

EFFECT OF BIOCONTROL AGENT (TRICHO-K) FOR THE CONTROL OF POWDERY MILDEW IN SANDALWOOD

MUTHU KUMAR, A.¹, SANDHYA, G. M.¹, MAMATHA, N.¹ AND DAKSHAYINI, G.²

¹Division of Forest Protection, Institute of Wood Science and Technology, Karnataka, India

²Department of Agricultural Microbiology, UAS, GKVK, Bengaluru, Karnataka, India

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Abstract–Sandalwood (*Santalum album* L.) is indigenous to India and belongs to the family Santalaceae. Sandalwood has a high demand for its highly valued essential oil and wood. In the nursery of Institute of Wood science and Technology (IWST), Bangalore Sandalwood seedlings were affected with Powdery mildew disease as the prevailing environmental / nursery conditions were more conducive for the disease development and spreading. Tricho - K, a bio-inoculant developed by IFGTB, Coimbatore was used to control the disease development. After the Tricho – K spray, the Disease Severity Percentage was reduced from 100% to 54.40 % and in younger leaves disease was not observed. *Trichoderma* sp. is one of the major plant growth-promoting fungi which have direct beneficial effects on plants by promoting growth, development, productivity, and resistance to abiotic stress. Tricho – K showed increased plant growth parameters like, Plant height, Collar diameter, Root length, Plant fresh weight and Plant dry weight in Sandalwood seedlings over the control plants at 30, 60 and 90 days after spraying.

INTRODUCTION

Sandalwood (*Santalum album* L.) is a commercially and culturally important plant species belonging to the family Santalaceae and the genus *Santalum*. Commercially valuable sandalwood is found naturally in all over the world. In India, it is mostly found in southern part of the country namely Karnataka and Tamil Nadu. It is second most expensive wood in the world. Sandalwood is one of the most important economic tree species harvested mainly for its heartwood and oil. There are many health benefits of sandalwood essential oil as it is used as antiseptic, anti-inflammatory, antiphlogistic, antispasmodic, astringent, carminative, diuretic, disinfectant, memory booster sedative and tonic substance. (Srinivasan *et al.*, 1992 and Coppen, 1995) reported that sandalwood and sandalwood oil are used in incense and medicine, as well as the wood is used in carving. Sandalwood was infected by several fungal, viral, Phytoplasmas and bacterial diseases.

Foliar diseases caused by fungi are a serious problem in forest regeneration and sometimes fungi may cause a heavy mortality in nursery. Many

fungal pathogens are carried over through seeds into forest nurseries and become established on seedlings (Singh *et al.*, 2017).

Powdery mildew is a fungal disease that affects a wide range of plants and tree species and takes away the plant nutrients. powdery mildew fungi belonging to the genera *Oidium*, *Erysiphe*, *Sphaerotheca*, *Podosphaera*, *Uncinula* and *Leveillula* (Sztejnberg *et al.*, 1989), And it is one of the easier plant diseases to identify, as its symptoms are quite distinctive. Infected plants display white powdery spots on the leaves and stems. The lower leaves are the most affected, but the mildew can appear on any above-ground part of the plant. As the disease progresses, the spots get larger and denser as large numbers of asexual spores are formed, and the mildew may spread up and down the length of the plant. Powdery mildew grows well in environments with high humidity and moderate temperatures. The potential significance of the genus *Trichoderma* as bio-control agent was first reported by Weindling in 1932 (Pandya *et al.*, 2011).

TRICHODEX is a biocontrol preparation based on an isolate of *Trichoderma harzianum* Rifai T39. It was isolated from cucumber canopy (Elad *et al.*,

1993). The success of *Trichoderma* as bio-control agents is due to their ability to survive under different unfavorable conditions, high reproductive capacity, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against plant pathogenic fungi and efficiency in promoting plant growth and defense mechanisms. These properties of *Trichoderma* have made a ubiquitous genus present in any habitat and at high population density (Misra and Prasad, 2003).

