

Full Length Research Paper

Biomass allocation and nitrogen distribution of *Pinus wallichiana* Jackson seedlings under different nitrogen fertigation levels

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Accepted 25 March, 2013

Pinus wallichiana seeds were sown in polybags filled with sterilized riverbed sand in 2010. Seedlings were thinned out to one seedling per polybag, which were treated weekly with Ingestad pretreatment nutrient solution for 4 weeks and then were fertigated with nitrogen levels of 0.99, 1.98, 2.97, 3.96, 4.95, 5.94, 6.93, 7.92, 8.91 and 9.90 mg and fixed levels of phosphorus, potassium, calcium and magnesium dissolved in 50 ml of water seedling⁻¹ week⁻¹ upto or until 28 weeks. Biomass allocation to needles, (separated by hand from shoot axis), stem (Includes hypocotyls and epicotyls) and roots increased significantly with the increase in nitrogen level and seedling age. At 7.92 mg level of nitrogen fertigation biomass allocation and nitrogen concentration partitioning at 28 weeks got increased by 190, 32 and 44%, and 179, 101 and 147% in needles, stem and roots, respectively as compared to control. Nitrogen concentration in shoot and root decreased at lower nitrogen level as seedlings age increased, but increased at higher nitrogen fertigation levels in needles, stem and roots. To the end of the experiment, nitrogen uptake per seedling increased, whereas, nitrogen use efficiency (NUE) decreased with the increasing nitrogen levels.

Key words: *Pinus wallichiana*, Biomass allocation, nitrogen partitioning, nitrogen uptake, nitrogen use efficiency (NUE).

INTRODUCTION

Pinus wallichiana Jackson (Kail pine) is a one of the important timber species of wealthy coniferous forests of Kashmir. It is found in moist and dry temperate forest types of Western and Central Himalayas from Kashmir to Bhutan with an altitudinal zonation of 1500 to 3700 m

a.m.s.l. *P. wallichiana* Jackson is a tall evergreen tree species with average height of 30 to 36 m and girth 2.5 to 3 m (Troup, 1921). The poor natural regeneration of this species has been a major concern and has thus, caused constant attention of researchers in Kashmir. A number

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of factors are considered responsible for the failure or patchy regeneration of this species, that is, early slow growth, poor regeneration and competition etc. may be the main causes for replacing this valuable timber species of Jammu and Kashmir natural forests by the other species like *Cedrus deodara* at an alarming rate.

Seedling quality, when defined as out planting performance is logically related to its mineral nutrient status. Nutrient loaded seedling performed better than conventionally produced seedlings when out planted especially in nutrient poor sites (Malik and Timmer, 1995; Xu and Timmer, 1999). Planting nutrient loaded seedlings is a more effective method of stimulating growth than post transplanting fertilizer application (Xu and Timmer, 1999). The improved seedling quality as a result of applied nutrients allows better interaction of planted seedlings with the planting site and therefore fuller expression of site potential (Fry and Poole, 1980). Fertilization is thus an essential component of producing high quality nursery stock for reforestation and enhances plant growth, nutrient storage reserves, root growth potential, and resistance to drought stress, freezing temperatures and diseases (Landis, 1985; Rook, 1991). Limited nitrogen supply usually inhibits shoot growth proportionally more than root growth (Ledig, 1983). Under high nitrogen condition; genotypes that allocate relatively more dry matter to shoots and may grow faster because they reinvest the high proportion of their assimilates in above ground biomass and vice-versa (Ledig, 1975). Leaf photosynthetic capacity has often been highly correlated with leaf nitrogen content. The reason for this includes the paramount role of nitrogen in light harvesting and CO₂ fixation (Evans, 1989).

Fertilization is one of the most important cultural practices for producing container seedlings when the limited volume seriously hinders seedling growth (Landis, 1989). and it also accelerate shoot and root growth, modify tissue nutrient contents and hence the amount of available reserves, improve post-transplant rooting and growth capacity, and increase resistance to water stress, low temperature and disease (Landis, 1985; Haase and Rose, 1997; Shaw et al., 1998; Grossnickle, 2000). However, such properties are of vital importance for successful seedling establishment under unfavorable conditions (Puttonen, 1997; Birchler et al., 1998). The morphological studies have shown that nutrient status of nursery seedlings played a significant role in post planting response. Keeping this in view, the present investigation has been undertaken to know the optimal range of nitrogen for *P. wallichiana* with the following objectives: the first is to study the pattern of biomass allocation in *P. wallichiana* seedlings and second is to study nitrogen partitioning, nitrogen uptake efficiency and nitrogen use efficiency (NUE) in *P. wallichiana* seedlings.

