Study on the Individual Impacts of Mineral nutrients N, P and K and their Combinations on the Physiology and Growth performance of *Pongamia pinnata* (L.) Pierre seedlings to assess Quality

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Abstract

The demand for raising energy plantation as a sustainable and dependable alternative indigenous energy source to commercial fuel is increasing due to the rapid depletion of fossil fuels and degradation of environment which resulted from over exploitation. The success of energy plantation is primarily dependent on the quality of tree seedlings planted in the field. In this context a study was conducted to determine the influence of mineral nutrients N, P, K and their combinations on the quality of *Pongamia pinnata* (L.) Pierre seedlings. Parameters like total leaf area, total chlorophyll, leaf starch concentration, CO_2 assimilation rate and total dry biomass production were selected and analysis was carried out in the nursery level. In general, nitrogenous nutrient treatments N, N+P, N+K and N+P+K significantly increased total leaf area, total chlorophyll, CO_2 assimilation rate and biomass production of *P.pinnata* seedlings compared to control. However the influence of non-nitrogenous nutrient treatments P, K and P+K caused only a marginal impact. Combination of N and K nutrients was found to have contributed maximum improvements in the seedling quality whereas P nutrient alone contributed least.

Keywords: *Pongamia pinnata* (L.) Pierre, seedling quality, nitrogenous and non-nitrogenous nutrient treatments

Introduction

Currently most of the emphasis on energy development is exclusively on electricity and oil and what is often neglected is the high dependence of developing countries on traditional biomass fuel. In developing countries 38% of the total energy is provided by the traditional biomass fuel and in countries like Bangladesh, Kenya and Paraguay it may be as high as 70 – 90% (Hall *et al.,* 1992). However the present pattern of development is oriented for rapid economic and commercial growth, which result in very large increase in the consumption of electricity and oil causing inequities within nation and among them, external debt and environmental degradation. Such commercial energy oriented developments are non-sustainable and there is a need to promote sustainable and dependable alternative indigenous energy sources to commercial fuel. The situation is clearly demanding the need for raising energy plantations as an alternative energy source.

This is important to evaluate the morphological and physiological quality of the seedlings in the nursery before transplanting them to the plantation site for achieving plantation success (Hawkins and Binder, 1990). The mineral nutrient application in the nursery is known to influence the growth and vigor of seedlings and they form significant output for enhancing productivity (Kannan, 1993). The nutrient deficiency is the most important factor limiting early tree growth on degraded soil (Jespersen, 1993). *Pongamia pinnata* (L.) Pierre is a tree species suitable for raising energy plantations as the wood of the tree is burned for cooking fuel considering the high calorific value of 4600 kcal/kg (National Academy of Sciences, 1980). The genus belongs to the family Leguminosae and the sub-family Papilionaceae. The present work was undertaken to study the morphological and physiological growth performance of *P. pinnata* in the nursery in response to the application of mineral elements N, P and K individually and in combinations.

Materials and Methods

One week after germination of *Pongamia pinnata* (L.) Pierre seeds, one seedling each was transplanted into each polythene container containing soil, sand and farm yard manure in the ratio 3:1:1, by volume. The container media had the following characteristics. pH- 5.1, bulk density- 1.19 g/cm^3, water holding capacity-35.63 %, organic carbon- 1.12%, available nitrogen (%)- 0.29 $\pm$ 0.07, available phosphorous (ppm)- 11.6 $\pm$ 1.38 and available potassium (ppm)- 20.3 $\pm$ 2.19.

The seedlings were arranged in triplicate blocks by using random block design. The nutrient(s) were supplied in the form of commercially available fertilizer(s), urea (46 % N_2), super phosphate (16 % P_2O_5) and murate of potash (59% K_2O). Mineral nutrients N, P and K were supplied alone or in combinations making a total of 8 treatments, including control. The amount of nutrient (s) supplied to the seedlings in different treatments were calculated and standardized by a preliminary trial and it was 0.3gm, 0.5gm and 0.3gm per seedling for N, P and K nutrients respectively. Watering was done twice a day with the help of a fine hose. Each container was displaced once in a week in order to prevent rooting into the sub soil, throughout the experiment.

