



Influence of Biofertilizers on Early Stage Seedling Growth, Biomass and Vigour of *Anthocephalus cadamba* (Roxb.) Miq.

Punamkumari Chauhan, L.K. Behera, M.B. Tandel, N.S. Thakur, R.S. Chauhan and C.A. Dholariya

College of Forestry, Navsari Agricultural University, Navsari-396 450, India
E-mail: punamcchauhan@gmail.com

Abstract: The present investigation was conducted to know the influence of biofertilizers on seedling growth and vigour in *Anthocephalus cadamba* (Roxb.) Miq., for which it was laid out in a Completely Randomized Design with three repetitions and seven biofertilizer treatments. An application of VAM @ 10 ml plant⁻¹ exhibited significantly maximum collar diameter (9.62 mm), root length (33.04 cm) and found at par with Azospirillum @ 10 ml plant⁻¹ at 150 DAT. However, Azospirillum @ 10 ml plant⁻¹ exhibited maximum shoot length (80.52 cm), number of leaves per plant (13.33) and total leaf area (cm²) per plant (1770.29 cm²), and found at par with VAM @ 10 ml plant⁻¹ having 76.50 cm, 13.25, 1692.92 cm² for same growth characters of *A. cadamba* seedlings at 150 DAT. Further, VAM @ 10 ml plant⁻¹ produced significantly maximum fresh weight, dry weight and seedling quality index of 96.58 g, 19.67 g and 1.77, respectively whereas the maximum root to shoot ratio of 0.33 and the lowest improved sturdiness quotient of 7.53 were observed in seedlings treated with Pseudomonas @ 10 ml plant⁻¹, which was statistically at par with VAM @ 10 ml plant⁻¹ at 150 DAT. Overall result showed that application of VAM @ 10 ml plant⁻¹ improved the growth and vigour of *A. cadamba* seedlings, so this treatment can be used for raising and production of good quality seedlings in the forest nursery.

Keywords: *Anthocephalus cadamba*, Biofertilizers, Seedling quality index, Seedling vigour, VAM

Anthocephalus cadamba (Roxb.) Miq., synonymously named as *Neolamarckia cadamba* (Roxb.) Bosser and *Anthocephalus chinensis* (Lam.) A. Rich ex Walp. and popularly known as Kadamb or Kadam tree belongs to Rubiaceae family. The Kadam is a large tropical tree with broad crown and straight cylindrical bole having average height about 15 meters and in the favourable climatic condition it attains a height of 20 meters or more with a clean bole of about 9 meter and a diameter of 40 to 60 cm. The tree is particularly aesthetic and deciduous in nature however sometimes evergreen and semievergreen in nature (Bijalwan et al 2014). *A. cadamba* is native to South and Southeast Asia, including India, Indo-Malayan region, Java, Sumatra, China, Indonesia, Malaysia, Bangladesh, Sri Lanka, Cambodia, Papua, New Guinea, Philippines and Singapore. In India, it occurs in the Sub-Himalayan tract from Nepal eastward to West Bengal and Assam, Bihar, Chhattisgarh, Madhya Pradesh, Andhra Pradesh and evergreen forest of Karnataka to Kerala (Anonymous 1985). The species is considered suitable for soil conservation, agroforestry, jhum and land reclamation. The rotation period or harvesting of Kadam depends upon the production purposes, normally for pulpwood and matches, harvesting can start at 4-5 years whereas for wood production, felling of trees can start approximately from the age of 10 years (Bijalwan et al 2014). *A. cadamba* wood used in the ceiling

boards, light construction work, packing cases, planking, carving and turnery. It makes good veneers and plywood suitable for the manufacture of grade IV commercial plywood and tea chest plywood. It is highly suitable for the manufacture of pencils, match boxes and splints and also suitable for making printing and wrapping paper (Chaturvedi et al 2017). There is high demand for the species in pencil industry, plywood and match splints and adopted in plantation, particularly in agroforestry with a spacing of at least 5 m × 5 m or 6 m × 6 m and farm forestry practices in humid tropics for industrial and income generation (Bijalwan et al 2014). Further, *A. cadamba* has minimum shade effect and no allelopathic effect on the agricultural crop advocates its suitability for the agroforestry purpose.

