DOI: 10.17707/AgricultForest.62.3.09

Shourav DUTTA [1]

NODULATION STATUS AND NITROGEN FIXING POTENTIAL OF AKASHMONI *(Acacia auriculiformis)* SEEDLINGS IN TROPICAL MIXED PLANTATIONS

SUMMARY

A study was carried out to assess biological nitrogen fixation and observe nodulation status of *Acacia auriculiformis* (Akashmoni) at mixed plantations in the Seed Research Laboratory and nursery of the Institute of Forestry and Environmental Sciences, Chittagong University (IFESCU), Bangladesh. The plantations consist of one pure planting plot (100% A) and eleven mixed planting plots (1A:1S, 1A:1G, 1A: 1G: 1S, 1A:2S, 1A:2G, 1A:3S, 1A:3G, 2A:1S, 2A:1G, 3A:1S and 3A:1G) of the three common plantation tree species of Bangladesh namely *Acacia auriculiformis* (A), *Swietenia macrophylla* (S) and *Gmelina arborea* (G). In Seed Research Nursery, seedlings of three species were raised in a randomized blocks with three replicates of twelve treatment plots. Nodule number, color, size, shape, form, structure, nodule fresh weight, oven-dry weight and total nitrogen accumulation in the soil of *A. auriculiformis* seedling were recorded. At the age of 10 month, highest nodulation (143 number) of *A. auriculiformis* were recorded in the mixed plot (1A:2S) in comparison with pure 100%A plot. Fresh and oven dry weight of roots of the *A. auriculiformis* seedlings were found significantly ($p<0.05$) highest in 1A:2S mixed plot. The findings of this study suggest that in comparisons with pure plot, A. auriculiformis fix better nitrogen in mixed plantations.

Keywords: *Acacia auriculiformis*, Bangladesh, Mixed Plantation, Nitrogen fixation, Nodule, Symbiosis.

INTRODUCTION

One of the major management objectives of tropical forests of Bangladesh was to replace the heterogeneous natural forests by the mixed plantations of valuable timber species (Dutta *et al*. 2014). The management of mixtures of two dominants is more difficult; they can usually only be managed on specific sites and often result in the suppression of one of the species (FAO 1992, Hossain 2008). Nitrogen fixing trees in tropical environments appear to offer both high growth rate and soil enrichment (Binkley and Giardiana 1997). Nitrogen fixing trees may increase the supply of available nitrogen in the soil, benefiting both N–fixing and non–N fixing trees (Binkley *et al.* 2000). The success of mixed species plantations depends on species attributes and site factors (Forrester *et al.* 2005)

[1] Shourav DUTTA, (corresponding author: shourav.forestry@gmail.com), Institute of Forestry and Environmental Sciences, University of Chittagong, Chittagong-4331, BANGLADESH.
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

that affect the balance of positive and negative interactions between the species (Boyden *et al*. 2005). Nitrogen fixation by NFS is supposed to improve soil N availability (Bouillet *et al.* 2008, Khanna 1997, Voigtlaender *et al.* 2012), alleviating N limitation and facilitating the growth of the target species in N_2 limited soils (Forrester et al. 2007). In addition, nitrogen fixing species may enhance phosphorus (P) availability through the rhizosphere acidification due to nitrogen fixation (Hinsinger *et al.* 2011), increasing the amount of P that cycles in mixed-species plantations compared to monocultures (Forrester *et al.* 2005). For increasing the effectiveness of the biological nitrogen fixation, studies are needed to carry out on the nodulation and nitrogen fixation in the existing legume flora in different parts of the world (Mahmood and Iqbal 1994). In Bangladesh, information on comparative seedling growth and nodulation of legumes are very scanty (Aryal *et al.* 1999). There is a wide range of nitrogen fixing plants that have been used in forestry with an objective of raising soil nitrogen levels and subsequently improving the growth of the non–nitrogen fixing forest species (Turvey and Smethrust 1983). But still research on nitrogen–fixing species in general has lagged behind that on food, feed, and forage crops.