The fertilizer application was done only once in the nursery and it was at the time of yellowing or detachment of cotyledons. The exact amount and type of nutrient(s) supplied were completely made soluble in water, so that each 200 ml. contained the required amount of nutrient(s). Water alone was done for control treatment. The observations on each of the parameters via total leaf area (TLA), total chlorophyll, leaf starch concentration, CO_2 assimilation rate and total dry biomass in the experimental seedlings were taken at every 30 days of regular intervals, till the end of the nursery phase. The first observations were made on 15th day after mineral nutrient application. Total leaf area (TLA) was estimated by using Licor 3100 Leaf Area Meter. Total chlorophyll was calculated by using Arnon's (1949) formula-

Total chlorophyll = $(20.2 \times A_{645}) + (8.02 \times A_{663})$

The starch was estimated by using the method of Pucher *et al.* (1948) as described by Whelan (1955). CO_2 assimilation rate was measured by using a portable carbondioxide analyzer with a leaf chamber (Model CI-301- CO_2 analyzer CID Inc-USA). Dry weight measurements were recorded with the help of Sartorious electronic balance (MCI Analytic AC 210 P. Germany). Statistical analysis was done by using Scheffee's Multiple Mean Comparison Test.

Result and Discussion

Estimation of total leaf area (TLA) is an essential component of plant growth analysis (Kvet *et al.,* 1971). Effect of mineral nutrient applications on the TLA of *Pongamia pinnata* seedlings in the nursery is depicted in table 1. Mineral nutrient applications greatly influenced the TLA and in most cases it followed a steady trend as the sampling period advances. All treated seedlings tended to exhibit an increased TLA over control. The maximum average total leaf area was noticed in seedlings supplied with combination of N+K nutrients and the minimum in P nutrient alone treated seedlings and were 58.60 % and 12.26% increase respectively over control. This inference noted in this study generally agrees with the observations of Vose *et al.* (1988) where the leaf area index increased significantly in Loblolly pine following nitrogen fertilization, while phosphorous addition did not affect significantly. Scheffee's Multiple Mean Comparison Test reveals the entire nitrogen nutrient treatments either alone (N) or its combination with P (N+P) or K (N+K) or both (N+P+K) significantly ($p<0.05$) enhanced the average total leaf area of seedlings, however the differences were not significant in non-nitrogenous (P, K and P+K) treatments with respect to control seedlings. Significant positive correlation attributed to the average total leaf area of *P. pinnata* seedlings in response to nitrogenous treatments strongly matches with the report of Prasad and Rawath (1992) in *Eucalyptus tereticornis* where nitrogenous fertilization significantly enhanced the leaf area and other growth parameters while the effect of phosphorous and potassium was marginal.

Table 1: Influence of mineral nutrient applications on the total leaf area (TLA) of *Pongamia pinnata* seedlings in the nursery.

Treatment	Total leaf area (TLA)/plant at different days after treatment (Sq .cm/plant)					Average TLA(Sq.cm/ plant)
	15	45	75	105	135	
N	730.83 c	847.17 c	1021.33 c	1109.17 de	1415.67 de	1024.83 c
P	301.83 a	403.83 a	491.83 a	570.00 ab	614.00 a	476.30 a
K	386.17 b	486.67 b	505.33 a	599.33 b	669.83 ab	529.47 a
N+P	687.33 c	718.17 c	800.67 b	839.00 c	1021.83 c	813.40 b
N+K	715.50 c	844.00 c	1013.5 c	1236.00 e	1532.17 e	1068.23 c
P+K	431.00 b	503.50 b	548.33 a	607.33 b	788.17 b	575.67 a
N+P+K	695.17 c	808.50 c	844.50 b	1043.67 d	1327.17 d	943.80 bc
Control	313.67 a	399.83 a	430.50 a	457.67 a	519.67 a	424.27 a
SE ±	9.04	11.00	11.53	11.05	13.02	66.62

A, b, c, d, e – Mean values within each column followed by different letter (s) differ significantly and the values share same letter (s) are not significantly different at $p<0.05$

Effect of mineral nutrient applications on the total chlorophyll content at different sampling period is shown in table 2. Nitrogenous treatments N, N+P, N+K and N+P+K always tended to cause a considerable increase in total chlorophyll content compared to control, whereas non-nitrogenous treatments P, K and P+K were not much affected.