Forest nurseries play a vital role in the development of plantations of forest trees. There is large demand for quality forest planting materials, so it has become imperative that forest nurseries should be managed professionally to produce the desirable quality of seedlings (Shreedher and Mohan 2016). The improved nursery seedlings stock can be obtained by application of biofertilizers. Biofertilizers are group of microorganisms consisting of bacteria, fungi, algae etc. These alone or in combinations are known to be increasing plant growth by way of various biochemical activities in the soil such as Nitrogen fixation, breakdown of organic matter, secretion of plant growth hormones and

increase of available mineral nutrients in soil. They are also helpful to build up other beneficial microorganisms and in turn improve soil health. The biofertilizers also control soil or root borne diseases, parasitic nematodes and maintain soil structure. An application of these microorganisms to seed, soil or composting areas with the objective of increasing the numbers of such microorganisms and accelerating certain microbial processes to augment the extent of the availability of nutrients in a form which can be easily assimilated by plants (Sharma and Chaubey 2015). Looking into the economical as well as environmental importance of Kadam and to produce quality planting materials in nursery, the present trial was carried out to study the effect of biofertilizers on seedling growth, biomass and vigour in kadam, *A. cadamba* (Roxb.) Miq. in early stage.

MATERIAL AND METHODS

The present investigation was conducted at Net House, College of Forestry, Navsari Agricultural University (NAU), Navsari, (Gujarat) from December 2019 to August 2020. For the experiment, orange coloured ripened fruits of Kadam were collected from healthy, well developed trees distributed in the campus of NAU, Navsari. The collected fruits were allowed to rot for three to four days and pulp was then washed off by hand in a small bucket or pot of water, after tiny seeds settled at the bottom were taken out and dried well (Vijayaraghavan 2014). These seeds were sown in germination bed containing soil and sand for raising seedlings. Germinated seedlings growth was very slow for which they kept in the same condition for two months and then transplanted into the 10" × 8" size perforated black polythene bags containing growing media of soil, sand and FYM (2:1:1) having a seedling height of about 5-6 cm. After one month of establishment, different biofertilizers such as T₁: Control (No biofertilizer), T₂: Azotobacter, T₃: Azospirillum, T₄: Acetobacter, T₅: PSB (Phosphate Solubilising Bacteria), T₆: Pseudomonas and T₇: VAM (Vesicular Arbuscular Mycorrhiza) at the rate of 10 ml per plant were directly applied to soil in the polythene bag. Various growth parameters such as shoot length, collar diameter, number of leaves per plant, total leaf area per plant, root length, total fresh and dry weight of plant were recorded at 150 DAT (Days After imposing Treatment). Further, seedling vigour indices like root: shoot ratio, sturdiness quotient (SQ) and Seedling Quality Index (SQI) were calculated. The data obtained from the experiment were processed and fed to the data sheet in MS Excel and subjected to statistical analysis using DOS based software developed by Department of Agricultural Statistics, NAU, Navsari in experimental design CRD (Completely Randomized Design). The ANOVA was constructed for

further inference. The appropriate standard error of mean [SEm (±)] was calculated in each case and critical difference (CD) at 5 per cent level of probability was worked out to compare the treatment means, where the treatment effects were significant (Panse and Sukatme 1985).

RESULTS AND DISCUSSION

The findings of present study showed that effect of biofertilizers such as VAM and Azospirillum on growth and development of Kadam seedlings was found considerably maximum as compared to other treatments with respect to shoot length, collar diameter, number of leaves plant⁻¹, total leaf area plant⁻¹, root length, fresh and dry biomass (Table 1). Among different biofertilizers, Azospirillum @ 10 ml plant⁻¹ (T₃) showed maximum shoot length (80.52 cm), number of leaves per plant (13.33), total leaf area per plant (1770.29 cm²) in *A. cadamba* seedlings and found at par with VAM @ 10 ml plant⁻¹ (T₇) at 150 DAT. However, application of VAM @ 10 ml/plant (T₇) exhibited the maximum collar diameter (9.62 mm) followed by Azospirillum (8.90 mm) @ 10 ml plant⁻¹ (T₃) at 150 DAT. Further, maximum root length (33.04 cm) was recorded in VAM @ 10 ml plant⁻¹ (T₇) and found at par with Azospirillum (32.20 cm) @ 10 ml plant⁻¹ (T₃). Similarly, VAM @ 10 ml plant⁻¹ (T₇) exhibited significantly maximum fresh and dry weight of plant (96.58 g) and (19.67 g) followed by Azospirillum @ 10 ml plant⁻¹ (T₃) (89.18 g) and (18.25 g) respectively in *A. cadamba* at 150 DAT.

