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IN-VIVO NITRATE REDUCTASE ACTIVITY IN THE *MYRICA ESCULENTA* BUCH. HAM. D.DON SEEDLINGS UNDER NURSERY CONDITIONS

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Abstract: *Myrica esculenta* locally known as *kafal*, is a dioecious, moderate sized, evergreen tree species. It is a characteristic associate of *Quercus leucotrichophora* and *Rhododendron* species between 1000-2200 m above sea level and valued for its wild edible fruits used in different preparations. An experiment was conducted under pot culture conditions to study the effects of different nitrogen fertilizer doses (*i.e.* 20; 40; 20 and control without fertilizer) on the *in-vivo* nitrate reductase activity (NRA) in different plant parts. Nitrogen doses were applied in two equal split between fifteen days intervals. Monthly nitrate reductase activity was estimated in different plant parts viz., leaf, stem and root for a period of twelve months. It was observed that maximum NRA was recorded in the 80 kg N/ha followed by 40 kg N/ha, 20 kg N/ha and minimum in control treatment in different plant parts as well as in total plant also. On the seasonal NRA a higher NR activity was recorded during rainy followed by summer and lowest in winter season. Seasonal effects were significantly different as compared to seasons x treatments. However, on monthly analysis basis, months and treatment effects in leaf, stem, root and total plant NR activity was significantly different among each other. However, for all the parameters studied months x treatments were found significantly different at 5% level.
Keywords: Fertilizer doses, *In-vivo* nitrate reductase activity, *Myrica esculenta*; Seasonal changes.
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INTRODUCTION

Forest soil is generally deficient in nitrogen (0.01 to 0.02%) and its non-availability is the limiting factor for the growth and development of plant kingdom. It is required substantially in larger quantity than other elements and occupies a prominent position in plant nutrition. The N reduced by the plant parts is incorporated into proteins and subsequently degraded and mobilized to other developing parts. Nitrate is one of the major sources of N in higher plant, translocated to the shoot, store in vacuole and assimilate into reduced N products (Black *et al*. 2002; Jabeen and Ahmad, 2011). The process of nitrate uptake, translocation and assimilation are interdependent and closely regulated in higher plants (Huber *et al*., 1996; Sivasankar and Oaks, 1996). Nitrate reductase (NR) catalyzes the first committed step of nitrate assimilation (Crawford *et al.,* 2000). Because of importance of N to plant productivity, nitrate assimilation and allocation patterns have been studied in many genera of tropical annuals and perennials plants. The importance of shoots and roots in nitrate assimilation was earlier assessed by the various workers in different tree species (Pandey, 2002; Mir; 2012, Bhat, 2012; Shams, 2013; Kumar, 2013 and Chaukiyal *et al*., 2014). Among different trees *Myrica esculenta* is a small moderate sized evergreen, 3-15 m. high, found in sub tropical Himalayas from Ravi eastwards to Assam and in Khasi Jaintia, Naga and Lushai hills at altitudes of 900 to 2100 m. The bark is reported to be used in Khasi hills as a fish poison (Chopra, 1958). The fruit of the plant are edible and have a pleasant sourish sweet taste used in the preparation of a refreshing drink called *sherbet*. The tree gives seasonal employment to locals at the time of fruiting. In a roughly estimated data one *gram sabha* (approximately having 500 families) earned Rs. 5, 00000 in one season. This tree not only gives the employment to rural peoples but also fixes atmospheric nitrogen (Pokhriyal *et. al.,* 1993) in the soil and ultimately enriches the soil nitrogen status. This is an important tree of the hill area; the natural regeneration of the tree is poor due to hard seed coat. Due to its multifarious uses, the nitrogen assimilating behavior is to be studied in respect to application of different doses of fertilizer to increase the growth and nitrogen uptake in different month of the year so that this species can be recommended for the various afforestation programmes.

EXPERIMENTAL

Myrica esculenta seedlings were grown in the nursery of Plant Physiology Discipline Botany Division Forest Research Institute Dehradun. The seeds were sown in perforated plastic trays filled with soil mixture (2:1:1 ratio soil, sand and farmyard manure) and after attaining the height of 8-10 cm were transplanted in the soil mixture filled earthen (30 cm diameter) pots. Plants were fertilized with N as urea @ 20, 40 80 Kg N/h^{-1} and control (0kg N/ha) without nitrogen treatment in two equal split doses. Watering was done as and when required. Nitrate reductase (NR) activity was estimated for 12 months regularly. In- vivo *Nitrate reductase activity* (*NRA)*: In July onwards uniform plants were uprooted, washed carefully, wrapped in filter paper and brought to laboratory. After taking the growth data the plant parts viz. leaf, stem and root were separated, chopped into small pieces of about 2- 3 mm length and width. From chopped material 0.5g of the parts was taken in a flat bottom culture tubes (30 ml capacity), containing 3 ml phosphate (0.2M pH 8.0) buffer and 3 ml substrate (0.15M KNO_3) and embedded in ice trays (Chaukiyal *et al*., 2014). These tubes were evacuated with the help of vacuum pump for about 2 minutes. The process was repeated until the plant tissues are fully submerged into the incubation medium. These tubes were transferred into a incubator shaker for one hour at 30^0C in dark for incubation. The tubes were removed and immersed into a boiling water bath for 4 minutes to stop the reaction and effective removal of the nitrate accumulated in the plant tissue. The amount of nitrate reduced into nitrite as end product during enzyme activity was determined by the method earlier described by Evans and Nason (1953). A required amount of an aliquot was pipetted in clean test tube, 1 ml sulphanilamide (1%, sulphanilamide $C_6H_8N_2O_2S$ in normal hydrochloric acid) was added, and shaken well, followed by 1 ml of NEDD (0.01% N, 1- Nepthyl ethylene diamine dihydrochloride) and mixed thoroughly. Colour was allowed to develop for 25 minutes and final volume was made 6 ml with the help of distilled water. A change in colour intensity was estimated at 540 nm in Perkin Elmer UV/VS Lamda 2S Series Spectrophotometer immediately.