In the nursery of Institute of Wood science and Technology (IWST), Bangalore Sandalwood seedlings were affected with Powdery mildew disease as the weather was most congenious for the disease development and spreading. As a control measures, Tricho – K was used to control the disease development which was procured from the Institute of Forest Genetics and Tree Breeding (IFGTB) Coimbatore. Tricho – K was the bio-inoculant composed of *Trichoderma* and Phosphate solubilizer. Bio-control agents such as *B. subtilis*, *T. harzianum* and Arbuscular mycorrhizal fungi (AM) have the ability to control powdery mildew, provide plants with growth promoting substances and play an important role in phosphate solubilizing and supplying P to plants (Eman *et al.*, 2020).

Therefore, this study was aimed to evaluate the Bio-control efficiency of Tricho – K on powdery mildew disease.

MATERIALS AND METHODS

An experiment was conducted to check the bio-control efficiency of Tricho – K on Powdery mildew disease. This experiment was conducted in the nursery under the Division of Forest protection, IWST, 18th cross Malleshwaram, Bengaluru-03.

1. Collection of liquid bio-fertilizer samples

Liquid formulation of Tricho – K was procured from the Institute of Forest Genetics and Tree Breeding, Coimbatore. The samples were maintained at ambient room temperature *i.e.*, 28±2⁰C and used for further work.

2. Bio-control test on powdery mildew pathogen (Antagonistic study)

In prior to Tricho – K application to the nursery, an experiment was conducted, just to test the bio-control efficiency of the Tricho – K on powdery mildew pathogen.

Antagonistic study was carried out by culturing

dual inoculants on solidified nutrient agar medium to find out compatibility among microorganisms. Tricho – K was streaked at one end of the nutrient agar plate and incubated for 24 h. Powdery mildew pathogen was plated at another end (opposite to Tricho – K growth). The plates were incubated for 48 h and observed for the growth of Powdery mildew pathogen and Tricho - K.

3. Development of Formulation

100 ml of Tricho-K is diluted with 200 ml of water in 1:2 ratio, and sprayed to the powdery mildew affected plants. And plant disease incidence (PDI) and plant disease severity (PDS) observations will be taken before and after the sprays.

4. Effectiveness study

Effectiveness of the Tricho – K inoculant formulation was tested on Powdery mildew affected plants at the intervals of 30, 60 and 90 days in a greenhouse study.

4.1 Maintenance and Observations

During the application of Tricho – K inoculant formulation to the powdery mildew affected plants, the seedlings were evenly watered in a regular way to maintain the moisture at field capacity and other usual care was taken to protect the plants from pests and diseases up to 90 days. And the corresponding growth parameter readings were recorded.

4.2 Plant Disease Incidence (PDI)

The Plant disease incidence represents the percentage of disease affected plants in a proportion indicating the prevalence of disease in a given area or host population.

$$\text{Plant Disease Incidence} = \frac{\text{Number of affected plants}}{\text{Total number of seedlings in a lot}} \times 100$$

Tricho –K sprays were given to the affected plants at 3 days interval consecutively for the period of 3 months. After that plant disease incidence and plant disease severity observations were taken.

4.3 Plant Disease Severity (PDS)

Disease severity is more appropriate in diseases like rusts, downy and powdery mildews, leaf spots and other similar disease. Disease severity tells about the extent of damage caused by the disease. It can be proportion or percentage of diseased leaves in a plant, diseased stalks or a tiller or diseased seedlings in a field, for foliar diseases severity was recorded

using below severity grade (0-4) as described by Mohanan and Sharma (1985).

Percent Disease Index (PDI) was estimated separately using the formula described by wheeler (1969) as given below,

$$\text{PDI} = \frac{(n_1 \times 0) + (n_2 \times 1) + (n_3 \times 2) + (n_4 \times 3) + (n_5 \times 4)}{\text{Total number of leaves observed} \times \text{Maximum grade used}} \times 100$$

(n_1, n_2, n_3, n_4 and n_5 indicate total number of leaves falling under severity scale 1, 2, 3, 4 and 5 respectively).