Estimates of N_2-fixation for tropical evergreen forests are extremely rare and highly variable (Cleveland *et al.* 1999). Over 600 tree species have been reported to fix nitrogen (MacDicken, 1994). In Bangladesh, a total of 98 genera and 332 sub–generic taxa of nitrogen–fixing species, both wild and cultivated have been recorded under the families Caesalpiniaceae (20, 60), Mimosaceae (14, 39) and Fabaceae (64, 233) (Khan *et al.* 1996). *Acacia auriculiformis* and *Albizia* spp. are two most common nitrogen fixing species commonly used in road side and forest plantation, agroforestry, community forestry and homestead plantation programs in Bangladesh. Various exotic nitrogen fixing plants with native nitrogen fixing trees are planted all around the country. A common expectation is that an N_2-fixing species will enhance nitrogen availability to the other and accelerate nitrogen cycling, so that total growth will be greater. Recently N_2–fixing tree species such as *Acacia auriculiformis, Albizia* spp., *Leucaena leucocephala* etc are planted with non–N_2 fixing tree in the homegarden of Bangladesh (Alam *et al.* 2005). *Acacia auriculiformis*, *Swietenia macrophylla* and *Gmelina arborea* are the three major plantation tree species proved successful in trials and in large scale plantation programs of Bangladesh (Hossain and Hoque 2013). The present study was aimed to investigate the optimum mixing and benefits of nitrogen fixation of N_2-fixing *A. auriculiformis* with non-nitrogen fixing species (*S. macrophylla* and *G. arborea*). The present attempt was also made with a view to studying the nodulation potential and nitrogen fixing ability of *A. auriculiformis* used in the mixed plantations of Bangladesh.

MATERIAL AND METHODS

Experiment site

The study was conducted in the nursery seed bed of Seed Research Laboratory of the Institute of Forestry and Environmental Sciences, University of Chittagong

(IFESCU) campus, Chittagong, Bangladesh. The experimental design was carried out over a period of ten months from February to November, 2015 in the IFESCU nursery. The experimental site (nursery) lies approximately at the intersection of 91°50´ east longitude and 22°30´ north latitude (Khan *et al*. 2004) The nursery site enjoys a tropical monsoon climate characterized by hot, humid summer and cool, dry winter (Mahmud *et al.* 2005). The average monthly mean temperature varied 29.75°C maximum and between 21.14°C minimum (Ahmed, 1990). The annual rainfall in the nursery is 2500–3000 mm which mostly takes place between June and September (Gafur *et al*. 1979). Relative humidity was generally the lowest (64%) in February and highest (95%) in June–September (Mahmood *et al.* 2005).

Experimental plot design

Seeds of *Acacia auriculformis* (Akashmoni), *Swietenia macrophylla* (Mahagony) and *Gmelina arborea* (Gamar) were collected from the Bangladesh Forest Research Institute (BFRI), Chittagong, Bangladesh in the month of February, 2015 and seedlings were raised in the nursery bed of Institute of Forestry and Environmental Sciences, University of Chittagong (IFESCU) with standard nursery techniques (Fig. 1). The soil samples collected from the barren hills of the Chittagong University Campus was sieved well (< 3mm) and then fill up the seed bed. The hills consist of moderate to strongly acidic soils (Osman *et al.* 1992) and an average soil pH 5.5 (Badruddin *et al.* 1989). The soil used in the nursery was moderately coarse to fine textured (Mahmood *et al.* 2005). Seedlings were raised in a randomized blocks with three replicates of twelve treatment plots. One pure planting plot of *A. auriculformis* along with ten mixed planting plots of two species (either *Acacia* × *Swietenia* or *Acacia* × *Gmelina)* was established. One mixed planting plots of three species [*Acacia* (A) × *Swietenia* (S) × *Gmelina* (G)] were established. Pure planting plot was P_1 (100% A) and eleven mixed planting plots were –

a) M_1 (1A:1S), M_2 (3A:1S), M_3 (1A:3S), M_4 (2A:1S), M_5 (1A:2S);
b) N_1 (1A:1G), N_2 (3A:1G), N_3 (1A:3G), N_4 (2A:1G), N_5 (1A:2G);
c) M_6 (1A: 1G: 1S).