MATERIALS AND METHODS

The experiment was conducted at the Wadura, Sopore, Faculty of

Forestry, SK University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir. The site is located at 34°17' N latitude and 74°33' E longitude with altitude of 1524 m a.m.s.l. Ingested and Lund (1979, 1986) and Ingested (1987) developed a method to regulate relative growth rates of seedlings by controlling nutrient supply. The technique is based on the concept that, it is the nutrient amount supplied per unit time (relative addition rate) and not the nutrient concentration in the growing medium that is important in controlling nutrient uptake and the subsequent growth. The Kail pine seeds were collected from Bandipora Forest Division, Kashmir, soaked in cold water for 24 h and treated with Captan (50% WP) at 2.5 g kg⁻¹ seed. The seeds were sown in polythene bags (10 × 15 cm) filled with sterilized river bed sand and kept in a mist chamber for germination in the last week of February, 2010. Three seeds were then sown in each polybag. Weeding was carried out manually as and when required. Polybags were irrigated once a week when fertilizer was not applied. Germinates were thinned out to one per polybag, 3 weeks after germination and fertigated at weekly interval with Ingestad pretreatment nutrient solution (Ingestad and Lund, 1979) that included all major nutrients by weight proportions of nitrogen, phosphorus, potassium, calcium and magnesium (in 100:13:65:7:8.5 ratio), 25 ml seedling⁻¹ for 1 month as pretreatment and each application supplied 1.982 mg nitrogen, 0.258 mg phosphorus, 1.288 mg potassium, 0.139 mg calcium and 0.168 mg magnesium seedling⁻¹. The pretreated seedlings were shifted from mist chamber to nursery and treated weekly with nitrogen fertigation which was continued till the termination (upto 28 weeks of age) of experiment.

The nitrogen levels were 10 and total fertigation treatments were 12 viz., 0 (with and without fertigation), 0.99, 1.98, 2.97, 3.96, 4.95, 5.94, 6.93, 7.92 8.91 and 9.90 mg N seedling⁻¹. All the treatments, except without fertigation treatment, were given fixed levels of other nutrients viz., P, K, Ca and Mg seedling⁻¹ week⁻¹. Nutrient fertigation was applied weekly at 50 ml seedling⁻¹ week⁻¹. The control treatment received only plain water. The experiment was conducted with a randomized complete block design with three replications and per treatment level was represented by 21 seedlings at each of the replications. Nine seedlings from each treatment were selected randomly and harvested at 4 weeks interval to measure physical parameters on monthly basis, that is, dry weight of needles, stem and roots. Total nitrogen (Jackson, 1973) was also measured on those same samples. Nitrogen uptake was determined as per procedure of Burgess (1991). NUE was estimated by dividing seedling biomass at a sample date by the amount of nitrogen taken up at that date (Prescott et al., 1989).

Statistical analysis

The experimental data were statistically analyzed using the two-way analysis of variance (ANOVA) approach following Gomez and Gomez (1984). The differences between treatments and the interaction between experimental factors (for example, nitrogen and seedling age) were tested at the 0.05 significance level. Data analysis was carried out using the software IBM SPSS Statistics.

RESULTS AND DISCUSSION

Pattern of biomass allocation in *P. wallichiana* seedlings

P. wallichiana seedlings allocated more carbohydrates to shoot at higher levels of nitrogen fertigation. Increase in nitrogen fertigation upto 7.92 mg nitrogen level