RESULTS AND DISCUSSION

Overall maximum NR activity was observed in 80 kg N/ha followed by 40 kg N/ha, 20 kg N /ha and lowest in control (Figure 1a). The treatments effects were not observed significantly different but months and months x treatments interactions were significant at 5 % level (Table 5). Fluctuations in per gram NR activity were also recorded between 200 to 700 n moles NO_3^- reduced g^{-1} fresh wt h^{-1}. On the basis of seasonal study higher NRA was observed in rainy followed by winter and minimum in summer season. However, only treatments were observed significantly different at 5 % level. Among different seasons, 40 and 80 kg N/ha in rainy; 20 and 80kg N/ha in winter; 20 kg N/ha and control in summer season and in pooled treatment 20 and 40 kg N/ha were observed significantly different from others. However, all the seasons were significantly different among each other (Table 1a).

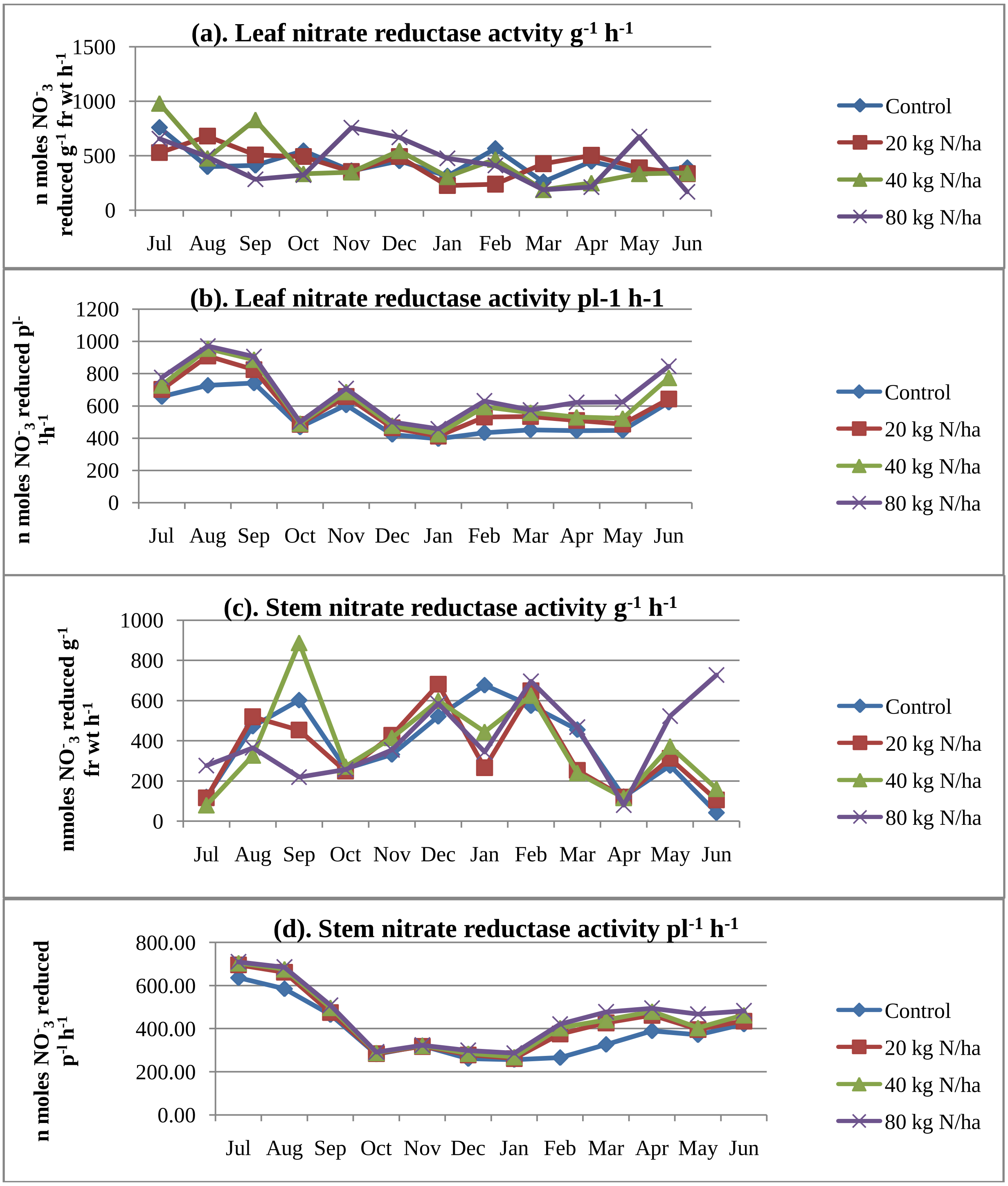
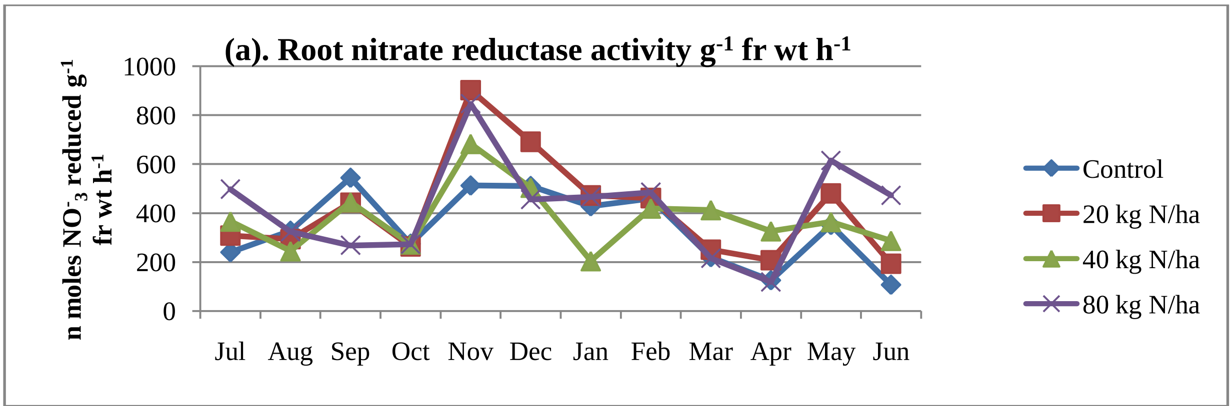
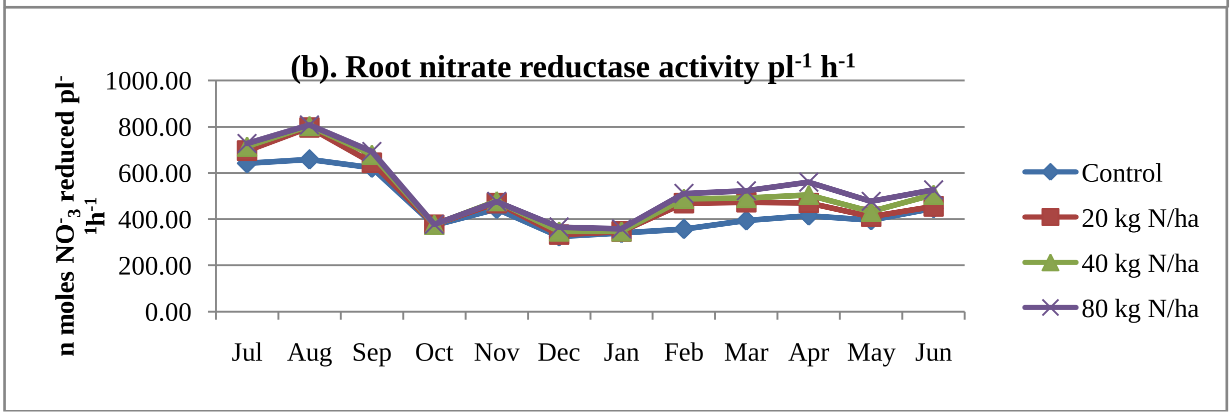


Figure 1 (a to d): Leaf and stem nitrate reductase (n moles NO^{-}_3 reduced g^{-1} fr wt h^{-1} and pl^{-1} h^{-1}) activity in *M. esculenta* seedlings as affected by different nitrogen treatments.