The increase in shoot length and collar diameter can be credited to VAM and Azospirillum application in the media as it helps in increasing nutrient and water uptake capacity which are necessary for the better growth and also helps in maintenance of good physical and chemical properties of the media (Chiranjeevi et al 2018). The growth of seedlings in early stages in nursery normally depends upon the growing media: its type, nature along with the types of inoculants or biofertilizers applied. The growth of seedlings in early stages also depends upon the tree species type and growth characteristics. Normally, certain biofertilizers enhance the growth of seedlings due to their symbiotic and positive interaction with the seedlings (Duponnoisa et al 2005, Wu et al 2010). The rhizosphere is a dynamic soil environment formed by living plant roots and their associated microorganisms and fauna. Among different microbes, plant growth promoting microbes like AM fungi, Azospirillum, Azotobacter, phosphobacteria in rhizosphere are able to exert a beneficial effect upon plant growth. These microbes have multiple functions like nitrogen fixation, phosphorus solubilization and mobilization (Sen and Paul 1957) and stimulating root development by producing metabolites like IAA and other growth hormones (Lynch 1990). Normally, the

effect of plant growth promoting microbes on the growth performance varied with the treatments and the host plant species (Shreedher and Mohan 2016). In the present study, all the growth parameters were found maximum in VAM and Azospirillum treatments. It may be due to the stimulatory effect exerted by these biofertilizers. AM have great importance as it efficiently enhances the nutrient uptake in infertile soils, water uptake and drought resistance in plants as well as improves the disease resistance (Nowak 2004). Similarly, Azospirillum has been attributed to several mechanisms including secretion of phytohormones (auxins and gibberellins), biological nitrogen fixation and enhancement of mineral uptake of plants (Mrkovack and Milic 2001).

Present findings of growth parameters of seedlings in Kadam inoculated with VAM and Azospirillum are in accordance with the reports of Budi and Christiana (2012), Shreedher and Mohan (2016). Budi and Christiana (2012) recorded maximum plant height, collar diameter, shoot and root dry weight when treated with Arbuscular Mycorrhizal Fungi (AMF) in *A. cadamba* seedlings whereas Shreedher and Mohan (2016) found in seedlings of *Neolamarckia cadamba* treated with VAM and Azospirillum in combination exhibited maximum shoot length, collar diameter, leaf area ratio. Similarly, inoculation of VAM and Azospirillum enhanced the seedling growth in other tree species. As reported by Vairamani and Rajendran (2021) in seedlings of *Casuarina junghuhniana* inoculated with the combined application of *Azospirillum* + *Paenibacillus polymyxa* + AM fungus produced the maximum growth and biomass; Mohan and Rajendran (2014) in *Feronia elephantum* treated with combined inoculation of *Azospirillum* + AM fungi + *Pseudomonas* showed shoot length, root length, collar diameter and biomass increased above 77.47 per cent than control; Parveen and Kumar (2020) used *Azospirillum*

brasilense + *Bacillus polyxyrna* + VAM in production of quality seedlings of *Acacia nilotica*. Further, Mohan and Rajendran (2017) observed increased shoot length, root length, collar diameter and biomass in combined inoculation of *Azospirillum* + AM fungi + *Pseudomonas* in *Aegle marmelos* whereas in *Casuarina equisetifolia* after six months of inoculation of biofertilizers, significantly maximum root length, shoot length, collar diameter, root weight and shoot weight were recorded in combination of *Frankia* + *Azospirillum* + *Phosphobacterium* (Saravanan et al 2012). Moreover, AM treated seedlings increased in plant biomass and height to the extent of 34 and 24 per cent respectively in *Dalbergia sissoo*, 126 and 50 per cent in *Acacia auriculiformis*, 48 and 24 per cent in *D. latifolia* and 100 and 112 per cent in *A. nilotica* (Uniyal and Thapar 1995). Similarly, Chiranjeevi et al (2018) also found the maximum seedling height (24.13 cm), root length (16.33 cm), seedling girth (0.63 cm), number of leaves per plant (18.86), fresh weight (26.9 g) and dry weight (3.07 g) in aonla when treated with VAM as an inoculant.