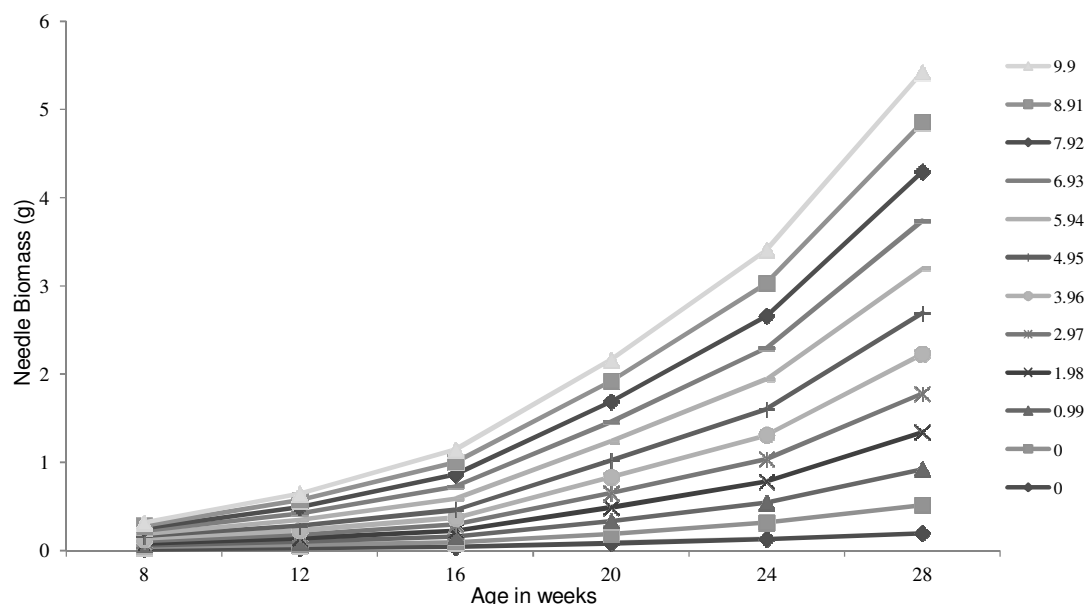


Figure 1. Effect of nitrogen fertilization and seedling age on allocation of biomass (g) to needles.

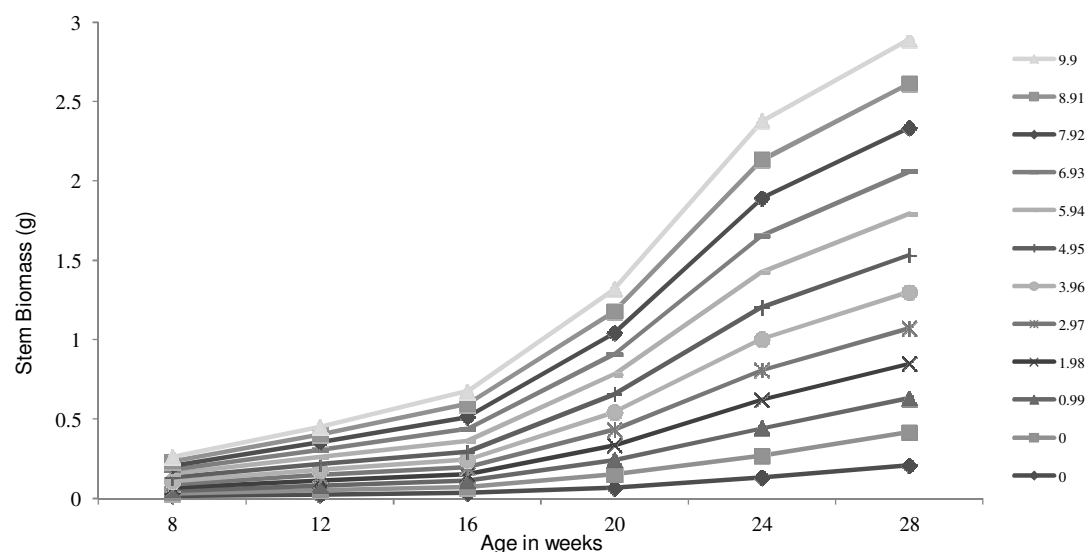


Figure 2. Effect of nitrogen fertilization and seedling age on allocation of biomass to stem (g).

