KAVAKA 43: 41-45 (2014)

Effect of bio-inoculants on seed germination and disease control of commercially important fast growing native tree species in nursery

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(Submitted in September, 2014; Accepted on November 25, 2014)

ABSTRACT

Different bio-inoculants (Arbuscular Mycorrhizal (AM) fungi and Plant Growth Promoting Rhizobacteria (PGPR)) were tested individually and in combinations on seed germination and survival of seedlings of four fast growing native tree species *Ailanthus excelsa, Gmelina arborea*, *Melia dubia* and *Neolamarckia cadamba* against the plant pathogenic fungus, *Fusarium oxysporum* causing root rot in nurseries. It was observed that application of bio-inoculants individually and in different combinations showed increased seed germination, better survival and growth of seedlings of all the four tree species even in the presence of the pathogen, *F. oxysporum*. Maximum percentage of seed germination was recorded in combined application of all four bio-inoculants along with *Fusarium* in all the four tree species. This was followed by AMF + Azo + *Fusarium* in *Ailanthus excelsa, Gmelina arborea* and *Neolamarckia cadamba* and AMF + Azoto + *Fusarium* in *Melia dubia*.

Keywords: Bio-inoculents, potting medium, AM fungi, disease control

INTRODUCTION

Forest is the second largest land use in India next to agriculture. The forest and tree cover of India is assessed at 78.29 million hectares which constitute 23.81% of the country's geographical area, ranging from the Himalayan Temperate to the Dry Deciduous forests (FSI, 2011). The country's per capita availability of forest area is 0.065 hectare and the productivity is 1.37 m^3 per hectare as against the world average of 0.64 hectare and 2.1 m^3, respectively (Joshi and Singh, 2003). While the demand for forest products and services in India is increasing, the area and quality of forests is declining due to encroachments, excessive collection of timber, fuel wood and other non wood forest produces, forest fires, over grazing, pests and diseases etc. The forestry sector is facing increasingly difficult challenges.

Among many native tree species, *Ailanthus excelsa* Roxb., *Gmelina arborea* Roxb., *Melia dubia* Cav. and *Neolamarckia cadamba* (Roxb.) Bosser are some of the economically important and fast growing ones planted by the Indian farmers and state forest departments in different parts of the country. These tree species are industrially important and useful to mankind in many ways. The wood of *Ailanthus excelsa* is extensively used in making matchwood boxes and match splints. The timber of *Gmelina arborea* is mainly used in construction and furniture industry. The wood of *Mebia dubia* is primarily used in plywood and packaging industry. The wood of *Neolamarckia cadamba* is mainly used in pencil making and pulp wood industry.

The effects and potentialities of various beneficial micro-organisms as bio-inoculants in agriculture have been well documented (Subba Rao, 1993; 1995; Dash and Gupta, 2011; Brahmaprakash and Sahu, 2012). They are eco-friendly, renewable, cost effective and pollution free. Therefore, the use of these beneficial microorganisms as bio-inoculants provides an effective alternative to the chemical fertilizers. They can also serve as important tools in forestry programmes including reclamation of degraded lands, mined overburdens and other problematic areas so as to make these programmes more effective (Grove and Le Tacon, 1993).

Raising of high quality elite seedlings is necessary to establish a good plantation (Durvey and Landis, 1984). The apparent result of the beneficial microorganisms may not be evident under all natural conditions because of insufficient population naturally occurring in the soil (Powell and Daniel, 1978). Therefore, application of most suitable beneficial microorganisms becomes imminent in nursery condition. Since very limited reports are available on use of AM fungi, *Azospirillum, Azotobacter* and Phosphobacteria on forest tree species, especially the economically important fast growing native tree species, the study with an aim of elucidating the information on the efficacy of these bio-inoculants on seed germination and disease control of selected commercially important four different tree species *viz*., *Ailanthus excelsa, Gmelina arborea, Melia dubia* and *Neolamarckia cadamba* in nursery.