Measurement of Plant growth parameters

The observations namely, Plant height, Collar diameter, root length, fresh weights of shoot and root and dry weights of shoot and root were recorded at 30, 60 and 90 days after spraying. The Plant height was measured from the base of the plant to the terminal growing point of the main stem and expressed in centimeters (cm). The collar diameter was measured with the help of digital vernier calliper and recorded in millimeter. The seedlings will be removed from poly bags without damaging the roots and the root length will be measured from the collar region to the tip of the root and expressed in centimeters (cm). The fresh weights of plants were recorded from the freshly collected seedlings and were determined by using an electronic weighing balance and expressed in grams. Plant dry weight was recorded after drying the sample in the oven at 60°C till it gained a constant weight.

RESULTS AND DISCUSSION

An attempt was made to study the efficacy of Tricho – K liquid formulation on Powdery mildew control and plant growth parameters in Sandal (*Santalum album* L.) under greenhouse condition.

Antagonistic effect of Tricho – K

Before spraying Tricho- K formulation, antagonistic study was done between Tricho – K and Powdery mildew pathogen by plating these two microorganisms on nutrient agar medium. It was observed from the plate that, these two microorganisms were non-compatible with each other and they have showed antagonistic interaction.

Similar results were reported by (Monte, 2001) Antagonists of plant pathogenic fungi have been

used to manage plant diseases and 90% of such applications have been carried out with different strains of Trichoderma.

Plant Disease Incidence (PDI)

$$\text{Plant Disease Incidence} = \frac{\text{Number of affected plants}}{\text{Total number of seedlings in a lot}} \times 100$$

Number of affected plants = 50

Total number of plant assessed = 50

$$\text{Plant Disease Incidence} = \frac{50}{50} \times 100$$

Plant Disease Incidence = 100 %

At the initial stage (before spraying) all 50 plants were affected with powdery mildew disease. The plant disease incidence was observed to be at 100%. Tricho – K sprays were given to the affected plants at 3 days interval consecutively for the period of 3 months. After that plant disease severity observations were taken.

Plant Disease Severity (PDS)

Table 1. Disease severity analysis on severity grade (0-4)

Sl. No.	Description	Disease Severity Grade
1	No symptom	0
2	1-10 % leaf area affected	1
3	11-25 % leaf area affected	2
4	26-50 % leaf area affected	3
5	More than 50 % leaf area affected	4

Percent Disease Index (PDI) was estimated separately using the formula described by wheeler (1969) as given below,

$$\text{PDI} = \frac{(n_1 \times 0) + (n_2 \times 1) + (n_3 \times 2) + (n_4 \times 3) + (n_5 \times 4)}{\text{Total number of leaves observed} \times \text{Maximum grade used}} \times 100$$

(n_1, n_2, n_3, n_4 and n_5 indicate total number of leaves falling under severity scale 1, 2, 3, 4 and 5 respectively).

$n_1 = 66, n_2 = 85, n_3 = 97, n_4 = 115, n_5 = 91$

Sum of all ratings = 988

Total number of leaves observed = 454

Max. Disease grade = 4

$$\text{PDI} = \frac{(66 \times 0) + (85 \times 1) + (97 \times 2) + (115 \times 3) + (91 \times 4)}{454 \times 4} \times 100$$

PDI = 54.40

After the Tricho – K spray, the Disease Severity



Fig. 1. Spraying of Tricho – K on Powdery mildew affected sandalwood seedlings



Fig. 2. Plant Disease Severity grade (0-4)

Percentage was reduced from 100% to 54.40 % and in younger leaves disease was not observed. Leaf shredding was also reduced. Disease severity was less observed on the upper surface of the leaves. This study supports the investigations done by (Elad *et al.*, 1993). TRICHODEX is a biocontrol preparation based on an isolate of *Trichoderma harzianum* Rifai T39.

It was isolated from cucumber canopy (Elad *et al.*, 1993) Spraying with *T. harzianum* T39 reduced the severity of cucumber powdery mildew, the control of disease was 75–97% in young leaves, but the efficacy declined with the aging of the leaves and the further development of the powdery mildew epidemic. Control of other powdery mildews by AQ10 was reported previously (Hofstein and Fridlender, 1994; Pasini *et al.*, 1997).