The present result of higher biomass production with application of VAM and the same findings was supported by Shreedhar and Mohan (2016) in Kadam where seedlings inoculated with AM fungi and PGPR exhibited higher biomass production. VAM normally enhances the surface area of roots which helps in absorption of available nutrient and water to larger extent as compared to other biofertilizer. So it may ultimately enhance the growth and biomass production of seedlings (Saini 2019). Furthermore, the increase in seedling biomass production may be strongly correlated with improved accumulation of N due to *Azospirillum* and *Azotobacter* and P due to AM fungi and PSB inoculation (Ratha Krishnan et al. 2004). Moreover, Kandasamy et al (1987) recorded, in *Ailanthus excelsa* and two other species inoculation with AM fungus, increased the

Table 1. Influence of biofertilizers on the growth parameters of *A. cadamba* seedlings at 150 DAT

Treatments	Shoot length (cm)	Collar diameter (mm)	Number of leaves/plant	Total leaf area /plant (cm ²)	Root length (cm)	Fresh weight /plant (g)	Dry weight /plant (g)
Control	65.40	7.47	12.17	1372.25	26.47	72.75	13.99
Azotobacter	68.22	8.43	11.42	1384.68	28.02	77.96	16.99
Azospirillum	80.52	8.90	13.33	1770.29	32.02	89.18	18.25
Acetobacter	74.73	8.45	12.25	1502.76	29.00	81.58	17.89
PSB	56.12	6.86	12.08	1469.79	27.66	49.61	9.54
<i>Pseudomonas</i>	57.31	7.61	10.42	1326.90	26.42	48.60	8.42
VAM	76.50	9.62	13.25	1692.92	33.04	96.58	19.67
Mean	68.40	8.19	12.13	1502.80	28.95	73.75	14.96
SEm(±)	1.78	0.13	0.27	43.85	0.68	1.77	0.42
CD (0.05)	5.38	0.40	0.80	133.02	2.06	5.35	1.26

shoot and root dry weights of 62.08 per cent and 43.18 per cent, respectively. Further, Balasubramanian and Srinivasan (1995) found that seedlings inoculation with four AM fungi in *A. excelsa*, *Tectona grandis* and *Dalbergia sissoo* significantly increased the total biomass; Madan et al (1995) reported that seedlings inoculated with AM fungal showed better shoot and root dry weight in *A. excelsa*, *Pongamia glabra* and *Cassia siamea*; Rahangdale and Gupta (1998) also recorded that *Gmelina arborea* and other five tree species exhibited significant increase in root and shoot biomass when treated with AM fungi.

Results also revealed that seedlings treated with PSB @ 10 ml plant⁻¹ and Pseudomonas @ 10 ml plant⁻¹ recorded lower value than control in most of the growth parameters. This may be due to the nature and functions of both biofertilizers and their effect on the growth characters of treated seedlings. Further, both these biofertilizers avail the P to host seedlings in most of conditions but might compete with the host plants for N as food supplements in the present situation as a result of which growth parameters were inhibited and found lower. Present findings were supported by Saini (2019) in *Swietenia macrophylla* seedlings treated with PSB @ 10ml plant⁻¹ and Pseudomonas @ 10 ml plant⁻¹ resulted in lower value of growth parameters as compared to control at 90 DAT.

The vigour parameters such as root: shoot ratio, sturdiness quotient and seedling quality index showed significant variation among seven treatments in the seedlings of *A. cadamba* at 150 DAT (Table 2). Among various treatments, Pseudomonas @ 10 ml plant⁻¹ (T6) produce maximum root: shoot ratio (0.33) and found at par with T₇, T₂, T₄ and T₅ in the seedlings of *A. cadamba*. Further, the lowest SQ value was recorded in Pseudomonas @ 10 ml plant⁻¹ (7.53) which was statistically at par with VAM @ 10 ml plant⁻¹

(7.95). Whereas, VAM @ 10 ml plant⁻¹ (T₇) was recorded significantly maximum SQI (1.77) followed by T₂, T₄, T₃ at 150 DAT.