Each plot was 200 cm × 60 cm in size with 30 seedlings at a spacing of 20 cm × 20 cm (seedling to seedling distance: 20 cm). Within each mixed plot of 1A:1S or 1A:1G, seedlings of *Acacia* were planted with *Swietenia* or *Gmelina* alternatively. In 2A:1S or 2A:1G plot, two seedlings of *Acacia* followed by one seedlings of either *Swietenia* or *Gmelina* sequentially. Similarly, in 1A:2S or 1A:2G plots one seedling of *Acacia* followed by two seedlings of *Swietenia* or *Gmelina* sequentially. Again, within each mixed plot of 3A:1S or 3A:1G, three seedlings of *Acacia* followed by one seedlings of *Swietenia* or *Gmelina* sequentially. Similarly, in 1A:3S or 1A:3G plot, one seedling of *Acacia* followed by either three seedlings of *Swietenia* or *Gmelina* consequently.

(a) (b)

Fig. 1. All the experimental plots in the Seed Research Nursery (a: Two months old seedlings, b: Five months old seedlings).

Harvesting and data collection in the seed research laboratory and nursery

Five randomly selected seedlings of each species were harvested carefully from each mix plot and ten seedlings from pure plantation plot at 10 months after germination. The harvested seedlings were washed in tap water and then in distilled water to clean the root regions off all soil particles. Fresh weight of the root of *A. auriculformis* was measured after removal of all water from the root portion of the washed seedlings. Dry weight of roots was recorded after oven dried at 70°C for 72 hours.

Evaluation of nodulation

For *A. auriculiformis* nodule numbers per seedling were recorded after measuring the root weight. Then nodules were separated from individual plants and further washed carefully to remove all the soil particles (Solaiman, 1999). The root nodules were counted and their fresh weight was recorded. Nodule status, shape, color and structure were recorded. Nodule numbers in roots were recorded and nodule score was evaluated using the following table (Table 1):

Table 1. Classification criteria used to evaluate the nodulation in the roots (Corbin 1977)

Soil level	Nodule number in roots	Nodule score
0 – 5 cm	0	0
>5 cm	0	
0 – 5 cm	<5	1
>5 cm	0	
0 – 5 cm	5 - 10	2
>5 cm	0	
0 – 5 cm	>10	3
>5 cm	0	
0 – 5 cm	>10	4
>5 cm	<5	
0 – 5 cm	>10	5
>5 cm	>10	

Effectiveness of nodules was represented as follows (Peoples, 1989):

Nodule Score	Representation
4 - 5	Excellent nodulation; excellent potential for N_2-fixation
3 - 4	Good nodulation; good potential for fixation
2 - 3	Fair nodulation; N_2 fixation may not be sufficient to supply the Nitrogen demand of the crop/plant.
0 - 2	Poor nodulation, little or no N_2-fixation.

Collection of soil samples and determination of nitrogen

In the nursery, soil samples were collected from each plot at different positions from a depth of 30–35 cm near each seedling. Soil samples were then air dried, ground in agate mortar and passed through a 2 mm mesh sieve. The soil samples were analyzed in the chemistry laboratory of institute of forestry and environmental sciences, Chittagong University (Ifescu), Bangladesh to determine total nitrogen. The total nitrogen content was determined by the Kjeldahl method (Bremner, 1965).