In total leaf NRA, two peaks one big in the month of August and other small was observed in November. Nitrogen treatment effects were observed significantly different however maximum per plant NRA was recorded in the 80 kg N/ha followed by 40 kg N/ha, 20 kg N/ha and least in control. On the monthly average basis higher activity was observed in August followed by November and least in the month of December and January (Figure 1b). Total leaf per plant NRA was observed significantly different at 5% level in respect to months, treatments and months x treatments (Table 5). On the seasonal average values basis, maximum NRA was recorded in rainy season followed by summer and winter. The treatments effects were significantly different at 5 % level over seasons and seasons x treatments. All nitrogen treatments individually as well as in pooled values were observed significantly different as compared to control. Whereas, rainy season was observed significantly different as compared to summer and winter (Table 1b). In stem two peaks one higher and another lower were observed during the month of August and February in all the treatments. Higher NRA was recorded in the 80 kg N/ha followed by 40 kg N/ha, control and minimum in 20 kg N/ha (Fig 1 c). A fluctuation in the NRA was also recorded during study period. The data were observed significantly different at 5% level in respect to months, treatments and their interactions months x treatments (Table 5). On the average seasonal basis higher activity was recorded in winter followed by rainy and least in summer season and all the N treatments in all the seasons were observed significantly different over the control at 5 % level. However, seasons were significantly different among each other **(**Table 2a). In total stem NRA two peaks moderately one higher in the month of July and another smaller in April was recorded. Maximum NRA was recorded in the 80 kg N/ha followed by 40 kg N/ha, 20 kg N/ha and minimum in control (Fig 1d). On seasonal average values basis higher activity was recorded during rainy followed by summer and least in winter season. Among all months and seasons, the control treatment showed lowest NR activity as compared to other. Whereas, the 80 kg N/ha treatment always showed higher NR activity. Stem per plant monthly average NR activity was also observed significantly different at 5% level in respect to months, treatments and months x treatments (Table 5). Seasonally, all N treatments in all the seasons and season pooled values were observed significantly different at 5% level **(**Table 2b)**.**





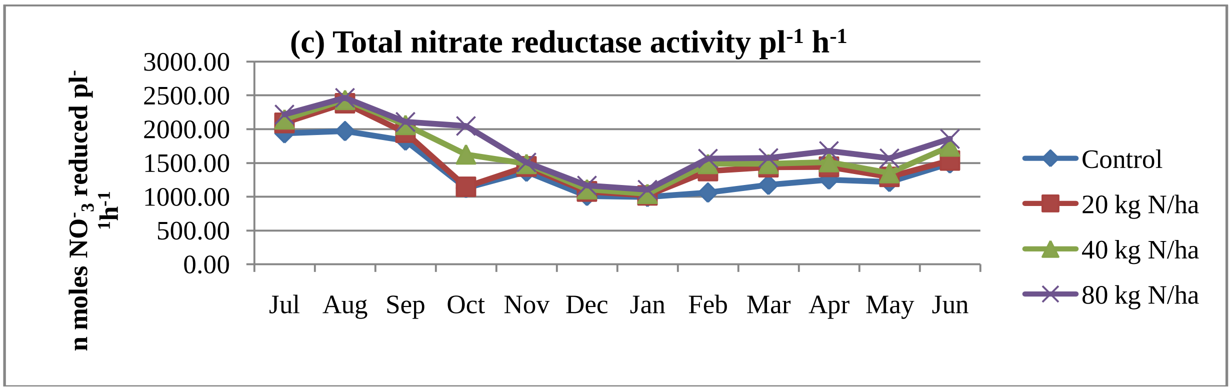


Figure 2 (a to c). Root nitrate reductase (n moles NO^-_3 reduced g^{-1} fr wt h^{-1} and pl^{-1} h-1) activity in *M. esculenta* seedlings as affected by different nitrogen treatments.

In root NRA (per gram per hour) two peaks one larger and another smaller were observed in the month of November and May for per gram NRA. However lowest activity was recorded in the month of April and June in control treatment. Under different N treatments maximum NR (902.13 n moles NO_3^- reduced g^{-1} fresh wt. h^{-1}) activity was observed in the month of November in 20 kg N/ha and minimum (106.38 n moles NO^-_3 reduced g^{-1} fresh wt. h^{-1}) in control treatment (Fig 2 a). Comparing N treatments effects, higher per gram root NRA was recorded in the 80 kg N/ha followed by 20 kg N/ha, 40 kg N/ha and least in control. However, the range between high and low activity remained between 200 and 500 n moles NO_3^- reduced g^{-1} fr wth^{-1}. Statistically data was observed significantly different at 5% level in respect to months, treatments and months x treatments (Table 5). On seasons wise, maximum root NRA was recorded in winter followed by rainy and minimum in summer season. Seasonal analysis showed a higher activity in 80 kg n/ ha as compared to 20 kg N /ha, 40 kg N /ha and control respectively. Statistically, only treatments were observed significantly different at 5 % level whereas, seasons and seasons x treatments were non-significant. In rainy season 20 and 40 kg N/ha were observed significantly different as compares to control and 80 kg N/ha. The seasonal variations were significantly different with each other (Table 3a). The total root NRA, moderately a higher and another lower peak was recorded in the month of August and April respectively (Fig 2 b). Among different N treatments, maximum NRA (807.76 n moles NO_3^- reduced g^{-1} fresh wt. h^{-1}) was recorded in the month of August in 80 kg N/ha and minimum (333.36 n moles NO_3^- reduced g^{-1} fresh wt. h^{-1}) in December in control treatment. Among different N treatments, higher per plant NRA activity was observed in 80 kg N/ha followed by 40 kg N/ha, 20 kg N/ha and lowest in control. The differences were significant at 5% level with respect to months, treatments and months x treatments (Table 5).

