

Effect of Different Levels of Artificial Mycorrhizal Inoculation on Growth of Pine Tree Seedlings

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Abstract

Mycorrhizal inoculation of pine seedlings for efficient water and nutrient uptake and consequent production of healthy, vigorous seedlings with high survival rate has been achieved in East Africa for over four decades by use of soil from mature pine stands. However, due to high soil transportation costs, many growers use locally collected soil leading to production of seedlings of ill-health. This study was done to determine the effect of different levels of artificial mycorrhiza on growth of *Pinus patula* Schldl and Cham. seedlings. Different levels of RHIZATECH[®]TM inoculants (0g, 16.5g, 33.0g, and 49.5g) were added to soil in 120 potting tubes at 2 cm depth and arranged in a completely randomized design (CRD) having four treatments replicated thrice. Seedling height (Ht), root collar diameter (RCD) and Dry weight (DW) were measured as response variables, three weeks after inoculation. The data were subjected to one-way ANOVA to test the effect of different levels of the mycorrhizal inoculation on the growth parameters at 5% probability levels using SAS software and means separated by Tukey's (HSD) test. There was significant increase in height growth (24.34%; $p=0.0045$), RCD (32.9%; $p < 0.05$) and DW (62.72%; $p < 0.05$) of the seedlings three weeks after pricking out due to the addition of mycorrhizal inoculant. This study recommends the application of 49.5 g of RHIZATECH[®]TM inoculum to potting soil in standard *Pinus patula* nursery tubes at a depth of 2 cm for increased height growth (24.34%), root collar diameter growth (32.9%), biomass (62.7%) and the production of *P. patula* seedlings of high vigour.

Key words: Mycorrhizae, Forest soil, Pine seedlings

Résumé

L'inoculation mycorhizienne des semis de pin pour une absorption efficace de l'eau et des nutriments et la production conséquente de semis sains et vigoureux avec un taux de survie élevé a été réalisée en Afrique de l'Est depuis plus de quatre décennies en utilisant le sol de peuplements matures de pin. Cependant, en raison des coûts élevés de transport du sol, de nombreux producteurs utilisent du sol collecté localement, ce qui conduit à la production de semis en mauvaise santé. Cette étude a été menée pour déterminer l'effet de différents niveaux de mycorhizes artificielles sur la croissance des semis de *Pinus patula* Schldl et Cham. Différents niveaux d'inoculants RHIZATECH[®]TM (0g, 16,5g, 33,0g et 49,5g) ont été ajoutés au sol dans 120 tubes de culture à une profondeur de 2 cm et arrangés dans un dispositif entièrement randomisé (CRD) avec quatre traitements répétés trois fois. La hauteur des semis (Ht), le diamètre au collet (RCD) et le poids sec (DW) ont été mesurés comme variables de réponse, trois semaines après l'inoculation. Les données ont été soumises à une ANOVA à un facteur pour tester l'effet des différents niveaux de l'inoculation mycorhizienne sur les paramètres de croissance à un

niveau de probabilité de 5% en utilisant le logiciel SAS et les moyennes séparées par le test de Tukey (HSD). Il y a eu une augmentation significative de la croissance en hauteur (24,34%; $p=0,0045$), du diamètre au collet (32,9%; $p < 0,05$) et du poids sec (62,72%; $p < 0,05$) des semis trois semaines après la transplantation en raison de l'ajout de l'inoculant mycorhizien. Cette étude recommande l'application de 49,5 g d'inoculum RHIZATECH[®] TM au sol de culture dans des tubes de pépinière standard de *Pinus patula* à une profondeur de 2 cm pour augmenter la croissance en hauteur (24,34%), la croissance du diamètre au collet (32,9%), la biomasse (62,7%) et la production de semis de *P. patula* de haute vigueur.

Mots-clés : Mycorhizes, Sol forestier, Semis de pin

Introduction

The use of mycorrhizal inoculation of nursery soil has been reckoned worldwide as a necessity for enhanced pine seedling growth in tree nurseries for nearly eight decades (McComb, 1943; Lamb and Richards, 1974, Aučina *et al.*, 2007; Cram and Dumroese, 2012) and is becoming an increasingly common practice in the nursery industry. Whether nurseries are producing native, ornamental or agricultural plants, adding mycorrhizae can lower mortality rates and reduce water and fertilizer consumption. In East Africa, Kenya included, the production of healthy, vigorous pine seedlings with high survival rate has been achieved for over four decades by use of soil collected from mature pine stands (i.e. “pines soil” or “forest soil”).