Plant biometric observations

Plant bio-metric observations were taken at the intervals of 30, 60 and 90 days after spraying. The following biometric observations will be made on seedlings, selected at **random** from **control** and **treated** seedlings.

Plant height

The efficacy of Tricho – K on sandalwood saplings was depicted in Table 2.

Plant growth and vigour are indicated by the plant height. Maximum plant height (mean value) was recorded in Tricho – K treated plants (14.32 cm, 14.87 cm and 17.26 cm) at 30, 60 and 90 days respectively. And the least plant height of 13.82 cm, 14.38 cm and 16.94 cm was observed from the plants

Table 2. Plant height

Sl. No.	Plant height (cm)					
	30 DAS		60 DAS		90 DAS	
	Control	Treated	Control	Treated	Control	Treated
1	12.5	13	14	14.5	17	18.5
2	13	13	12.4	13	16.5	16
3	14	14.2	14.3	15	18	17.3
4	13.7	15	16	17.2	19.2	19
5	15.2	16.5	16.5	16.8	18.1	19.6
6	14	12	11.8	12.5	14.6	14
7	16.0	16.5	16.5	17	18.6	18.5
8	14.6	15	14.8	15.2	16.4	18
9	11	13.5	13.5	11.5	13.4	13.2
10	14.2	14.5	14	16	17.6	18.5
Mean value	13.82	14.32	14.38	14.87	16.94	17.26

*DAS – Days After Spraying

kept as control at 30, 60 and 90 days respectively after spraying. Recently, some researchers reported the effect of *Trichoderma* isolates directly on the plant growth parameters in some commercial crops (Shanmugaiah *et al.*, 2009; Bal and Altintas, 2008; Babeendran *et al.*, 2000) and our results pertaining to present investigations are matching with their findings. Seedling height, plant fresh and dry weight, and root length were increased significantly by applying *Trichoderma* isolate.

This study supports the findings of (Ashish Kumar *et al.*, 2019) and it was observed that different isolates of *Trichoderma* varied significantly in influencing the plant height of chilli plant. Results similar to this study were also found by Pereira (2017) for *T. asperellum*, when used as growth promoter for seedlings of *Pinus taeda*, 150 days after planting. Amaral *et al.* (2017) assessed seedlings of *Jacaranda micrantha*, using the same fungal species,

90 days after planting.

Collar diameter

The effect of Tricho – K liquid formulation on Collar diameter at 30, 60 and 90 days after spraying is depicted in the Table 3.

Plants treated with Tricho – K, recorded maximum collar diameter (1.60 mm, 2.06 mm and 2.46 mm) when compared to control (1.38 mm, 1.79 mm and 2.42 mm). The increased collar diameter was observed from Tricho - K received plants than the control at 30, 60 and 90 days after spraying (1.38 mm, 1.79 mm and 2.42 mm). Corroborating the results observed in this study, Amaral *et al.* (2017) concluded that the isolates of *Trichoderma asperelloides* and *Trichoderma virens* caused a significant increase in collar diameter of seedlings of *J. micrantha*, 90 days after planting.

Table 3. Collar diameter

Sl. No.	Collar diameter (mm)					
	30 DAS		60 DAS		90 DAS	
	Control	Treated	Control	Treated	Control	Treated
1	1.11	1.37	1.26	1.8	1.98	2.03
2	1.02	1.20	1.21	1.79	1.82	1.98
3	1.84	1.99	1.95	2.01	2.71	2.17
4	1.10	2.05	2.00	2.17	2.24	2.35
5	1.50	1.43	1.61	1.55	2.32	2.97
6	1.88	1.91	1.97	2.00	2.97	2.14
7	1.65	1.70	2.02	2.38	2.44	2.86
8	1.21	1.27	2.25	2.31	2.73	2.59
9	1.31	1.29	1.71	1.74	2.34	2.11
10	1.10	1.78	1.91	2.81	2.58	3.36
Mean value	1.38	1.60	1.79	2.06	2.42	2.46

Root length

The effect of Tricho – K formulation on root length of *Santalum album* saplings is depicted in Table 4. The efficacy of Tricho – K on sandalwood saplings was depicted in Table 4.