Seasonally average values among different N treatments were higher in 80 kg N /ha followed by 40 kg N /ha, 20 kg N /ha and lowest in control treatment. However, on season wise maximum values were observed in rainy followed by summer and minimum in winter season. Statistically only treatment effects were significantly different at 5 % level and both seasons and seasons x treatments interactions were non-significant. In winter season all N treatments were observed significantly different as compared to control and rainy season showed significantly higher value than summer and winter (Table 3 b). In case of total NRA per plant (leaf + stem + root), a larger peak was recorded in the month of August and another smaller in April for all nitrogen treatments (Fig 2 c). Among different N treatments, maximum values were recorded in 80 kg N/ha followed by 40 kg N/ha, 20 kg N/ha and minimum in control throughout the study period. On the seasonal basis, maximum per plant total plant NRA was recorded during rainy followed by summer and minimum in winter season. The nitrogen fertilizer effect was significantly higher as compared to control. Total NRA fluctuated in different seasons were observed between 1100 to 2400 n moles pl^{-1} h^{-1}, in rainy season followed by 1100 to 1800 n moles pl^{-1} h^{-1} in summer and between 1000 to 1600 n moles pl^{-1} h^{-1} in winter season. The changes in monthly observations were significantly different at 5 % level with respect to months, treatments and months x treatments (Table 5). However, only season and season x treatment interactions were significant at 5 % level. In rainy season 20kg N /ha and control, in winter 20 and 40 kg N/ha and in summer 40 and 80 kg N /ha were observed significantly different as compared to remaining other treatments. In pooled treatment 40 and 80 kg N/ha showed higher values as well as significant difference in control and 20 kg N /ha. However, the seasonal effects were significantly different among each other (Table 4).

The primary input of nitrogen in to the ecosystem is through the process of biological nitrogen fixation. Nitrogen fixing plants can utilize atmospheric as well as soil nitrogen, thereby, benefitting the associated vegetation regularly. The process of nitrogen utilization is a complex phenomenon since many climatic and edaphic factors influence the amount of nitrogen availability. Among influences of seasonal changes on different plant parts higher NRA was recorded in leaf > stem > root in rainy; leaf> root > stem in winter and root > leaf > stem respectively in summer season in all N treatments. Singh and Pokhriyal (2002) also reported higher NRA in leaf followed by root and stem in different seed sources of *Dalbergia sissoo*. Higher NRA in leaf may act to preferentially of partition newly assimilated N in the developing leaves and shoot tips (Black *et al*., 2002) whereas, difference in nitrate induced NRA between leaves and roots also have been reported for other tree species i.e., red oak (*Quercus rubra*) and red ash (*Fraxinus pennsylvanica*) (Traux *et al*., 1994). It shows that leaf is the major part of nitrogen assimilation as compared to stem and root. Black *et al*. (2002) also explained that different tissue localization of nitrate reduction and assimilation. Some species reduce nitrate primarily in the leaves whereas; other species localize nitrate reduction and assimilation in the roots. Further, NRA decreased with leaf age (Chaukiyal *et al*., 2014) and leaf NRA increased with nitrate availability (Dykstra, 1974).

Table 1. Seasonal variations analysis for leaf NR activity in *Myrica esculenta* seedlings as influenced by different nitrogen treatments

Character	Seasons	Bar Diagrams			
Leaf NRA g^{-1} fr.wt h^{-1}	Rainy	N-40 (655.31)^{C}	N-20 (551.06)^{B}	N-0 (528.19)^{B}	N-80 (438.29)^{A}
	Winter	N-80 (578.19)^{C}	N-0 (422.87)^{B}	N-40 (416.48)^{B}	N-20 (327.12)^{A}
	Summer	N-20 (412.23)^{C}	N-0 (360.63)^{B}	N-80 (310.63)^{A}	N-40 (277.65)^{A}
	Pooled T	N-40 (449.82)^{C}	N-80 (442.37)^{B}	N-0 (437.23)^{B}	N-20 (430.14)^{A}
	Pooled S	Rainy (543.21)^{C}	Winter (436.17)^{B}	Summer (340.29)^{A}	
	Two-way analysis		Season (S)	Treatment (T)	S x T
		P value	0.99	0.00	0.13
		F value	0.03	6.64	1.77
		Significant	NS	S	NS
Character	Seasons	Bar Diagrams			
Leaf NRA pl^{-1} h^{-1}	Rainy	N-80 (787.29)^{B}	N-40 (765.16)^{B}	N-20 (730.38)^{B}	N-0 (648.12)^{A}
	Winter	N-80 (573.17)^{B}	N-40 (546.13)^{B}	N-20 (516.34)^{B}	N-0 (464.61)^{A}
	Summer	N-80 (665.53)^{C}	N-40 (596.09)^{B}	N-20 (543.49)^{B}	N-0 (491.65)^{A}
	Pooled T	N-80 (675.33)^{B}	N-40 (635.79)^{AB}	N-20 (596.74)^{A}	N-0 (534.80)^{A}
	Pooled S	Rainy (732.74)^{B}	Summer (525.06)^{A}	Winter (574.19)^{A}	
	Two-way analysis		Season (S)	Treatment (T)	S x T
		P value	0.09	0.00	0.99
		F value	2.30	10.08	0.07
		Significant	NS	S	NS

Table 2. Seasonal variations analysis for stem NR activity in *Myrica esculenta* seedlings as influenced by different nitrogen treatments