Table 2: Influence of mineral nutrient applications on the total chlorophyll content of *Pongamia pinnata* seedlings in the nursery.

Treatment	Total chlorophyll content at different days after treatment (mg/gram leaf tissue)					Average total chlorophyll (mg/gram leaf tissue)
	15	45	75	105	135	
N	7.26 c	9.03 d	6.27 b	6.48 b	6.07 b	7.02 c
P	6.41 a	7.71 b	5.06 a	5.23 a	5.12 a	5.91 a
K	6.72 ab	6.83 a	6.02 b	5.71 a	5.54 ab	6.16 a
N+P	6.85 bc	8.42 c	5.55 a	6.48 b	5.82 b	6.62 b
N+K	7.17 c	8.66 cd	7.96 c	7.39 c	6.98 c	7.63 c
P+K	6.73 ab	7.60 b	5.20 a	5.62 a	5.05 a	6.04 a
N+P+K	6.94 bc	8.85 cd	7.43 c	7.65 c	6.75 c	7.52 c
Control	6.41 a	6.42 a	5.33 a	5.61 a	5.23 a	5.8 a
SE±	0.018	0.006	0.007	0.022	0.051	0.219

A, b, c, d – Mean values within each column followed by different letter (s) differ significantly and the values share same letter (s) are not significantly different at $p<0.05$

Average total chlorophyll content indicated that almost all the nutrient treated seedlings exhibited increased total chlorophyll content than the control and the maximum average increase was noticed in seedlings which received combination of N+K nutrients whereas the least was with P nutrient alone applied seedlings which were 31.55% and 1.90% increase respectively over control. A linear relationship between chlorophyll and nitrogen content in plants was inferred by Linder (1980) and present observations are also in agreement with this inference. Significant increase in the chlorophyll content recorded in *P. pinnata* seedlings treated with nitrogenous nutrients in the present study may be attributed to their role in the synthesis of many important proteins including chl - protein complexes essential for chlorophyll synthesis.

Table 2: Influence of mineral nutrient applications on the total chlorophyll content of *Pongamia pinnata* seedlings in the nursery.

Treatment	Total chlorophyll content at different days after treatment (mg/gram leaf tissue)					Average Total chlorophyll (mg/gram leaf tissue)
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P	6.41 a	7.71 b	5.06 a	5.23 a	5.12 a	5.91 a
K	6.72 ab	6.83 a	6.02 b	5.71 a	5.54 ab	6.16 a
N+P	6.85 bc	8.42 c	5.55 a	6.48 b	5.82 b	6.62 b
N+K	7.17 c	8.66 cd	7.96 c	7.39 c	6.98 c	7.63 c
P+K	6.73 ab	7.60 b	5.20 a	5.62 a	5.05 a	6.04 a
N+P+K	6.94 bc	8.85 cd	7.43 c	7.65 c	6.75 c	7.52 c
Control	6.41 a	6.42 a	5.33 a	5.61 a	5.23 a	5.8 a
SE±	0.018	0.006	0.007	0.022	0.051	0.219

A, b, c, d – Mean values within each column followed by different letter (s) differ significantly and the values share same letter (s) are not significantly different at $p<0.05$

Contradictory to the trend of TLA and total chlorophyll content, leaf starch concentration of *P. pinnata* exhibited a different trend. The supply of mineral nutrient N alone or its combination with P or K or both resulted in lesser accumulation of starch whereas supply of P or K or both together caused an increased starch accumulation (table 3). The highest average starch accumulation was noticed in seedlings treated with combination of P+K nutrients and the lowest average was in seedlings that received combination of N+K nutrients and which were 31.58% increase and 26.78% decrease respectively over control seedlings. Scheffee's Test clearly revealed none of the treatments caused any significant variation ($p<0.05$) in the average starch content compared to control. However the variations in starch content were significant ($p<0.05$) in seedlings which received P nutrient alone and their combination with K over seedlings that received combination of N+K and N+P+K. Lower starch accumulation in nitrogenous nutrient treated seedlings compared to non-nitrogenous nutrient treated seedlings and control may be due the better utilization of starch in respiration and growth (Kannan and Kailash, 1996).