Statistical analysis

Analysis of variance (ANOVA) and tests for means ($p < 0.05$) were run using the means of each variable from each of the three replicate plots. The statistical evaluations of the data obtained as a result of research was carried out though variance analysis according to randomized blocks experimental design. In order to determine the difference between the averages, the Duncan's multiple range test (DMRT) was utilized. All the data collected were analyzed statistically by using spss (Aryal et al. 1999, khan et al. 2004).

RESULTS AND DISCUSSION

Nodulation status of A. auriculiformis seedling

Nodules of *A. auriculiformis* were elongated to ovate with/ without branching and pink/ brown in color. *A. auriculiformis* nodules were found both in primary and secondary roots of the seedlings (Fig. 2). Nodule number differed significantly among pure and mixed plots (Table 2).

(a) (b) (c) (d) (e)

Fig. 2. Biological nitrogen fixation of *A. auriculiformis* through nodulation (a: Seeds of *A. auriculiformis*,b: Germination of seeds in seed bed, c: Three months old seedlings under nursery condition, d: Species interactions among *A. auriculiformis, S. macrophylla* and *G. arborea*, e:Nodule formation of A. auriculiformis seedlings both in primary and secondary roots).

Table 2. Nodule number, nodulation status, color, shape, branching status and distribution of nodules of A. auriculiformis in pure and mixed planting plots at 10 months after germination.

Treatments	Nodule number	Nodule condition	Color	Shape	Branch/ Cluster	Distribution in roots
P_1	28.34^{abc*}	Sparse	Pink	ovate to obovate	Branch and Cluster	Primary and Secondary
M_1	29.35^{abc}	Sparse	Brown to dark brown	elongate to elongate	Branch	Secondary
M_2	21.67^{abc}	Sparse	Pink	elongate to elongate	Branch	Primary
M_3	38.67^{bc}	Moderate	Brown	elongate to ovate	Cluster	Primary
M_4	32.34^{abc}	Moderate	Brown to dark brown	elongate to ovate	Branch and Cluster	Secondary
M_5	58.67^{c}	Abundant	Brown	elongate to elongate	Cluster	Primary and Secondary
M_6	18.35^{ab}	Sparse	Pink	ovate to obovate	Branch and Cluster	Secondary
N_1	14.3^{ab}	Sparse	Pink	elongate to elongate	Brach	Secondary
N_2	18.36^{ab}	Sparse	Brown	elongate to ovate	Cluster	Secondary
N_3	20.67^{ab}	Sparse	Dark Brown	ovate to obovate	Cluster	Secondary
N_4	12.32^{ab}	Sparse	Pink	elongate to ovate	Branch and Cluster	Primary
N_5	6.68^{a}	Sparse	Pink	elongate to elongate	Branch	Secondary

(*) Means followed by the same letter(s) in the same column are not significantly different at $p < 0.05$ (DMRT).

Nodules of *A. auriculiformis* in pure and mixed plots were found pink or brown in color. (Fig. 3). Nodule size varied from 2.6 × 3.3 mm in N5 to 5.2 × 6.3 mm in M5. A. auriculiformis possessed nodules both in single and aggregate forms. Variations in nodule size, form and structure were observed among the treatment plots (Table 3).

a) b) c) d)

Fig. 3. Nodulation in *A. auriculiformis* seedlings (a: Nodules in Cluster, b: Ovate to obovate nodules, c: Elongate to ovate nodules, d: Nodules in lateral roots).

Table 3. Nodule size and Structure of ten months old seedlings of A. auriculiformis grown under nursery condition

Treatments	Nodule size(mm)	Form	Nodule structure
P_1	3.1 × 3.6	S*	Advance indeterminate
M_1	2.8 × 3.4	A	Primitive indeterminate
M_2	3.2 × 3.7	S & A	Advance indeterminate
M_3	3.5 × 4.2	S	Advance determinate
M_4	3.7 × 4.4	A	Primitive indeterminate to Advance determinate
M_5	5.2 × 6.3	S & A	Advance determinate
M_6	2.7 × 3.9	A	Advance determinate
N_1	3.7 × 4.5	S	Advance indeterminate
N_2	4.2 × 4.9	S & A	Primitive indeterminate to Advance determinate
N_3	3.8 × 4.6	A	Advance indeterminate
N_4	3.6 × 3.8	S	Primitive indeterminate to Advance determinate
N_5	2.6 × 3.3	A	Primitive indeterminate

*S: single and A: aggregated.