Mycorrhizae, first identified in 1840, are fungi that form symbiotic relationship with roots of a wide variety of terrestrial plants in which an external mycelium of a fungus supplies soil derived nutrients to a plant root (Smith and Read, 1997a). Their role in the symbiotic partnership is providing a fine hyphal network capable of extending the range of root hairs (Allen, 1991). They are highly evolved non-pathogenic symbiotic associations between roots of most vascular plants and certain specialized soil fungi (Basidiomycetes, Ascomycetes and Zygomycetes) that colonize the cortical tissues of roots during periods of active plant growth both in natural environment and in cultivation (Miller and Jastrow, 1994; Smith and Read, 1997b; Read, 1999). Whereas most conifers form ectomycorrhizal (ECM) fungi, studies by Berch *et al.* (1993) indicate that some conifers like western Redcedar (*Thuja plicata* Donn ex. D. Don) can form vesicular arbuscular mycorrhiza (VAM), and thus the possibility that other conifers like pines could be capable of forming such mycorrhizae.

Mycorrhizal fungus provides the host plant with nutrients, such as phosphate and nitrogen, and increases the abiotic (drought, salinity, heavy metals) and biotic (root pathogens) stress resistance of the host. In return for their beneficial effect on nutrient uptake, the host plant transfers between 4 and 20% of its photosynthetically fixed carbon to the mycorrhizal fungus (Wright *et al.*, 1998). In some cases, mycorrhizae increase a plant's access to nutrients such as phosphorous and zinc, which are important for the growth of plants. The availability of these nutrients is very low in tropical soils. In addition, mycorrhizae have been found to increase nitrogen-fixation for nodule-forming plants. Forest reforestation programs with pine or other mycorrhizal tree species are successful because the seedlings used as planting stock usually have mycorrhizae from the nursery which ensure good seedling survival and growth. These symbiotic associations form naturally, and contribute significantly to growth and development of trees.

However, recent research results show that we can improve upon nature by manipulating the fungal symbionts towards more economically beneficial mycorrhizal associations of trees (Sieverding, 1991).

Traditionally, soils from established pine plantations have been used as sources of mycorrhizal inoculum in nurseries. The soils have normally been added as part of the nursery pot mix. However, with the proliferation of tree nurseries occasioned by increasing demand for wood products, obtaining the “pine soil” has become expensive due to transportation costs thus leading to growers resorting to use of any soil type for potting and this has led to production of pine seedlings of ill-health. Buying commercially produced mycorrhizal inoculants may be initially expensive but cost-effective in the long run because it is much labour-intensive than collecting forest soil, and the inoculants may be used to infect fresh soil for further mycorrhizal pine root inoculation. In addition, mycorrhizal inoculation also has advantages over forest mycorrhizae because it is generally free from contaminants and appears to be more virulent. This study was done to determine the effect of different levels of artificial mycorrhizal inoculation on growth of *Pinus patula* Schldl. and Cham. seedlings in tree nursery. The specific objectives of the study were to determine the effect of different levels of mycorrhizal inoculation on height growth, root collar diameter (RCD) growth, and dry-weight of *Pinus patula* seedlings.

Materials and Methods

Study Area. The study was conducted in Uasin Gishu County (Kenya) at the University of Eldoret’s Tree Nursery, situated at a Latitude of 0°31’N and Longitude of 35°16’E., and lying at an altitude of 2073 metres above sea level with a mean annual rainfall of 960 mm, mean annual maximum temperature of 24 °C – 26 °C and mean annual minimum temperature of 6 °C – 10 °C (GOK, 1997).

Source of Mycorrhizal Inoculums and Experimental Design. The inoculum used in this study, RHI-ZATECH[®]TM, was obtained from Dudutech Company (Naivasha, Kenya). RHIZATECH[®]TM contains spores, colonized root fragments, and other propagules of Arbuscular Mycorrhizal Fungi (AMF) in a granular carrier at a concentration of 50 propagules per cm³ of product (Dudutech, 2015).

