has anti-gonadotropic, anti-inflammatory,-bacterial, -viral and -hepatotoxic properties. Keeping in view its medicinal importance and increasing exploitation from the natural sources, an attempt was made in the present study to evolve a nutrient management technology for optimizing and enhancing the biomass as well as yield of quercetin.

MATERIALS AND METHODS

Rasana (*Pluchea lanceolata* (DC) Clarke) plants were grown in the experimental field at Hamdard University, New Delhi, India, located at $28^0 38'$ N, $77^0 11'$ E and 228 m asl. The weather conditions of the location are semi-arid with sandy loam soil. The soil characteristics were: sand 63.8%, silt 18.2%, clay 17%, organic carbon 0.34%, available S 0.002%, N 0.07%, P 42.0 and K 117.0 µg g^{-1}soil and pH7.8. The treatments included different doses of fertilizers after 30 days when the plants were at full vegetative growth (15 cm tall). The fertilizer treatments included three levels of N applied in the form of urea, 60, 100 and 150 kg ha^{-1} (containing 46% N), and 30 kg $ha^{-1}$of gypsum that contains 13% S along with neem cake at the rate of 20 or 40% of urea . One hundred kg of commercial grade urea containing 46% N was coated with 20 or 40 kg of neem cake in a fertilizer mixing drum. Neem cake blended urea was prepared by dissolving coal-tar in kerosene in 1:2 w/v ratio and added to 100g urea and mixed thoroughly. Ground and sieved neem cake was then added to urea at 20 or 40% and mixed by shaking. The neem cake contains 4.7% N and thus the amount of N added through neem cake was only 2 and 4%, respectively of total N applied per treatment; this is, however, an organic N which mineralizes slowly (Sharma and Prasad 1996). Benzene hexachloride (BHC) was applied during preparation of beds to protect the plants from termites. Hand weeding was done regularly. One uniform irrigation was provided before sowing and subsequently when required. The experiments were laid out in randomized block design plots with three replicates of each treatment. The individual plot size was $9m^2 (3\times3m)$ laid with proper beds along with necessary irrigation channels.

The sampling was performed 30,45 and 60 days after fertilizer treatment. Twelve plants were taken out randomly from each plot, washed properly and cut at root-shoot level to evaluate growth parameters such as dry weight of shoots and leaf area, Chlorophyll a and b, protein content, and photosynthetic rate (Pn) were determined in the third leaf from the top. Total soluble protein content in leaf was estimated by Bradford method (1976). Chlorophyll a and b were estimated according to Hiscox and Israelstam (1978) by using Dimethyl sulphoxide (DMSO). The rate of photosynthesis was measured by using IRGA (Infra-red gas analyzer, Li-COR 6400, Lincoln, Nebraska, USA) and carried out under controlled light (photosynthetic active radiation: 1000 µmol m^{-2} s^{-1}) and CO_2 (incoming CO_2 concentration: 360 µmol mol^{-1}) conditions. The data of different treatments was analyzed using Tukey's test, usually referred to as honestly significant difference (HSD). The significance of treatments between the pairs are given in brackets at the base of the tables.

Quercetin analysis

Reverse phase HPLC (Perkin- Elmer) was performed with solvents A (2% HOAc) and B (MeOH-HOAc-H_2O, ratio: 18:1:1) in the ratio of 30:70 at a flow rate of 1.0 ml min^{-1}as mobile phase maintained by binary pump (Perkin-Elmer-LC250). Separation was done in C_{18} peco sphere column with functional surface of octadecy1 dimethy1 silane (particle size, 3µm, column dimension, 83×4.6mm). A diode array UV detector (Perkin-Elmer-LC235) was used at 245nm, sensitivity 0.5 AUFS (absorbance unit full scale) and bandwidth 5.The plotting and peak area calculations were accomplished with an integrator (Perkin-Elmer-LCI 100). The extracted and subsequently dried samples (1mg) were dissolved in 1ml of MeOH for HPLC analysis. The aliquots were filtered with 0.22 µ syringe filter. Filtrate (20 µl) was injected in the HPLC column. The areas of the relevant peaks of each sample were interpolated with the standard curve to determine the quantity of quercetin (in µg mg^{-1} dry wt). From stock solution (1mg ml^{-1}) of quercetin prepared in methanol, dilutions of 2-20 µl were taken in duplicates and analyzed independently by HPLC and a standard curve was plotted between concentration and peak area. The injected quantities and peak area showed good linearity.