Table 3: Influence of mineral nutrient applications on the leaf starch content of *Pongamia pinnata* seedlings in the nursery.

Treatment	Starch concentration at different days after treatment (mg/gram leaf tissue)					Average starch content (mg/gram leaf tissue)
	15	45	75	105	135	
N	10.65 cd	14.07 ab	18.46 ab	20.20 b	21.87 cd	17.05 ab
P	11.22 d	19.78 cd	29.81 d	26.93 c	27.16 e	22.98 b
K	11.03 cd	18.83 c	27.24 d	22.66 b	19.06 bc	19.76 ab
N+P	10.35 c	12.16 a	20.86 bc	21.93 b	21.75 cd	17.41 ab
N+K	5.38 a	11.47 a	16.19 a	15.24 a	15.84 a	12.82 a
P+K	15.12 e	23.25 d	26.91 d	25.72 c	24.22 d	23.04 b
N+P+K	10.83 cd	16.26 bc	17.24 ab	16.50 a	16.33 ab	15.43 a
Control	8.52 b	14.85 ab	22.23 c	21.86 b	20.11 c	17.51 ab
SE $\pm$	0.13	0.59	0.52	0.45	0.42	1.26

A,b,c,d,e – Mean values within each column followed by different letter (s) differ significantly and the values share same letter (s) are not significantly different at $p<0.05$

CO_2 assimilation rate of *P. pinnata* seedlings in response to different nutrient treatments is shown in table 4. Seedlings provided with nitrogenous treatments N, N+K, N+P and N+P+K generally exhibited higher CO_2 assimilation rate compared to P, K and P+K treated seedlings and control. The average highest CO_2 assimilation rate was noticed in seedlings received combination of N+K nutrients whereas the lowest in P nutrient alone supplied seedlings which were 88.97 % increase and 2.01 % decrease respectively over control. Multiple mean comparison test reveals the improvements in CO_2 assimilation rate contributed by nitrogenous nutrient treatments were significant over CO_2 assimilation rate contributed by non-nitrogenous treatments and control, whereas the variations were insignificant among control and non-nitrogenous treatments. The present study fully shares the trend in this respect made by Sharma *et al.* (1989) in citrus plants, where highest CO_2 assimilation rate was observed in plants receiving N+P or N+K nutrients and minimum values of this was observed in plants receiving P+K nutrients. The results also are in similar lines with the report of Mehouachi *et al*. (1993) in potato plants.

Table 4: Influence of mineral nutrient applications on the CO_2 assimilation rate of *Pongamia pinnata* seedlings in the nursery.

Treatment	CO_2 assimilation rate at different days after treatment (µ moleCO_2/sq.cm/sec)					Average CO_2 assimilation rate (µmole CO_2/sq. cm/sec)
	15	45	75	105	135	
N	7.75 d	6.48 c	5.04 b	6.26 bc	4.53 b	6.01 b
P	5.24 a	3.91 ab	3.43 a	3.63 a	3.33 a	3.91 a
K	5.46 a	4.25 b	3.71 a	5.62 bc	3.61 a	4.53 a
N+P	7.34 c	6.16 c	5.15 b	5.52 b	3.94 ab	5.63 b
N+K	7.88 d	6.87 c	7.17 c	8.48 d	7.29 c	7.54 c
P+K	5.12 a	4.13 b	3.83 a	3.72 a	3.26 a	4.01 a
N+P+K	6.87 bc	6.68 c	8.06 c	6.49 c	7.68 c	7.16 c
Control	6.23 b	3.04 a	3.34 a	3.93 a	3.42 a	3.99 a
SE ±	0.16	0.13	0.16	0.13	0.16	0.17

A, b, c, d – Mean values within each column followed by different letter (s) differ significantly and the values share same letter (s) are not significantly different at $p<0.05$