The root: shoot ratio is an important measure for seedling survival. It relates the water absorbing area of roots to the transpiring area of shoot. A good ratio indicates a healthy plant (Jaenicke 1999). It may be due the fact that nitrogen deficiency in soil media may result in an increased root: shoot ratio (Harris 1992). Similar finding was recorded in *Swietenia macrophylla* seedlings treated with Pseudomonas at 90 DAT (Saini 2019). Moreover, kadam seedlings recorded higher value of root: shoot ratio when treated with AMF and Azospirillum (Shreedher and Mohan 2016). The result was further supported by Firuzsalari et al (2012) where significant difference in root: shoot ratio was found maximum in treatment involving Nitragin (a local biofertilizer containing Azospirillum, Azotobacter and Pseudomonas as its content).

The lowest value of SQ signifies the sturdiness i.e. ability to withstand in stress condition of the seedlings in terms of field establishment. Present finding of minimum SQ value was in accordance with the result of *S. macrophylla* seedlings inoculated with Pseudomonas (Saini 2019). Further, Shreedhar and Mohan (2016) reported that the biofertilizer inoculated seedlings showed better sturdiness quotient value as compared to uninoculated seedlings. Moreover, Maharana et al (2018) reported the lowest SQ of 11.75 in treatment inoculated with Azospirillum + Novel in *Gmelina arborea* seedlings whereas Chandra and Ujjaini (2002) recorded lowest result in forest tree seedlings treated with AM fungi + organic manure.

Seedling Quality Index is considered a promising integrated measure of morphological traits and a good indicator of seedling quality as its computes robustness and biomass distribution of seedlings as compared to individual growth parameters like shoot length, collar diameter etc. (Binotto et al. 2010). In the trial, VAM @ 10 ml plant⁻¹ was recorded significantly maximum SQI (1.77) at 150 DAT. Higher the value of SQI, better the ability of the seedling to survive in the field. VAM inoculated seedlings exhibited better result in terms of SQI which may be due the availability of nutrients such as N and P increased with the application of it. However, the high percentage of root colonization in AM fungi treated seedlings is found to be directly correlated with an improved growth and physiology. Further, the presence of AM fungi significantly increases root surface area by production of extensive hyphae, increase transpiration, reduce leaf temperature and restrain the decomposition of chlorophyll and improves the seedling quality (Ajeesh et al 2015). Moreover, inoculation of VAM in seedlings of woody species at nursery stage improves the seedling growth and

Table 2. Influence of biofertilizers on vigour parameters of *A. cadamba* seedlings at 150 DAT

Treatments	Root:shoot ratio	Sturdiness quotient	Seedling quality index
Control	0.26	8.75	1.11
Azotobacter	0.31	8.09	1.50
Azospirillum	0.29	9.04	1.46
Acetobacter	0.30	8.84	1.47
PSB	0.30	8.17	0.82
Pseudomonas	0.33	7.53	0.80
VAM	0.31	7.95	1.77
Mean	0.30	8.34	1.28
SEm(±)	0.01	0.15	0.03
CD (0.05)	0.03	0.45	0.09

survivability (Rashmi and Bhavana 2015). According to Shreedhar and Mohan (2016), the higher SQI value was reported in *N. cadamba* seedlings when inoculated with AM fungi and PGPRs. The results for *A. cadamba* seedlings inoculated with VAM produced maximum SQI and this finding was further supported by Sumana and Bagyaraj (2002) in neem treated with VAM (maximum SQI of 0.85), Raj et al. (2010) in teak seedlings inoculated with VAM (maximum SQI of 0.44) and Maharana et al. (2018) in seedlings of *Gmelina arborea* treated with AM + PSB + Novel (maximum SQI of 1.82). Moreover, current findings are in line with the findings of earlier researchers on native tree species like, *Tectona grandis* (Ayswarya 2008, Rajeshkumar et al 2009), *Melia azadirach* (Rajeshkumar et al 2009) as well as exotic like *Eucalyptus* hybrid (Sastry et al 2000), *Simarouba glauca* (Ratha Krishnan et al 2004) and *Acacia auriculiformis* and *A. mangium* (Tamilselvi 2005).

CONCLUSION

VAM inoculated seedlings of *Anthocephalus cadamba* resulted in maximum SQI value, collar diameter, fresh as well as dry biomass with higher shoot length, number of leaves and total leaf area with improved SQ value than other biofertilizer treatments and control. Thus, VAM inoculation is found best for the growth and vigour of seedlings of *A. cadamba* in early stage and can be used for production of quality seedlings of *Anthocephalus cadamba* in forest nursery.

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