RESULTS AND DISCUSSION

Dry weight of plants recorded after 45days of treatment showed increase to the extent of 32.68% with 100 kg urea ha^{-1}. Together with 30 kg gypsum ha^{-1} 48.06%improvement was observed. Urea, 100 kg ha^{-1}, blended with 20% of neem cake resulted in 45.91% increase in dry weight, addition of 30 kg gypsum ha^{-1}, caused 82.15% increase (Table 1). Application of urea 60,100 and 150 kg ha^{-1} alone resulted in 158, 178 and 169% increase in leaf area in 45-day-old plants, respectively. The increase was 207 and 198%, respectively with 100 kg ha $^{-1}$ urea blended with 20 or 40% neem cake. Urea 100kg ha^{-1} blended with 20% neem cake along with 30 kg gypsum ha^{-1} resulted in 235% increase (Fig.1). The increase in chlorophyll a content as influenced by 100 and 150 kg urea ha^{-1} was 127 and 112%, respectively. Corresponding figures for

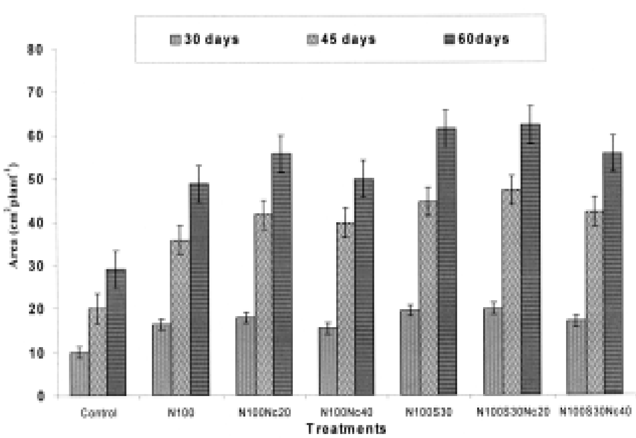

Fig. 1. Leaf area of *Pluchea lanceolata* after treatment with nitrogen, sulphur and neem cake.

chlorophyll b were 87 and 81%. Urea blended with 20 or 40% neem cake proved better. With 20%, chlorophyll a content increased by 174% and chlorophyll b by 99% after 45-days. Gypsum (30g ha^{-1}) along with 20% neem cake-blended urea resulted in increment in chlorophyll a content by 200 and in b by 122%. Similar trend was observed for other parameters. Soluble protein (Table 2), sulphur content (Table 3), photosynthetic rate (Table 4) and quercetin content (Fig. 2), all showed enhancement under different combination of fertilizers used.

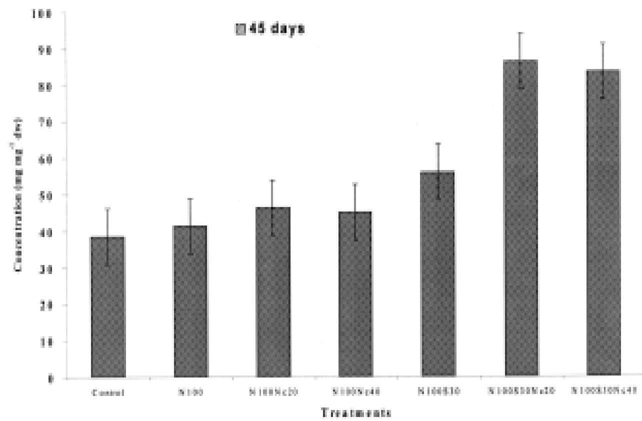

Fig. 2. Quercetin content in *Pluchea lanceolata* after treatment with nitorgen, sulphur and neem cake.

Efficient N utilization is an essential goal in crop management. One of the recent achievements is the use of inhibitors which retards nitrification for sufficiently longer time leading to reduction in the leaching loss of nitrate-N and improving N uptake (Jat and Pal 2002). The effect of nitrification inhibitors, neem cake and dicynamide (DCD) on the efficiency of applied prilled urea nitrogen in a maize-wheat cropping system has been reported by Sharma and Prasad (1996). Walters and Malzer (1990) observed that N use efficiency increased by using inhibitors when the potential for nitrate leaching was high. Nitrification inhibiting properties of neem seed and its cake after oil extraction have been recorded by Prasad *et al.* (2001). Neem cake coated urea has also proved superior to prilled urea for rice (Jat and Pal 2002). Our results with *Pluchea* prove that urea applied with neem cake not only significantly enhanced the growth performance but also the yield of secondary metabolite, quercetin. Still better yield of quercetin could be achieved when N was combined with S along with neem cake.