MATERIALS AND METHODS

Seed source and potting medium

Healthy seeds of *Ailanthus excelsa* were obtained from Seed Technology Division, Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore; while seeds of *Gmelina arborea* were obtained from Genetics Division, Tamil Nadu Forest Department (TNFD), Coimbatore, Tamil Nadu and seeds of *Melia dubia* and *Neolamarckia cadamba* were obtained from Seed Division, Kerala Forest Research Institute (KFRI), Peechi, Thrissur, Kerala.. The seeds of *Gmelina arborea* and *Melia dubia* were pre-treated by soaking in cool water overnight. The seeds of *Ailanthus excelsa* and *Neolamarckia cadamba* were not given any pretreatment. Potting medium used in the present study was a mixture of solar sterilized sand: soil: farmyard manure in the ratio 1:2:1. It was analyzed for its physico-chemical characteristics such as pH, Electrical Conductivity (EC), macro (Nitrogen, Phosphorus and Potassium) and micro nutrients (copper,

zinc, iron and manganese) by following standard procedures. It was found that the pH was 7.3 and Electrical Conductivity 0.14. The status of macro nutrients were found low and it was recorded that the available nitrogen was 180 kg/ha; phosphorus was 19 kg/ha and potassium was 225 kg/ha. The micro nutrients were found low to moderate and it was found that copper was 1.8 ppm; zinc was 1.54 ppm; iron was 10.8 ppm and manganese was 11.08 ppm.

Bio-inoculants

The bio-inoculants were obtained from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. The soil base inoculum of Arbuscular Mycorrhizal (AM) fungi consortium consisted of *Glomus mosseae* and *G. fasciculatum* at the rate of 4 spores per gram of soil. Peat soil based inocula of *Azospirillum, Azotobacter* and phosphobacterium consisted of *Azospirillum brasilense, Azotobacter chroococcum* and *Bacillus megaterium,* respectively, each with a population count of 10^8 bacterial cells per gram of peat soil. The commercial chemical fertilizer, Diammonium Phosphate (DAP) was obtained from the market in Coimbatore.

Nursery experiment

The experiment was conducted in Experimental Nursery of Institute of Forest Genetics and Tree Breeding, Coimbatore. Healthy seeds of *Ailanthus excelsa, Gmelina arborea* and *Melia dubia* were selected for uniformity. The seeds of *Neolamarckia cadamba* were separately weighed to get 0.01g units. About 300 seeds were obtained in each 0.01g unit. The seeds of all these species were surface sterilized in mercuric chloride (0.1%) for one minute and washed four times with sterilized distilled water. The seeds were then sown in trays (30 x 20 x 5cm) containing sterilized potting medium layered with bio-inoculants/DAP at 0.75 cm below the top surface. Total of 14 treatments involving the fungal pathogen (*Fusarium oxysporum* alone), dual inoculation of the fungal pathogen and bio-inoculants such as AM fungal consortia (*Glomus mosseae* + *G. fasciculatum*), *Azospirillum brasilense, Azotobacter chroococcum* and phosphobacteriium (*Bacillus megaterium*); triple and combined inoculation of all the bio-inoculants and the fungal pathogen was made. Another treatment involving chemical fertilizer viz., Diammonium phosphate (DAP) alone and the fungal pathogen was made. The seedlings grown in poly bags without application of the fungal pathogen and the bio-inoculants were kept as control.

Based on the size of the seeds, twenty five seeds each of *Ailanthus excelsa* and *Gmelina arborea*, thirty seeds of *Melia dubia* and three hundred seeds of *Neolamarckia cadamba* were sown in each tray above the layer of bio-inoculants (AM fungi 10gm per tray and PGPRs 5gm per tray) about 0.5 cm below from the surface. The mycelial and spore suspension of the pathogen, *Fusarium oxysporum* (10^7CFU/ml) @ 10 ml per tray was applied. The trays were watered twice daily and maintained. Observations on seedling germination were recorded from 2 weeks after sowing and post emergence mortality periodically up to the termination of the experiment (4 months for *Melia dubia* and 2 months for the other three species).