enhances mean needles (0.232 g), stem (0.132 g) and root (0.179 g) biomass (Figures 1 to 3) in Kail pine seedlings. There was no significant difference among the higher doses. There was significant difference in seedling mean needle, stem and root biomass in case of nitrogen-free and control treatment. The effect of age on different components of seedling (needle, stem and root) biomass was conspicuous with highest (0.452, 0.241 and 0.265 g) observed at 28 weeks age, which were 17, 11 and 6 times more than the seedling needle, stem and root biomass at 8 weeks age, respectively. With increase in

age from the 8 to 28 weeks, the biomass showed significant increase. Interaction between fertilization and seedling age of Kail pine was maximum at the 9.90 mg nitrogen treatment and seedling age of 28 weeks shown in seedling biomass of needle (0.566 g), stem (0.280 g) and roots (0.320 g). Ledig (1983) suggested that under high nitrogen condition, plants allocate relatively more biomass to shoots and may grow faster by inverting a high proportion of their photosynthetic capital to shoots. The shift in relative total plant dry weight from the roots to the shoots might be due to redirection of the relative

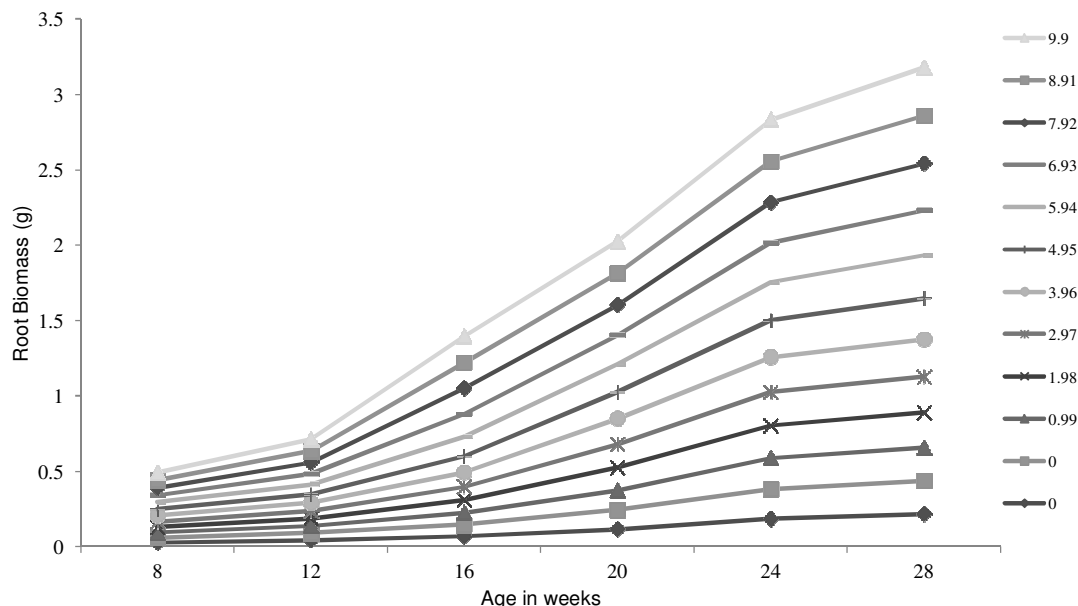


Figure 3. Effect of nitrogen fertilization and seedling age on allocation of biomass to roots.

proportion of total plant nitrogen from the root system to leaves (Grime, 1979; Rose and Biernacka, 1999). Genetic variability is higher within individual of long leaf pine seedlings than among stands or sources, both in years and nitrogen rate (1 to 3 mg) root biomass increased about 30%, compared with the 230% increase in shoot biomass which may be due to this factor that root biomass appears less plastic in response than shoot biomass (Boyer, 1990). Our results were confirmed with Jackson et al. (2007) who revealed that shoot (2.06 g) and root biomass (1.52 g) increased at 4 mg nitrogen seedling⁻¹ week⁻¹ in long leaf pine (*Pinus palustris*) for a period 30 week. Similar findings have been reported for sugar (*Pinus lambertiana*) and Jeffrey pine (*Pinus jeffreyi*) by Walker (2001). However, our results were also supported by Li et al. (1991) who revealed that nitrogen had significant effects on seedling growth and biomass allocation to needles, stem and roots.