Potting tubes were quarterly filled with forest soil from a pine plantation, the mycorrhizae (in different amounts) was then added in the potting tubes; the mycorrhizae was 2cm from the top of the potting tubes. One hundred and twenty (120) potted *Pinus patula* Schldl. and Cham. seedlings, previously raised in pine forest soil for 60 days after pricking out, were transferred to potting tubes containing soil inoculated with different amounts of mycorrhizae per tube, and forming the following treatments replicated three times: Control (0.0 g), Treatment 1 (16.5 g), Treatment 2 (33.0 g), and Treatment 3 (49.5 g). There were ten (10) seedlings per treatment, and four treatments (including control) replicated three times, giving a total of 120 seedlings arranged in a completely randomized design (CRD) and watered twice a day (in the morning and in the evening). All the seedlings were placed at the same place where they got enough sunlight uniformly. The seedlings were placed in an east-west direction to ensure that they received enough sunlight for their growth. It is important to note that unlike other commercial sources of mycorrhizal inoculum that contain both bacteria and nutrients, the inoculum used in this study contained only bacteria. Thus there was no need to have a sterilized control.

Pine Seedling Height, Root-collar diameter and Dry-weight measurements. Measurements of root-collar

diameter (cm) and height (cm) of the seedlings were taken after every three (3) days using Vernier calipers and a 30 cm ruler respectively, three (3) weeks after mycorrhizal inoculation. A month after treatment with mycorrhizae the seedlings were uprooted for determination of dry weight (g). Potted seedlings were placed in a trough full of water after removing the tube and observed to ensure all the soil around the roots was completely loose. The soil was then carefully washed away from the roots. The seedlings were oven dried for 24 hours at a temperature of 45°C after which their weights were measured using an electronic weighing balance.

Data Analysis. Means of height, root-collar diameter and seedling dry-weight were determined and summarized according to treatments and presented as bar charts. The data were subjected to one-way ANOVA to test the effect of different levels of mycorrhizal inoculation on the height and root-collar diameter at 5% probability levels using SAS® software, and Tukey's Studentized Range (HSD) test was used to separate significant means.

Results

Effect of different amount of mycorrhizal inoculation on height growth of *Pinus patula* seedlings. Mycorrhizal inoculation of *Pinus patula* seedlings by 49.5g of the inoculum achieved the highest height growth of 6.8 ± 0.3 cm and this represented 24.34% height growth increase compared to 5.44 ± 0.2 cm, being the height of control seedlings (Table 1). The effect of different levels of mycorrhizal inoculation on height growth of the seedlings was significant ($p = 0.0045$), with treatment 3 (addition of 49.5g of RHIZATECH®™ inoculum) achieving the highest percent height increment (24.3%) in *P. patula* seedling growth (Tables 1 and 2).

Effect of different amount of mycorrhizal inoculation on root-collar diameter (RCD) growth of *Pinus patula* seedlings. Mycorrhizal inoculation of *Pinus patula* seedlings by 49.5g of the inoculum achieved the highest root-collar diameter (RCD) growth of 0.21 ± 0.01 cm and this represented 32.9% RCD growth increase compared to 0.0 ± 0.0 cm in control seedlings (Table 1). The effect of different levels of mycorrhizal inoculation on RCD of the seedlings was significant ($p < 0.0001$), with treatment 3 (addition of 49.5g of RHIZATECH®™ inoculum) achieving the highest percent RCD increment (32.9%) in *P. patula* seedling growth (Tables 1 and 2).

Table 1. Mean ($\pm$ SE) height growth, root-collar diameter, dry weight and corresponding percent growth of potted *P. patula* seedlings inoculated with different amounts of RHIZATECH®™ inoculum

Treatment	Ht $\pm$ SE	% Incr. Ht.	RCD $\pm$ SE	% Incr. RCD	DW $\pm$ SE	% Incr. DW
Control (0.0g Inoculum)	5.446 ± 0.24^a	0	0.16 ± 0.01^a	0	0.15 ± 0.01^a	0
Treatment 1 (16.5g Inoculum)	5.96 ± 0.27^{ab}	9.62	0.17 ± 0.01^b	8.39	0.15 ± 0.01^a	0
Treatment 2 (33.0g Inoculum)	5.93 ± 0.26^{ab}	9.05	0.17 ± 0.01^b	6.45	0.22 ± 0.01^b	41.2
Treatment 3 (49.5g Inoculum)	6.76 ± 0.26^c	24.34	0.21 ± 0.01^c	32.9	0.24 ± 0.01^c	62.7

Ht-Height; **Incr.** – Increase in (e.g. height); **RCD**-Root collar diameter; **DW** –Dry weight; **a,b,c**: significant differences are indicated by different superscript letters

Effect of different amount of mycorrhizal inoculation on dry weight of *Pinus patula* seedlings. Mycorrhizal inoculation of *Pinus patula* seedlings by 49.5g of the inoculum achieved the highest biomass (dry weight) of the seedlings (0.24 ± 0.01 g), representing 62.7% of dry weight increment (Table 1). The effect of different levels of mycorrhizal inoculation on dry weight of the seedlings was significant ($p < 0.0001$) (Table 2).