All data were subjected to analysis of variance and the significant difference among the means were compared by Duncan's Multiple Range Test (DMRT) at P=0.05 level using SPSS (Version 10.0, SPSS Inc.) statistical software to determine the effects due to treatments. The germination percentage of seeds and survival percentage of seedlings in the experiment were arcsine transformed before subjecting to statistical analysis.

RESULTS

Data on seed germination and survival of all the selected four different fast growing native tree species *viz*., *Ailanthus excelsa, Gmelina arborea, Melia dubia,* and *Neolamarckia cadamba* seedlings inoculated with disease causing pathogen, *Fusarium oxysporum*, different bio-inoculants (individually and in combinations) and DAP (alone) is presented in **Table 1**. Maximum and highly significant seed germination per cent was observed in the treatment involving all the four bio-inoculants + *Fusarium oxysporum* as compared to uninoculated control, *Fusarium oxysporum* (alone) and DAP (alone) treatments in all the tree species. Among the other treatments, in general, no significant difference was observed in germination percentage of the seeds. The highest percentage survival of seedlings was noticed in uninoculated control treatment, followed by the treatment involving all the four bio-inoculants + *Fusarium oxysporum* and then by AMF + *Azospirillum brasilense* + *Fusarium oxysporum*, but between them there was no significant difference. These were followed by the treatment involving *Azospirillum brazilense* + Phosphobacterium + *Fusarium oxysporum* and Phosphobacterium + *Fusarium oxysporum*. The lowest percentage survival of seedlings was noticed in the treatment with *F. oxysporum* alone. In general, it was observed that the survival percentage of seedlings with dual inoculations of bio-inoculants was higher than those with single inoculation.

DISCUSSION

Among the nursery diseases, root rot is one of the most prevalent and highly destructive diseases causing heavy loss of tree seedlings. It is caused by different soil fungi such as *Fusarium* and *Rhizoctonia* which are quite prevalent in forest nurseries in India (Shivanna, 2003; Mohan *et al*., 2013). These feeder root pathogens infect immature and meristematic cortical tissues of roots and cause necrosis. Chemical control measures are generally used to manage these fungal diseases. However, ecological damage and pathogen resistance resulting from the use of chemical compounds are putting pressure on governments/growers to find environmentally safe alternative disease management strategies. There is therefore, increasing interest in biological control methods to suppress the growth of plant pathogens and to stimulate natural plant disease resistance.

Valuable data generated on induced suppression of soil-borne pathogens by AM fungi and PGPR have no doubt proved their potentiality in controlling plant pathogens (Dehne, 1982; Chakravarty and Mishra, 1986; Sanjay

Table 1. Effect of bio-inoculants on seed germination and disease control of seedlings of *Ailanthus excelsa, Gmelina arborea, Melia dubia* and *Neolamarckia cadamba* against the plant pathogen, *Fusarium oxysporum*