Character	Seasons	Bar Diagrams

Stem NRA g^{-1} fr. wt h^{-1}	Rainy	N-40 (391.84)^{C}	N-0 (364.18)^{B}	N-20 (334.39)^{B}	N-80 (279.78)^{A}
	Winter	N-0 (526.06)^{B}	N-40 (521.27)^{B}	N-20 (505.85)^{A}	N-80 (495.21)^{A}
	Summer	N-80 (449.82)^{C}	N-0 (224.11)^{B}	N-40 (222.69)^{B}	N-20 (198.22)^{A}
	Pooled T	N-80 (408.27)^{A}	N-40 (378.60)^{A}	N-0 (371.45)^{A}	N-20 (346.15)^{A}
	Pooled S	Winter (512.10)^{C}	Rainy (342.55)^{B}	Summer (273.71)^{A}	
	Two-way analysis		Season (S)	Treatment (T)	S x T
		P value	0.88	0.00	0.57
		F value	0.21	6.65	0.79
		Significant	NS	S	NS
Character	Seasons	Bar Diagrams			
Stem NRA. Pl^{-1} h^{-1}	Rainy	N-80 (548.95)^{B}	N-40 (541.27)^{B}	N-20 (529.21)^{B}	N-0 (491.66)^{A}
	Winter	N-80 (332.91)^{B}	N-40 (319.35)^{B}	N-20 (308.13)^{B}	N-0 (275.43)^{A}
	Summer	N-80 (480.77)^{B}	N-40 (446.61)^{B}	N-20 (429.80)^{B}	N-0 (376.78)^{A}
	Pooled T	N-80 (454.21)^{B}	N-40 (435.74)^{B}	N-20 (422.38)^{B}	N-0 (381.29)^{A}
	Pooled S	Rainy (527.77)^{C}	Summer (433.49)^{B}	Winter (308.95)^{A}	
	Two-way analysis		Season (S)	Treatment (T)	S x T
		P value	0.43	0.00	0.99
		F value	0.92	15.48	0.04
		Significant	NS	S	NS

Table 3. Seasonal variations analysis for root NR activity in *Myrica esculenta* seedlings as affected by different nitrogen treatments

Character	Seasons	Bar Diagrams			
Root NRA. g^{-1} fr.wt h^{-1}	Rainy	N-0 (346.63)^{B}	N-80 (340.42)^{AB}	N-40 (331.91)^{A}	N-20 (327.12)^{A}
	Winter	N-20 (631.91)^{B}	N-80 (563.29)^{B}	N-0 (477.65)^{A}	N-40 (452.65)^{A}
	Summer	N-80 (355.14)^{C}	N-40 (347.51)^{C}	N-20 (283.33)^{B}	N-0 (200.35)^{A}
	Pooled T	N-80 (419.62)^{B}	N-20 (414.12)^{B}	N-40 (377.36)^{A}	N-0 (341.54)^{A}
	Pooled S	Winter (531.38)^{C}	Rainy (336.52)^{B}	Summer (296.58)^{A}	
	Two-way analysis		Season (S)	Treatment (T)	S x T
		P value	0.52	0.00	0.60
		F value	0.75	12.05	0.76
		Significant	NS	S	NS
Character	Seasons	Bar Diagrams			
Root NRA pl^{-1} h^{-1}	Rainy	N-80 (651.60)^{B}	N-40 (643.66)^{B}	N-20 (628.47)^{AB}	N-0 (574.32)^{A}
	Winter	N-80 (428.04)^{B}	N-40 (415.66)^{B}	N-20 (404.65)^{B}	N-0 (367.20)^{A}
	Summer	N-80 (521.63)^{C}	N-80 (484.03)^{B}	N-80 (452.13)^{B}	N-80 (413.48)^{A}
	Pooled T	N-80 (533.76)^{A}	N-40 (514.45)^{A}	N-20 (495.08)^{A}	N-0 (451.67)^{A}
	Pooled S	Rainy (624.51)^{B}	Summer (467.82)^{A}	Winter (403.89)^{A}	
	Two-way analysis		Season (S)	Treatment (T)	S x T
		P value	0.31	0.00	0.99
		F value	1.22	17.08	0.05
		Significant	NS	S	NS