Tissue nitrogen concentration and relative nitrogen distribution in *P. wallichiana* seedlings

Increase in nitrogen fertilization upto 7.92 mg nitrogen level significantly enhances the mean needle (Figures 4 to 7) nitrogen percent (1.97), stem (1.02) and roots (1.42) in Kail pine. There was significant difference in mean seedling needle, stem and root nitrogen concentration for nitrogen-free and control treatments. As age advances mean nitrogen concentration significantly increased in different components [needle (1.53%), stem (0.79%) and roots (1.19%)] of seedlings, which was observed at 28 weeks age, nitrogen concentration was highest in needles, then in roots and lowest in stem. Interaction

between fertilization and seedling age of Kail pine again indicate that the maximum interaction occurred between 9.90 mg nitrogen level and the age of 28 weeks with nitrogen concentration in needle (2.08%), stem (1.14%) and roots (1.50%). As expected, with increase in the rate of nitrogen applied, the seedling nitrogen level increased significantly. The increase in total nitrogen content of *P. wallichiana* seedling with increased nitrogen concentration may be due to the combination of increase in dry weight and nitrogen concentration in tissues. Because nitrogen is a major constituent of enzymes, changes in tissue nitrogen concentration generally reflects a change in enzymes concentration. Both gross primary products and plant respiration represents biochemical processes that are catalyzed by nitrogen rich enzymes as the rate of these processes depend on the nitrogen content in tissues. Higher level of nitrogen availability generally increases the nitrogen concentration in leaves, which increases the growth of the plants (Mc Guire and Joyce, 1995). Fertilization can modify tissue nutrient contents and the amount of available reserves, improves post transplant rooting and growth capacity of seedling (Landis, 1985; Van den Driessche, 1992). Our results were similar to the optimal range of conifers since 2.5% of needle nitrogen concentration is the upper limit of optimum range for conifers as defined by Landis (1989) and Van den Driessche (1988) reported maximum survival of Douglass fir seedling with a 2.1% nitrogen concentration in needles at the time of planting. Burgess (1991) recorded 2.41% nitrogen in Douglas fir 14 weeks of age under 6% nitrogen fertilization rate, which subsequently decreased to 1.61% at 18 weeks of age, while in Western hemlock at same fertilization rate it increased from 1.94% at 14 week age to 2.23% at 18

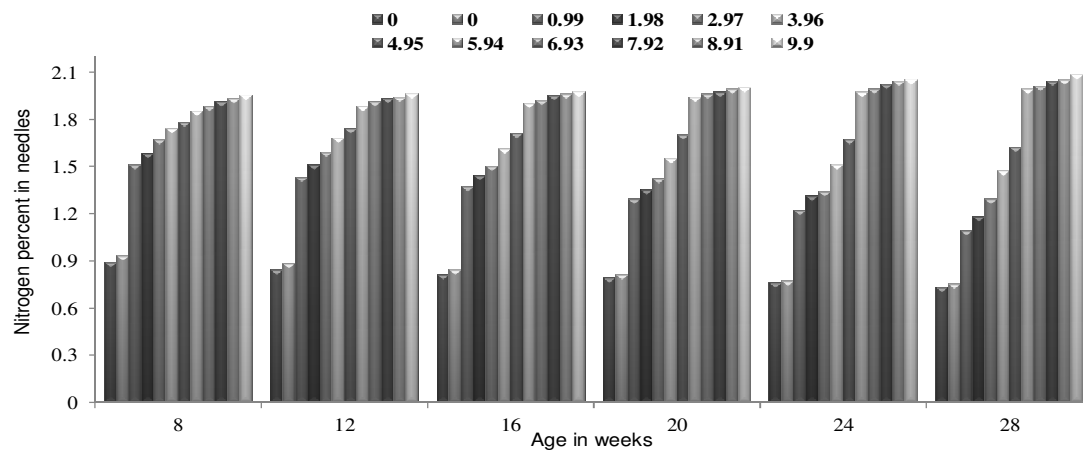


Figure 4. Effect of nitrogen fertilization and seedling age on nitrogen concentration of needles.