Maximum number (143) of nodule was recorded in M5 followed by 38 in M3 and 32 in M4. Nodule number was found minimum (6) in N5. A. auriculiformis seedlings showed excellent and good nodulation status in both pure and mixed plots. A. auriculiformis fixed more Nitrogen through nodule formation in their root systems with S. macrophylla than with G. arborea. A. auriculiformis seedlings showed excellent, good or fair nodulation status in mixed plots except N5 plot. Nodulation status found fair in N2 and N4 plots and poor in N5 plot (Fig. 4).

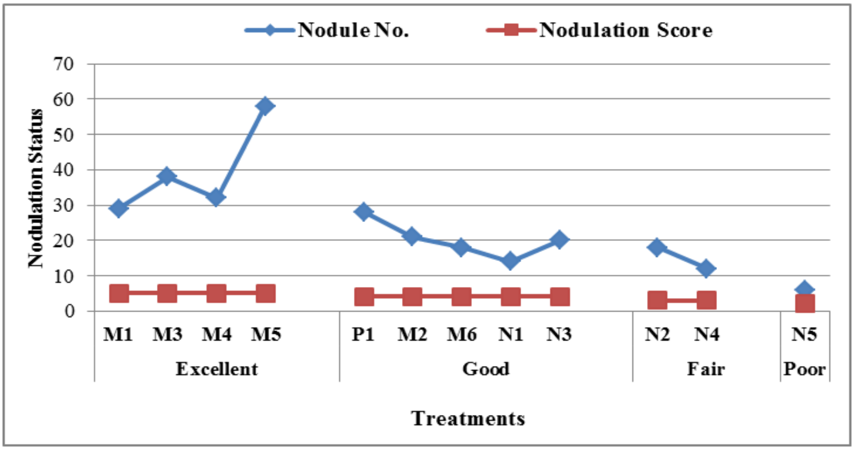

Fig.4. Treatment-wise nodulation status and nodulation score of A. auriculiformis seedlings.

At the time of *A. auriculiformis* seedling harvest, the highest root fresh weight (27.26 g) was recorded in M5 followed by 20.06 g in P1 and 19.07g in M3. Compared with pure plot, the fresh weight of root was significantly higher in mixed plots. The result of variance analysis for root dry weight and nodule fresh weight *A. auriculiformis* of revealed that the effect of planting pattern was significant ($p < 0.05$). Mean comparison using Duncan multiple range test (DMRT) showed that the highest root dry weight obtained in mixed plot M5. Compared with pure plot (10.06 g), the dry masses of root were also significantly higher in M5 (19.28g) followed by M2 (10.82 g). The result of analysis of variance revealed that planting patterns had significant effect on the nodule fresh weight of A. auriculiformis (Table 4).

Table 4. Comparative root fresh weight (g), nodule fresh weight (g), root dry weight (g) and total nitrogen (%) of ten months old *A. auriculiformis* seedlings.