The different pattern of NRA in different plant parts in different seasons in this species may be being as is a temperate species, winter is cold and in this period all leaves remained intact with tree whereas, *Dalbergia sissoo* sheds its leaves in winter and is a sub tropical species therefore, the pattern is altogether different. Overall seasonal basis maximum activity was observed in rainy followed by summer and minimum in winter season. Almost similar pattern have been reported earlier in *Acacia catechu* and *Dalbergia sissoo* by Pokhriyal *et al*., (1990 and 1991) and Chaukiyal and Pokhriyal (2005) in *Pongamia pinnata.* Among different treatments maximum NRA was recorded in 80 kg N/ha followed by 40 kg N/ha, 20 kg N/ha and minimum in control. In rainy season maximum NRA was gained by leaf (39-42%) followed by root (31-33%) and minimum in stem (27-28%). In winter higher values were recorded in leaf (40-45%) followed by root (31-34%) and least in stem (24-26%). Whereas, in summer higher NRA was in leaf (39-45%) followed by root (29-32%) and minimum (20-29%) in stem. Among season the overall maximum NRA pattern was recorded leaf > root > stem in all plant part (Figure 3).For rainy and summer season similar pattern was observed in *Pongamia pinnata* (Chaukiyal and Pokhriyal, 2005). Uniyal (1997) reported similar pattern for rainy season i.e. leaf > root > stem but different trend in winter (root> stem > leaf) and summer (root> leaf > stem) season. Whereas, Kumar (2005) reported different pattern in *Acacia catechu* as leaf > root > stem in rainy and leaf > stem > root in rainy and no pattern in winter season in different N treatments. Leaving some exceptional cases, among different seasons overall maximum NRA was observed in the rainy followed by summer and winter season. Almost similar type of observations was reported by Johnsen *et al*. (1991) for *Rubinia pseudoacacia* and Pokhriyal *et al.* (1993) for *Eucalyptus teriticornis*. In a survey of 555 woody species, only 41 % showed NRA in leaves (Smirnoff *et al*., 1984). Similar pattern was observed in *Pongamia pinnata* by Chaukiyal and Pokhriyal (2005). Recently a different pattern of NRA was reported in *Dalbergia sissoo* (Pandey, 2002; Bhat, 2012; Mir, 2012) and *Albizia lebbeck* (Thapliyal, 2002). It clearly indicates about the influences of season on NRA pattern of different tree species. Sellstedt (1986), reported that the partitioning of NRA among different plant parts varies with species, for instance *Alnus incana* shown high NRA in both root and stem tip but not in the leaves. Whereas, Beevers and Hageman (1969), Harper and Hageman (1972), Johnson *et al*.(1991) and Pokhriyal *et al*. (1993) reported that the major site of nitrate reduction is the leaves. Several workers (Gebauer and Stadler, 1990; Stadler and Gebauer, 1992; Downs, *et al*., 1993; Traux *et al*., 1994 and Black *et al.,* 2002) also explained that localization of nitrate reduction and assimilation is different from plant to plant species. Some species shown higher NR activity in leaves whereas, others in root. In other temperate tree species root NRA exceeds leaf (Sarjala *et al*., 1987; Gojon *et al*., 1991; Lee and Titus, 1992; Downs *et al*., 1993). These results support our findings that *M. esculenta*, a temperate tree species having higher nitrate reduction in leaf than root and stem (Figure 3). Similar types of results were also reported by Smirnoff *et al.* (1984) in leaves of some temperate tree species.

Table 4. Seasonal variations analysis for total per plant NR activity of *Myrica esculenta* seedlings as affected by different nitrogen treatments

Character	Seasons	Bar Diagrams			
Total NRA pl^{-1} h^{-1}	Rainy	N-80 (1987.85)^C	N-40 (1950.10)^C	N-20 (1888.07)^B	N-0 (1714.12)^A
	Winter	N-0 (1452.08)^C	N-80 (1370.15)^C	N-20 (1219.89)^B	N-40 (1039.40)^A
	Summer	N-80 (1655.27)^C	N-20 (1479.08)^B	N-0 (1417.55)^B	N-40 (1358.48)^A
	Pooled T	N-80 (1671.09)^C	N-20 (1529.01)^B	N-0 (1527.92)^B	N-40 (1449.33)^A
	Pooled S	Rainy (1885.03)^C	Summer (1477.60)^B	Winter (1270.38)^A	
	Two-way analysis		**Season (S)**	**Treatment (T)**	**S x T**
		P value	0.00	0.00	0.00
		F value	110.237	5.010	148.864
		Significant	S	NS	S

Table 5. ANOVA for the nitrate Reductase activity of *Myrica esculenta* plant parts

Nitrate Reductase Activity (NRA)								
Characters	**Leaf g^{-1} NR A**		**Leaf pl^{-1} NRA**		**Stem g^{-1} NRA**		**Stem pl^{-1} NRA**	
	F	**P**	**F**	**P**	**F**	**P**	**F**	**P**
Months (M)	30.409	0.00	527.076	0.00	69.767	0.00	1474.612	0.00
Treatments (T)	0.400	0.753	180.432	0.00	5.911	0.00	2217.169	0.00
M x T	11.659	0.00	106.100	0.00	11.436	0.00	913.445	0.00

Characters	**Root g^{-1} NR A**		**Root pl^{-1} NRA**		**Total NRA**	
	F value	**P**	**F value**	**P value**	**F value**	**P value**
Months (M)	75.796	0.00	1509.912	0.00	3789.99	0.00
Treatments (T)	7.078	0.00	531.032	0.00	786.65	0.00
M x T	9.245	0.00	187.581	0.00	226.33	0.00