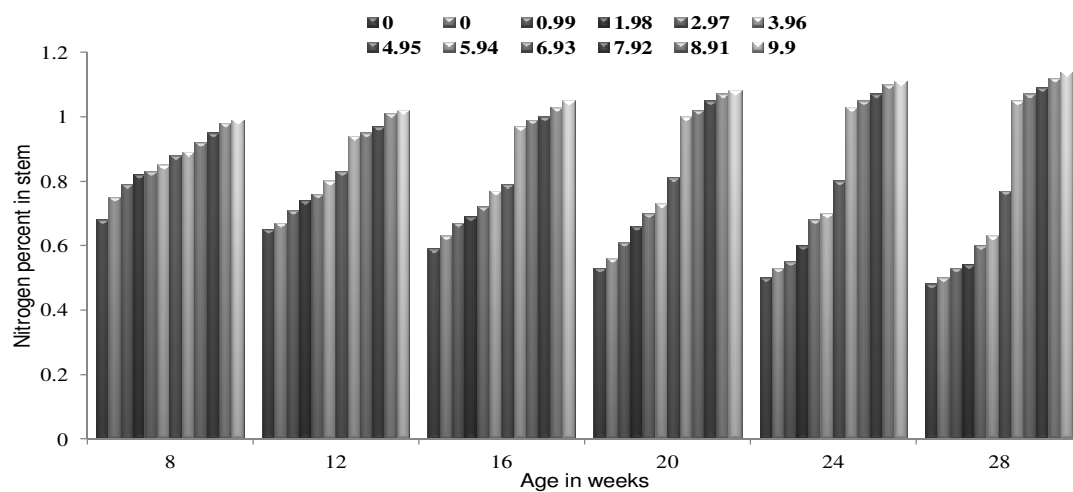


Figure 5. Effect of nitrogen fertilization and seedling age on nitrogen concentration of stem.

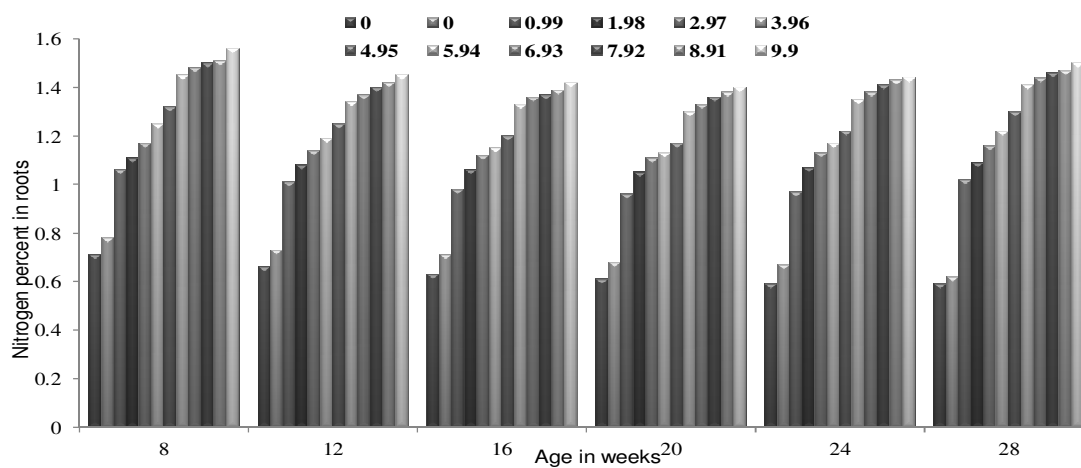


Figure 6. Effect of nitrogen fertilization and seedling age on nitrogen concentration of roots.