Treatment	*Ailanthus excelsa*		*Gmelina arborea*		*Melia dubia*		*Neolamarckia cadamba*	
	Germination %	Survival %	Germination %	Survival %	Germination %	Survival %	Germination %	Survival %
T1	51.57 abc	65.85 h	29.28 ab	90.00 f	19.43 a	80.00 d	43.47 b	83.37 f
T2	50.02 ab	5.37 a	28.41 a	8.03 a	19.43 a	10.00 a	42.51 b	15.93 b
T3	52.38 abcd	43.07 ef	39.98 ef	52.99 bcd	24.92 bcde	33.69 c	52.48 cde	24.18 cd
T4	54.79 abcd	35.83 cde	34.42 cd	50.31 bc	22.31 ab	28.86 bc	49.55 c	19.66 bc
T5	56.58 abcd	30.03 c	34.42 cd	59.85 cde	23.20 bc	31.93 bc	51.95 cde	20.65 c
T6	58.16 bcd	36.68 cde	33.62 bcd	75.70 ef	23.20 bc	27.71 bc	50.77 cd	22.23 c
T7	60.72 cd	55.94 gh	39.99 ef	84.15 f	24.92 bcde	37.91 c	57.46 f	27.02 de
T8	54.19 abcd	41.93 def	37.66 def	71.77 def	26.51 de	38.46 c	56.41 f	29.18 e
T9	51.59 abc	48.08 fg	35.26 de	71.35 def	25.69 cde	28.48 bc	55.65 ef	30.70 e
T10	53.21 abcd	31.37 cd	34.45 cd	76.20 ef	22.31 ab	28.86 bc	50.19 c	22.67 cd
T11	53.25 abcd	44.11 ef	37.58 def	81.59 f	23.20 bc	36.15 c	50.84 cd	21.51 c
T12	53.25 abcd	50.99 fg	36.85 def	76.96 ef	24.09 bcd	30.79 bc	54.28 def	20.59 c
T13	61.59 d	57.41 gh	40.76 f	84.15 f	27.34 e	40.38 c	57.85 f	30.56 e
T14	47.71 a	18.08 b	30.20 abc	37.52 b	25.74 cde	16.06 ab	23.63 a	6.43 a

Means within a column followed by the same letter(s) are not significantly different according to DMRT (P=0.05).
DAI - Days after inoculation.

T1 – Control
T2 – *Fusarium oxysporum*
T3 – AM fungi+*Fusarium*
T4 – *Azospirillum*+*Fusarium*
T5 – *Azotobacter*+*Fusarium*
T6 – Phosphobacterium+*Fusarium*
T7 – AMF+*Azospirillum* +*Fusarium*
T8 – AMF+*Azotobacter*+*Fusarium*
T9 – AMF+Phosphobacterium+*Fusarium*
T10–*Azospirillum*+*Azotobacter*+*Fusarium*
T11–*Azospirillum*+Phosphobacterium+ *Fusarium*
T12–*Azotobacter*+Phosphobacterium+ *Fusarium*
T13–AMF+*Azospirillum*+*Azotobacter*+ Phosphobacterium+*Fusarium*
T14 –Diammonium phosphate (DAP) + *Fusarium*

Arya and Kaushik, 2003). But, experiments have only usually tested single AM fungus. Also, there are certain pathogenic antagonists, *Pseudomonas, Bacillus* and other PGPR which cooperate with mycorrhizal fungi for biocontrol. The phytosanitary role of mycorrhizal fungi can be made more effective when integrated with other PGPR. Thus, for exploiting the prophylactic activity of bio-inoculants in a best way, the right combinations of factors should be found out, the most important being the selection of appropriate/efficient combination of bio-inoculants.

Maximum percentage of seed germination was recorded in combined application of all four bio-inoculants along with *Fusarium* in all the four species. This was followed by AMF + Azo + *Fusarium* in *Ailanthus excelsa, Gmelina arborea* and *Neolamarckia cadamba* and AMF + Azoto + *Fusarium* in *Melia dubia*. These findings are in accordance with those of Vanagamudi *et al*. (1993) who reported that seed inoculation of *Azadirachta indica* with AM fungi or *Azospirillum* or PSB significantly enhanced the seed germination of *Azadirachta indica*; Bhadauria *et al*. (2000) who reported that application of *Azospirillum* had positive effect on the seed germination of *Emblica officinalis*. Vijayakumari and Janardhanan (2003) have also found that inoculation with *Azospirillum*, PSB and AM fungi on *Bombax ceiba* improved seed germination. Singh *et al*. (2003) reported that germination was found to be improved with seed inoculation of *Trichoderma harzianum* + AM fungi and organic matter + *Azospirillum* combinations in *Gmelina arborea*.