Table 4. Root length

Sl. No.	Root length (cm)	
	90 DAS	
	Control	Treated
1	16.2	15
2	8.2	9
3	13.5	13
4	15.3	14
5	21.5	22
6	11.2	11
7	10.5	11.5
8	2.8	27.2
9	10.5	11
10	25.5	26
Mean value	16.04	15.97

There were significant differences in the root length of 2 different treatments were observed in Sandalwood. In case of plants highest root length (16.04 cm) was observed in control plants than the plants receiving Tricho – K formulation (15.97 cm). This study supports the investigations done by (Ashish kumar *et al.*, 2019) Although there was a reduction in root length of the plants treated with isolates of *T. asperellum* T-09 (seeds and substrate after planting) and T-52 (substrate after planting), these treatments did not compromise the final development of the seedlings, besides providing positive results on other variables, when applied in different ways.

Fresh weights of Plants

Plant fresh weights of Sandalwood plants sprayed with Tricho - K formulation are presented in the Table 5.

Highest shoot fresh weight (mean value) of plant was recorded by the plants which are sprayed with Tricho – K (0.95 g/plant) than the control i.e., 0.86 g/plant. This study confirms the investigation of (Ashish kumar, 2019) the maximum fresh weight of 129.78 g was recorded in treatment with *Trichoderma* isolate. The fresh weight of seedlings inoculated with ZT05 and E15 increased by 125.00% and 200.00%, respectively, compared to the control (Halifu, 2019).

Table 5. Fresh weights of Plants

Sl. No.	Fresh weights of Plants (g.)	
	Control	Treated
1	1.118	1.247
2	0.985	0.542
3	0.967	1.604
4	0.52	1.495
5	0.37	1.20
6	1.06	1.007
7	1.20	1.015
8	0.98	0.431
9	0.71	0.525
10	0.69	0.435
Mean value	0.86	0.95

Total dry weight

Total dry weight will be calculated by summing up the dry weights of the shoot as well as the root and expressed in grams per seedling.

Total dry weight = Shoot dry weight + Root dry weight

In case of plants with Tricho – K showed highest mean (average) total dry weight i.e., 0.31 g/plant than the plants which are kept as control (0.26 g/plant). The present study is in accordance with the study of (Ashish kumar, 2019) maximum dry weight of 32.25 g was recorded in seed treatment along with its three foliar sprays in *Trichoderma* isolate. The dry weight of seedlings inoculated with ZT05 and E15 increased by 28.57% and 44.44%, respectively, compared to the control. Contribution of *T. harzianum* E15 to seedling height, ground diameter, and total biomass was more significant. (Halifu, 2019).

Different isolates of *Trichoderma* sp. promote direct effects on plants, increasing their growth

Table 6. Total Dry weight

Sl. No.	Dry weights of Plants (g.)	
	Control	Treated
1	0.17	0.26
2	0.12	0.39
3	0.28	0.27
4	0.39	0.59
5	0.29	0.31
6	0.11	0.22
7	0.42	0.12
8	0.21	0.38
9	0.15	0.33
10	0.41	0.24
Mean value	0.26	0.31

potential and nutrient uptake, affecting the production of biomass (Shoreshet *al.*, 2010).

CONCLUSION

Tricho – K liquid formulation was sprayed to the sandalwood seedlings to control the powdery mildew disease at IWST nursery. It is evident from the results that the Tricho – K sprayed plants showed decreased plant disease incidence as well as plant disease severity after 3 months of spraying. And along with the disease control, Tricho – K was observed as plant growth promoter, as *Trichoderma* showed direct beneficial effects on plants by promoting growth, which was evident from the assessment of growth parameters like, plant height, collar diameter, plant fresh weight and plant dry weight (plant biomass) over the control.

Conflict of interest

The research findings in this article do not have any conflict of interest.

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