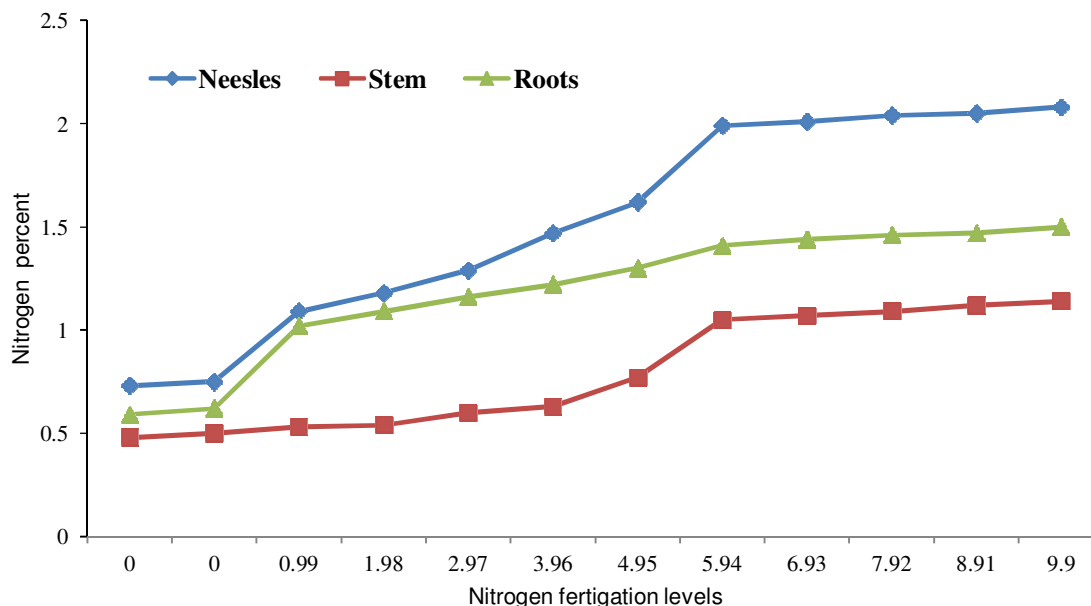


Figure 7. Nitrogen distribution in *P. wallichiana* seedlings at 28 weeks of age.

weeks age. So, the percentage of nitrogen in plants depends upon the nitrogen addition rate and species response towards the nitrogen fertilizers. Increasing trend of nitrogen content has also been reported by Dumroese et al. (2005) in long leaf pine under the application of 40 mg and 66 mg nitrogen seedling⁻¹.

Nitrogen uptake of *P. wallichiana* seedlings under different fertigation levels

The cumulative amount of nitrogen added by treatments varied from 19.82 to 198.2 mg seedling⁻¹ by the time the seedlings were 28 weeks old (Figure 8). Nitrogen uptake by *P. wallichiana* seedlings at the 9.90 mg nitrogen fertigation rate accumulated only 19.76 mg of nitrogen (9.97%). At basal dose (7.92 mg seedling⁻¹), *P. wallichiana* seedlings absorbed 60.9% of the nitrogen applied to medium. As concentration of nitrogen in treatments increased, the nitrogen uptake efficiency decreased from 60.92% in the treatment with 0.00 mg nitrogen to 9.97% at the 9.90 mg nitrogen which means it decreased as the nitrogen addition levels increased. The decrease in the nitrogen -uptake efficiency at initial nursery stage definition may be due to its intrinsically low relative growth rate. Matching plant growth with nutrient uptake and maintain stable internal nutrient concentration has been a topic of interest. In this regard, Ingestad (1974), and Ingestad and Lund (1986) improved fertilizer efficiency and prevent nutrient deficiency and toxicity in different studies and concluded that it occurs due to low or high nutrient addition levels. These results are consistent with that of Burgess (1991) who observed nitrogen uptake efficiency of 30.60 and 22.42% at highest

(6% addition) rate in Douglas fir and Western hemlock, respectively. Burgess (1991) further reported that although both Douglas fir and Western hemlock grew fastest under 6% nitrogen fertigation rate, Western hemlock was more efficient in nitrogen uptake. Contrary to these findings, Jackson et al. (2007) observed increase in total nitrogen content seedling⁻¹ of long leaf pine with increase in nitrogen fertigation rate. Burgess (1990) also reported similar results in Black and White Spruce seedlings.

NUE of *P. wallichiana* seedlings under different fertigation levels

Figure 8 reveals that total biomass of seedlings (oven dry weight) at 28 weeks of age varied from 618 mg at lowest nitrogen application rate to 1166 mg at highest addition rate. The cumulative amount of nitrogen added by treatments varied from 19.82 to 198.20 mg seedling⁻¹ up to the time seedlings were harvested at the 28 weeks of age. NUE in *P. wallichiana* seedlings decreased from lowest addition rate 95.19 mg of biomass per mg of nitrogen in the treatment 0.00 per mg nitrogen to 5.88 mg per mg nitrogen at the highest nitrogen level at 9.90 mg. NUE CO₂ gained per unit of nitrogen absorbed from medium by *P. wallichiana* seedlings was lowest (5.88%) at highest nitrogen addition rate (Figure 8) emphasizing the ability of slow growing species to retain nutrients with in plant body at nursery stage. Leaves with maximum photosynthesis rate may invest a large proportion of the leaf nitrogen in rubisco (Field and Mooney, 1986). Low NUE may be as a result of inefficient allocation of nitrogen among photosynthetic compounds, such that