The entire nutrient treatments generally caused an increased total dry biomass production in *P. pinnata* seedlings over control seedlings (table 5). Final measurements reveal that the enhancement in total dry matter production was significant in the nitrogenous nutrient treated seedlings compared to control and non-nitrogenous nutrient treated seedlings. Variations due to the application of P, K and P+K were insignificant over control. Maximum dry biomass was obtained in seedlings that received a combination of N and K nutrients while least with P nutrient alone received seedlings which were 87.85 % and 5.80 % increase respectively over control. Significant improvements in total leaf area, total chlorophyll content and CO_2 assimilation rate induced by nitrogenous nutrient treatments might be the reason which contributed more improvement in seedling quality and better dry biomass accumulation. Another attribution to the higher biomass production in nitrogenous nutrient treated seedlings could be due to the increased utilization of starch in respiration, which in turn reflected in enhanced growth and biomass whereas comparatively lower biomass in non-nitrogenous nutrients treated seedlings could be due to accumulation of starch instead of utilization in respiration and growth (Kannan and Kailash, 1996: Nguyen et al., 2003).

Table 5: Influence of mineral nutrient applications on the total dry biomass accumulation of *Pongamia pinnata* seedlings in the nursery.

Treatment	Average total dry biomass at different days after treatment (gm/plant)				
	15	45	75	105	135
N	4.20 b	8.87 c	12.60 b	17.16 b	21.96 b
P	2.34 a	5.29 ab	6.79 a	10.27 a	13.67 a
K	2.55 a	5.10 a	7.04 a	11.28 a	15.40 a
N+P	3.76 b	8.36 c	12.16 b	17.69 bc	21.75 b
N+K	3.93 b	8.38 c	13.18 b	19.59c	24.27 b
P+K	2.71 a	6.12 b	7.34 a	10.37 a	14.50 a
N+P+K	3.70 b	8.36 c	12.88 b	19.28 c	23.91 b
Control	2.78 a	4.66 a	6.71 a	9.62 a	12.92 a
SE ±	0.10	0.17	0.19	0.36	0.53

A,b,c – Mean values within each column followed by different letter (s) differ significantly and the values share same letter (s) are not significantly different at $p<0.05$

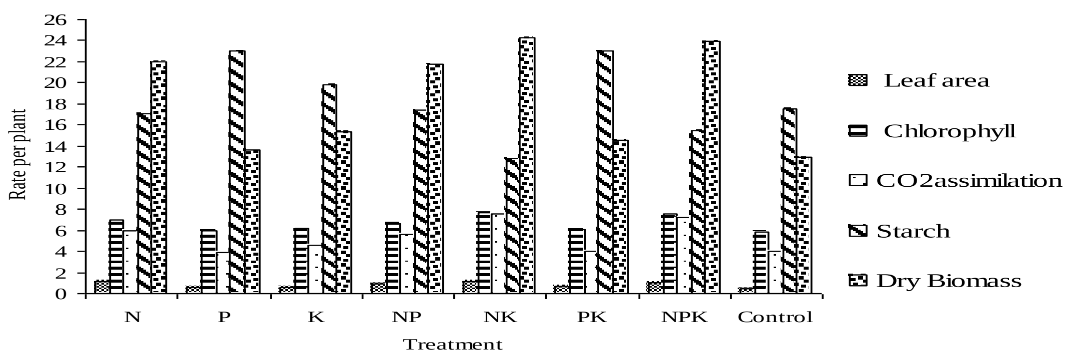

Fig. 1: Comparative evaluation of total leaf area (sq.ft /plant), total chlorophyll (mg/gm leaf tissue), CO_2 assimilation rate (µmoleCO_2/sq.cm/sec), starch content (mg/gm leaf tissue) and dry biomass accumulation (gm/plant) in *Pongamia pinnata* seedlings in response to mineral nutrient(s) applications.

Conclusion

The study clearly revealed the application of mineral nutrient N alone or its combinations N+P or N+K or N+P+K significantly improved seedling quality of *Pongamia pinnata* (L.) P*ierre* through better physiology and growth performance while improvements made by P or K nutrient either alone or their combinations were only marginal and insignificant. In the light of the present investigation, it is suggested that each plant species may be studied with respect to different mineral nutrient application in the nursery level to raise quality seedlings before large scale field application is made. Even more so caution is to be taken to know the right combination and right concentration for specific tree species so as to provide better results than untreated seedlings for plantation success.

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