Treatments	Root Fresh Wt. (g)	Root Dry Wt.(g)	Nodule Fresh Wt.(g)	Total N (%)
P_1	20.06^{cd*}	10.06^{cde}	1.19	0.29^{ab}
M_1	14.51^{abc}	7.64^{abcd}	1.54	0.36^{ab}
M_2	18.53^{bcd}	10.82^{de}	1.98	0.93^{c}
M_3	19.07^{bcd}	9.34^{bcde}	1.76	0.47^{ab}
M_4	14.11^{abc}	7.70^{abcd}	0.98	0.40^{ab}
M_5	27.26^{d}	19.28^{e}	2.01	0.22^{ab}
M_6	10.71^{abc}	7.03^{abcd}	1.50	0.38^{ab}
N_1	6.33^{a}	4.2^{a}	1.29	0.32^{ab}
N_2	9.66^{ab}	5.28^{abc}	0.86	0.56^{b}
N_3	7.74^{a}	4.83^{ab}	1.47	0.34^{ab}
N_4	8.42^{a}	5.25^{abc}	1.67	0.31^{ab}
N_5	6.49^{a}	4.62^{ab}	1.04	0.11^{a}

(*) Means followed by the same letter(s) in the same column are not significantly different at $p < 0.05$ (DMRT).

Highest values of total Nitrogen (0.93%) was measured in mixed M2 plot. Compared with pure plots, the total nitrogen were significantly higher in mixed plots M2 followed by N2 (0.56%) and M3 (0.47%). Among all the plots the sequence of total nitrogen (%) were M2 > N2 > M3 > M4 > M6 > M1 > N3 > N1 > N4 > P1 > M5 > N5 (Fig. 5).

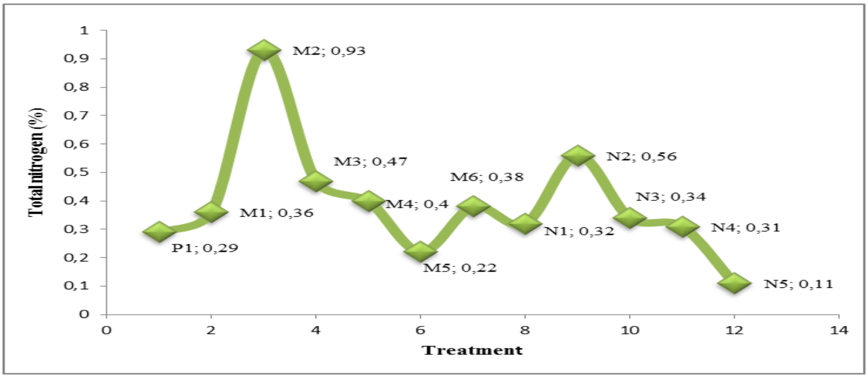

Fig. 5. Percentage of total nitrogen in the soil of twelve experimental plots

The present study reveals a total of 143 nodules per tree for *A. auriculiformis* in the mixture with *S. macrophylla* were comparatively more than Aryal *et al.* (1999) and Khan et al. (2004). Aryal *et al.* (1999) recorded 55 nodules per tree in a 14 months old plantation and Khan *et al.* (2004) recorded 61 nodules per tree in a 3 months old plantation for *A. auriculiformis* respectively. Compared with the findings of several other researchers, the findings of the present study were similar to that of Aryal *et al*. (1999) and Khan *et al*. (2004). The present findings also revealed that *A. auriculiformis* showed excellent nodulation behavior in the mixture with *S. macrophylla* than with *G. arborea.* The present investigation also recorded nodule fresh weight (2.01 g) for *A. auriculiformis* which was greater than Aryal *et al.* (1999). Aryal *et al.* (1999) recorded 1.34 g nodule fresh weight for *A. auriculiformis.*

A. auriculiformis (Akashmoni) is extensively planted in plantations all over the country for its short rotation, wider adaptability and faster growth. *S. macrophylla* (Mahagony) and *G. arborea* (Gamar) are two non–nitrogen fixing species extensively planted in forests, marginal lands, institutes, roadsides, railway sites, field borders and homesteads of Bangladesh. From the present investigation, it is evident that the mixed stands possess better effects than the pure stands in improving soil nitrogen.