Table 1. Dry weight (g/plant) of shoots of *Pluchea lanceolata* after application of urea or neem cake (Nc) blended urea in the persence of sulphur*.

	Neem cake 0			Neem cake 20%			Neem cake 40%		
Treatments kg ha^{-1}	**Days after treatment 30**	**45**	**60**	**Days after treatment 30**	**45**	**60**	**Days after treatment 30**	**45**	**60**
Control	7.2±0.5	8.56±0.4	9.3±0.8						
N 60	8.23±0.8	9.45±0.5	11.12±0.2	8.51±1.5	9.58±1.5	9.78±1.3	8.28±1.4	9.56±1.2	10.45±1.2
N 100	10.25±1.1	11.54±1.7	12.34±2.1	10.56±2.2	11.81±0.6	13.57±1.2	10.47±0.4	11.57±0.4	12.97±1.3
		(1)			**(2)**			**(3)**	
N 150	9.20±1.8	10.48±0.5	12.74±2.3	9.63±1.5	10.72±1.3	12.78±1.5	9.09±1.7	10.21±1.8	12.67±0.9
	S30 + Nc 0			**S30 + Nc 20%**			**S30 + Nc 40%**		
N 60	8.34±0.5	9.87±0.6	11.65±0.7	9.66±1.3	10.77±1.0	11.78±1.2	10.88±1.3	11.74±1.7	12.38±1.0
N 100	10.43±2.1	12.75±1.5	13.77±1.0	11.88±1.4	14.46±1.4	16.94±2.1	11.99±1.0	12.84±0.2	13.37±0.5
		(4)			**(5)**			**(6)**	
N 150	9.66±0.8	10.54±1.2	12.62±1.3	10.77±1.5	12.82±1.3	13.45±2.0	9.45±0.9	10.66±1.4	12.65±1.1

N = urea, S = sulphur, Nc = neem cake

*HSD = 2.41, at 5% level of significance all pairs of treatments are significant except (1) & (3), (1) & (6), (2) & (3), (2) & (4), (2) & (6), (3) & (4), (3) & (6), and (4) & (6).

Table 2. Protein content (mg g^{-1} fw) in the leaves of *Pluchea lanceolata* after application of urea or neem cake blended urea in the persence of sulphur*.

Treatments kg ha^{-1}	**Neem cake 0** Days after treatment 30	45	60	**Neem cake 20%** Days after treatment 30	45	60	**Neem cake 40%** Days after treatment 30	45	60
Control	24.15±1.2	33.73±1.0	30.00±1.1						
N 60	37.43±1.5	65.81±1.2	52.50±1.2	36.22±1.2	75.60±1.1	63.00±1.2	35.98±1.1	73.23±1.5	60.00±1.1
N 100	45.88±1.3	75.33±0.6	62.40±0.8	54.33±1.0	87.41±1.3	74.10±1.2	49.99±1.0	81.67±2.2	69.00±1.4
					(1)			**(2)**	
N 150	43.17±0.9	67.50±1.0	58.20±1.1	53.13±0.8	84.37±1.3	72.00±1.5	52.40±1.8	83.02±2.1	70.02±1.2
	S30 + Nc 0			**S30 + Nc 20%**			**S30 + Nc 40%**		
N 60	43.47±1.0	74.25±1.1	63.60±1.0	45.12±1.3	79.46±1.7	65.48±1.8	38.64±1.8	83.36±1.4	69.20±1.6
N 100	48.30±1.0	80.66±1.5	68.10±1.5	58.54±1.4	90.65±1.5	78.63±1.6	54.82±1.0	88.42±1.3	75.90±1.5
		(3)						**(4)**	
N 150	43.17±1.9	67.50±0.8	58.20±0.7	54.85±1.7	86.23±1.2	75.03±1.7	56.02±1.2	90.00±1.7	79.00±2.1

N = urea, S = sulphur, Nc = neem cake

*HSD = 3.84, at 5% level of significance all pairs of treatments are significant except (1) & (4), (2) & (3).

Table 3. Sulphur content (µmol g^{-1} dw) in the stem of *Pluchea lanceolata* after application of urea or neem cake blended urea in the persence of sulphur*.