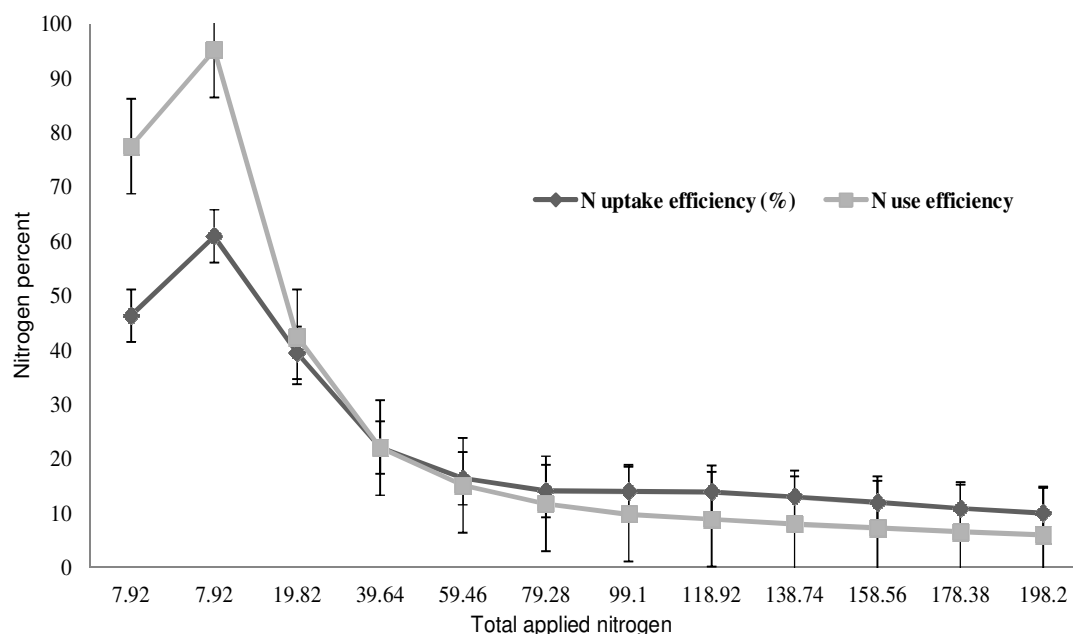


Figure 8. NUE and nitrogen uptake efficiency of *P. wallichiana* seedlings at the end of first growing season.

some compounds are present in excess, while the rate limiting compounds are underrepresented. For example, shade plants invert larger quantity of nitrogen in light harvesting pigments and proteins, but make only small investment in rubisco and others CO₂ processing enzymes (Bjorkman, 1981; Evans, 1989). Our results are consistent with Riech and Schoettle (1988) who reported that in white pine (*Pinus strobus*) seedlings NUE decreased with increasing concentration of nitrogen in tissues. Difference in NUE may also result from difference in allocation levels. Contrary to this, Masoodi et al. (2007) reported NUE of 57.47% in *C. deodara* and 100.14% in *Cupressus torulosa* at 9 mg nitrogen addition rate. However, both these species grow relatively faster than *P. wallichiana*.

Conclusion

All the species have an optimum range of nitrogen requirement. However, the optimum range for biomass allocation and nitrogen distribution in *P. wallichiana* seedling is 7.92 mg level of nitrogen addition rate for first growing season at nursery, at this rate optimum biomass and nitrogen content is recorded. However, the below nitrogen addition rates have non-significance (low concentration have non significance effect on response) and the above nitrogen levels adds minute biomass (more fertilizer was used and less gains was obtained) and nitrogen percent to Kail pine seedlings, which has statistically and economically no importance. NUE in *P. wallichiana* seedlings was found inverse of nitrogen

uptake efficiency.

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