Verma *et al.* (2008) have also observed that teak seed germination was maximum with application of *Azospirillum* and combination of AM fungi + PSB. Mafia *et al*. (2009) reported that PGPR promoted seed germination in *Eucalyptus* species and very recently Singh *et al*. (2011) reported that inoculation with *Bacillus licheniformis* and *Sinorhizobium saheli* had positive and synergistic effect on seed germination of *Acacia senegal*. The improved germination of seed with application of bio-inoculants may be due to modification of the soil environment surrounding the seed by AM fungi and PGPR. Similar finding was made by Zambrano and Diaz, (2008) who reported that *Glomus* sp. and *Azospirillum brasilense* had significant effect on the germination of *Gmelina arborea* seeds. The germination percentage of seed in DAP + *Fusarium* treatment of *Ailanthus excelsa* (47.71%) and *Neolamarckia cadamba* (23.63%) was found to be less than that of uninoculated controls (51.57% and 41.55%, respectively). This may be due to the chemical nature of the fertilizer affecting the small sized and soft coated seed of these tree species.

Many earlier studies indicated that bio-inoculants such as AM fungi and Plant Growth Promoting Rhizobacteria (*Azospirillum, Azotobacter* and Phosphobacterium) have significant effect on seed germination of many plant species (Singh *et al*.,2003; Verma *et al.,* 2008; Zambrano and Diaz, 2008; Singh *et al*., 2011). The better seed germination of all the selected tree species in the present study with application of bio-inoculants may be due to modification of the soil environment surrounding the seed by inoculation with AM fungi and PGPRs.

The survival percentage of seedlings was minimum in *Fusarium* alone treatment whereas it increased due to bio-inoculations significantly. The present findings are in accordance with those of Singh *et al*. (2003) who reported that maximum mortality was observed in *F. oxysporum* treatment and many treatment combinations involving AM fungi, *Azospirillum*, *Trichoderma harzianum* and

organic matter could effectively control the *Fusarium* wilt disease in *Gmelina arborea*. The findings of the study are also in agreement with the observations made by earlier researchers using AM fungi and other bio-inoculants against plant pathogenic fungi especially *Fusarium* and *Rhizoctonia* on different host plants like *Acacia nilotica* (Mohan and Verma, 1996; Kaushik *et al*. 2000), *Dalbergia sissoo* (Shukla *et al.,* 2007; Shukla and Gupta, 2009), *Pinus taeda* (Enebek *et al*., 1998), *P. roxburghii* (Singh *et al.,* 2008) and *Aquilaria agallocha* (Tabin *et al*., 2009). The reduction/suppression of disease incidence and improved survival of seedlings of all bio-inoculants applied tree species may be due to the inhibition of pathogen's spore germination by inoculated AM fungi and PGPR and improved nutrient uptake leading to increased host vigour (Kaushik and Mandal, 1991). Bio-inoculants (AM fungi, *Azospirillum, Azotobacter* and Phosphobacterium) alone have improved seed germination in all the four commercially important tree species and survival against plant pathogenic fungus, *Fusarium oxysporum* in nursery condition.

CONCLUSION

Application of bio-inoculants will not only give desired benefits in terms of better seed germination, high quality seedlings and out planting performance in the field, but also ensure the suppression of soil borne plant pathogens and maintenance of soil health which is essential for sustainable and eco-friendly forestry. Use of superior AM fungal and PGPR bio-inoculants in seedling and transplanting stage may be an excellent solution to strengthen seedlings to withstand dry land conditions and support the Trees Outside Forest (TOF) efforts, thereby promoting farmers, tree growers and wood based entrepreneurs of the country.

ACKNOWLEDGEMENTS

The authors are highly grateful to the Director, IFGTB, Coimbatore, for providing all the facilities and encouragement.

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