Treatments kg ha^{-1}	Neem cake 0			Neem cake 20%			Neem cake 40%		
	Days after treatment 30	45	60	Days after treatment 30	45	60	Days after treatment 30	45	60
Control	6.60±0.5	7.10±0.6	7.00±1.2						
N 60	7.20±1.4	9.00±1.6	7.85±1.5	8.00±0.8	10.20±1.4	9.45±1.0	7.00±1.0	8.89±0.2	7.65±0.8
N 100	11.45±1.6	13.97±1.4	12.85±1.4	12.05±0.5	14.22±1.0	13.75±1.2	11.75±1.8	14.00±1.7	13.15±0.4
		(1)			**(2)**			**(3)**	
N 150	10.10±1.0	13.43±1.6	12.27±1.2	10.90±1.5	13.73±1.7	13.00±1.2	11.80±1.3	14.05±0.8	13.65±0.5
	S30 + Nc 0			**S30 + Nc 20%**			**S30 + Nc 40%**		
N 60	10.90±2.1	13.86±1.2	12.97±1.5	11.15±1.0	14.93±1.2	12.25±1.6	8.78±1.4	10.08±1.7	9.20±1.8
N 100	15.00±1.7	17.49±1.9	16.90±1.4	16.45±1.7	18.00±1.8	17.45±1.5	15.25±1.9	17.02±1.9	16.45±1.5
		(4)			**(5)**			**(6)**	
N 150	12.15±2.0	14.35±1.9	13.75±1.8	13.00±1.2	14.55±1.3	13.75±1.4	15.40±1.7	17.44±1.5	16.80±1.2

N = urea, S = sulphur, Nc = neem cake

*HSD = 3.80, at 5% level of significance all pairs of treatments are significant except (1) & (2), (1) & (3), (2) & (3), (4) & (5), (4) & (6), and (5) & (6).

Table 4. Photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) in the leaves of *Pluchea lanceolata* after application of urea or neem cake blended urea in the persence of sulphur*.

	Neem cake 0			Neem cake 20%			Neem cake 40%		
	Days after treatment			Days after treatment			Days after treatment		
Treatments kg ha^{-1}	**30**	**45**	**60**	**30**	**45**	**60**	**30**	**45**	**60**
Control	6.21±1.0	8.00±1.8	7.75±.8						
N 60	9.62±2.1	16.00±0.7	14.57±1.0	9.87±0.5	18.64±1.6	17.36±1.2	9.31±1.2	18.16±1.3	16.58±1.5
N 100	11.30±1.1	19.04±1.5	17.05±1.2	12.29±1.5	21.52±1.4	19.60±1.2	10.55±0.5	19.76±1.0 **(1)**	18.29±1.2
N 150	10.74±2.3	18.40±1.3	16.50±1.1	11.61±1.4	20.00±1.2	18.91±08	11.24±1.4	19.20±1.5	18.67±1.5
	S30 + Nc 0			**S30 + Nc 20%**			**S30 + Nc 40%**		
N 60	9.93±0.2	17.60±0.8	15.50±0.5	10.24±1.5	19.76±1.0	18.52±1.2	10.06±1.4	19.96±1.2	17.82±1.3
N 100	12.23±1.5	20.00±1.1 **(2)**	18.21±1.5	13.35±1.1	22.16±0.3	20.53±1.2	12.48±1.1	20.56±0.7 **(3)**	18.60±1.6
N 150	11.61±1.2	19.36±1.6	18.13±1.5	12.91±1.2	21.12±1.5	19.76±1.6	12.73±1.0	20.88±1.4	19.29±1.5

N = urea, S = sulphur, Nc = neem cake

*HSD = 1.80, at 5% level of significance all pairs of treatments are significant except (1) & (2), (2) & (3).

Studies have demonstrated the synergistic effect of combined application of S and N (Jaggi *et al.*1977, Eriksen *et al.* 2002, Huang *et al.* 2002). Randall *et al.* (1981) observed increased yield of wheat grains with S application. Sulphur is reported to favour dry matter accumulation and yield components due to proper partitioning of photosynthates from source to Sink (Legha and Giri 1999). The present investigation also confirms that S X N interaction induces better response in comparison to nitrogen alone.

Integrated effect of both organic and inorganic fertilizers has proved quite promising not only in maintaining higher productivity but also in providing maximum stability in crop production in comparison to minerals N, P and K fertilizers alone (Pospisil *et al.* 2000, Clark and Richardson 2002). Our results demonstrate that application of urea along with organic supplements can significantly increase the growth performance coupled with increase in quercetin content. It is thus apparent that balanced application of organic supplement and inorganic fertilizers significantly improves growth performance and yield of secondary metabolite content in medicinal plants. With resurgence of herbal system of medicine, it is imperative that sustained efforts are made to enhance the yield of drug component. Our report is one of the few attempts in this direction.

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