Table 2. ANOVA of the effect of different amounts of mycorrhizal inoculation on height growth (Ht), root-collar diameter

Variable	Source	Df	Sum of Squares	Mean Square	F	<i>p</i>
Ht (cm)	Treatment	3	20.796	6.932	4.66	0.0045
	Error	90	133.874	1.487		
RCD (cm)	Treatment	3	0.034	0.0115	8.68	<0.0001
	Error	90	0.119	0.0013		
DW (g)	Treatment	3	0.033	0.0116	9.67	<0.0001
	Error	90	0.116	0.0012		

Ht-Height; RCD-Root collar diameter; DW –Dry weight

Discussion

Whereas many mycorrhizal-tree interaction studies have either been directed at elucidating infectivity and inoculation rate of host plants or Effect of mycorrhizal inoculation on nutrients in the soil and vice versa (Turjaman *et al.*, 2006; Tarbell and Koske, 2007; White *et al.*, 2008), this study was directed at determining the effect mycorrhizal inoculation on height, RCD and dry weight of *P. patula* seedlings under tree nursery conditions. Mycorrhizal inoculation of *Pinus patula* seedlings by 49.5g of RHIZATECH[®]TM inoculums increased height, RCD and dry weight of the seedlings.

Although past studies have revealed that seedling inoculation have an overall greater effect on relative height growth rates, dry biomass, and stem volumes seedlings and mature trees, and that the application of mycorrhizal fungi could increase plant growth, and shoot nutrient content in some tropical tree members of the family Apocynaceae, Dipterocarpaceae, Guttiferae, Thymelaeaceae, and Leguminosae under nursery and field conditions (Ortega *et al.*, 2004; Turjaman *et al.*, 2009; Onwuchekwa *et al.*, 2014;), this study provides new information on the amount of *P. patula* seedling growth increment that is attributable to RHIZATECH[®]TM inoculums under tree nursery conditions in the tropics. However, level of infectivity (or inoculation rate) that was achieved for every amount of inoculums used per potting tube remain unknown and, thus, is an area that requires further study. Also, effect of RHIZATECH[®]TM inoculums in quantities greater than 49.5 g per potting tube need to be determined.

Conclusion and Recommendation

The following conclusions were made: (i) Mycorrhizal inoculation of *Pinus patula* seedlings by 49.5g of RHIZATECH[®]TM inoculum achieved the highest height growth of 6.8 ± 0.3 cm after three weeks, represented 24.34% more height growth than that of non-inoculated seedlings. (ii) Mycorrhizal inoculation of *Pinus patula* seedlings by 49.5g of RHIZATECH[®]TM inoculum achieved the highest root-collar diameter (RCD) growth of 0.206 ± 0.005 cm after three weeks, represented 32.9% more RCD growth than that of non-inoculated seedlings. (iii) Mycorrhizal inoculation of *Pinus patula* seedlings by 49.5g of RHIZATECH[®]TM inoculum

achieved the highest biomass (dry weight) of the seedlings (0.244 ± 0.01 g) after three weeks, representing 62.7% more biomass (dry weight) than in non-inoculated seedlings. This study recommends the application of 49.5 g of RHIZATECH[®]TM inoculums to soil in standard *Pinus patula* nursery potting tubes at a depth of 2 cm from the top for increased height growth (24.34%), increased root collar diameter growth (32.9%) and increased biomass (62.7%), and thus for the production of *P. patula* seedlings of high vigour under standard tree nursery conditions in Kenya. This study further recommends the following for further research: the determination of level of infectivity (or inoculation rate) that can be achieved for every amount of inoculums used per potting tube; the determination of the effect of RHIZATECH[®]TM inoculums in quantities greater than 49.5 g per potting tube on height growth, RCD and biomass of *P. patula* seedlings; and the comparison of the performance of RHIZATECH[®]TM and other commercial inocula with respect to pine seedling inoculation and growth in tree nurseries.

Acknowledgement

This paper is a contribution to the 19th RUFORUM Annual General Meeting and Scientific Conference held 28th October-02 November 2023 in Yaoundé, Cameroon.

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