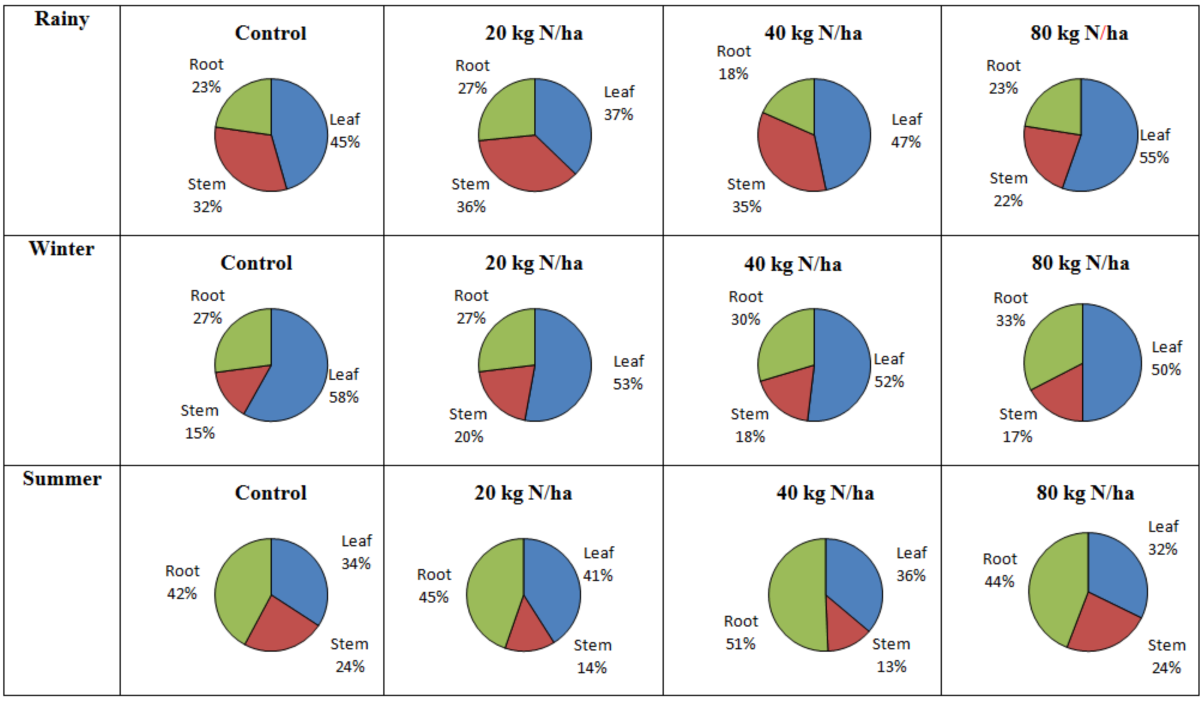


Figure 3. Percent allocation in leaf, stem and root NR activity as influenced by different nitrogen treatments and seasons in *M. esculenta* seedlings under nursery.

A fluctuation in NR activity was observed in all seasons in this species which is a common phenomenon in other species like *Eucalyptus tereticornis* and *Acacia nilotica* (Pokhriya *et al*., 1995) mixed planting; *Dalbergia sissoo* (Singh and Pokhriyal, 2002; Uniyal, 1997); Bhat, 2012; Mir, 2012); *Pongamia piñata* (Chaukiyal and Pokhriyal, 2005; Kumar, 2013); *Albizia lebbeck* and *Eucalyptus tereticornis* (Shams, 2013) mixed planting. Pokhriyal *et al.* (1995) explained that NRA is known to fluctuate in response to the changes in environmental conditions, and such fluctuation usually also influences the capacity of different plant parts to assimilate nitrate. The responsiveness of tree crop to N fertilizers is extremely variable and depends on species, soil as well as environmental conditions of that particular area (Williams and Haynes, 1995). To maximize the utility of nitrogenous fertilizers in non-legume nitrogen fixing tree crops, the nitrogen doses should be ideally applied in split form depending upon the requirement of plant. In this study the nitrate assimilation increased as the N fertilizer doses increased up to 80 kg N/ha means that this species can assimilate higher doses of fertilizer. It might be due to this species belong to temperate zone whereas, the experimental zone was in sub tropical conditions in which the growth was continued during winter season also, that's why the available N was immediately incorporated. More trials are required to be conducted on both the altitude to know actual demand of N in different places separately. The major aim of tree growers to obtain maximum economic returns on the cost of fertilizers applied in the initial stages of growth. But excessive application of it to be avoided as this leads to an economic loss of N and environmental problem. Initially, low N starter doses will be helpful boosting seedling growth, whereas, higher doses will inhibit the process of N assimilation and fixation on one hand and deplete it through volatilization, leaching and polluting atmosphere on the other.

CONCLUSION

The effect of different doses of N on *in-vivo* nitrate reductase activity was studied in different plant parts of one year old pot grown seedlings of *Myrica esculenta*. It was observed that comparatively higher NR activity was observed in leaves followed by root and minimum in stem. Among different N treatments higher NR activity was recorded in 80 kg N/ha followed by 40 kg N/ha; 20 kg N/ha and minimum in control. Nearly similar pattern was also observed in all plant part and total plant also. Though no single pattern was observed in all the seasons however, mostly maximum NR activity was observed in rainy followed by winter and minimum in summer season. Therefore, leaves rainy season and 80 kg N/ha is observed suitable to increase plant N R activity in the seedlings of *Myrica esculenta*.

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