In this study, the nodulation status of *A. auriculiformis* seedlings was measured at ten months after germination. In this respect, given the values presented in Table 2, it has been determined according to variance analysis applied that there were statistically significant differences among nodulation of this species in various treatments (p<0.05). According to the results of Duncan Multiple Range test implemented, it has been found that the nodulation behavior of *A. auriculiformis* in pure plots were lower than in mixed plots. The present study also indicates the increment of nitrogen in the soil through plantation of nitrogen fixing tree species.

Though monoculture of exotic species such as *A. auriculiformis* has some effect on native biodiversity of Bangladesh, mixed plantation of *A. auriculiformis* with valuable indigenous tree species particularly *G. arborea* may show marvelous results. *A. auriculiformis* may also be planted in degraded hilly areas because it was reported to thrive well on moderately acid, poorly fertile soil and also improves pH, physical and chemical properties of soil. The present investigation indicates three most common plantation tree species (*A. auriculiformis, G. arborea* ***and*** *S. macrophylla*) of Bangladesh which were similar to Das and Sarkar (2014) conducted in the Bhawal Sal forest, in the central region of Bangladesh. Das and Sarkar (2014) observed significant positive relationship between species growth, diversity and productivity in four mixed species plantations and the subject species were *A. auriculiformis, G. arborea, S. macrophylla* and *A. mangium.*

From the present investigation it was also found that nodule size of *A. auriculiformis* seedlings varied from 2.6 × 3.3 mm in N_5 plot to 5.2 × 6.3 mm in M_5 plot which was greater than Khan *et al*. (2004). Khan *et al.* (2004) recorded

3.2 × 6.1 mm nodules in three months old *A. auriculiformis* seedlings under nursery condition.

Nodule number and nodule mass or nodule weight per unit dry weight of the whole plant or root system are often used in trial comparisons; however, similar information can be obtained by visually scoring nodulation on a 0-5 basis taking into account nodule number, size, pigmentation and distribution. Nodule score is judged by the number of effective nodules in the crown-root zone (regarded as the region 5 cm below the first lateral roots) and elsewhere on the root system (Corbin, 1977). The present study indicates an excellent nodulation status of *A. auriculiformis* in plantation forests by increasing nodule number below (5 cm) the first lateral roots.

The present study reveals that mixed species plantations of *A. auriculiformis* with other non-nitrogen fixing species (*G. arborea* and *S. macrophylla*) have the potential to improve nodulation, nitrogen fixation and stand productivity over that of *Acacia* monoculture through catalytic effects on soils by *Acacia*, which is similar to that of Forrester *et al.* (2007). A study was conducted by Forrester *et al*. (2007) reported that nitrogen fixation and productivity were higher in mixed stands of *Acacia mearnsii* with non-nitrogen fixing *Eucalyptus globulus* than in *A. mearnsii* monocultures.

Effectiveness of nodules can generally be gauged by the degree of pink or red coloration of N_2-fixing bacteroid tissue inside each nodule (Corbin 1977). As a general rule, white or green nodules are inactive and would not be considered when classifying active nodulation (Corbin 1977, Peoples 1989). The present study revealed that nodules of *A. auriculiformis* in pure and mixed plots were pink or brown in color. So the result indicates all the nodules in the root system were active.

CONCLUSIONS

The results of present investigation showed biological nitrogen fixation and nodulation status of *Acacia auriculiformis* in mixed plantations in comparison to pure plantations. During the present study, fixation of biological nitrogen in the soil through the root system of *A. auriculiformis* seedlings was considered as the only way of nitrogen increment in the soil, rejecting all other factors such as rainfall, storm, thunder–bolt, fertilizer treatment etc. The present findings would be so much helpful to carry out further investigation on the growth and development of different nitrogen fixing species to both acidic and alkaline soils before recommending them in mixed plantation programs. These results are based on 10–month old seedlings. So, further researches are necessary to assess the exact performance of *A. auriculiformis* in fixation of biological nitrogen in the soil.

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