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Investigation on the efficacy of bio-product - A biocontrol agent on Patchouli (*Pogostemon cablin* Benth.)

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ABSTRACT

Fusarium sp. was found to be the most prominent among the fungi causing root rot diseases in Patchouli (*Pogostemon cablin* Benth.).Protection of the Patchouli from the diseases using chemical fungicides has been regular practice. However, constant uses will lead development of resistant to fungicides in the pathogens and also may herm to the beneficial rhizosphere microbes. The present work was undertaken to evaluate the efficacy of commercially available bio-control agent such as Bio-product (*Trichoderma viride, Trichoderma harzianum and Pseudomonus flouroscences*) on the control of *Fusarium sp.* root rot disease in Patchouli and accelerate growth. *In-vitro* experiment (dual culture technique) also exhibit significant inhibition of growth of *Fusarium sp.* by the above mentioned microbes. It was found to be very efficient in controlling the disease.

Key words: *Pogostemon cablin*, *Fusarium sp.*, antagonists, morphometric parameters.

INTRODUCTION

Pogostemon cablin Benth. is a perennial herbaceous plant belongs to the family Lamiaceae and is commonly known as patchouli. It is native to subtropical Himalayas, Southeast Asia and the Far East, and has been cultivated extensively in Indonesia,Malayasia,China and Brazil (Lawrence *et al*., 1981).The plant is a well-branched,erect or ascending pubscent herb or under shrub with quadrangular stem about 0.5 m to 1.2m tall.The leaves are simple, ovate to oblong ovate,pale to purplish green in colour. Inflorescence in axillary or terminal spike of contiguous whorls; flowers pale purple. The leaf surfaces are mostly covered with glandular and non-glandular trichomes.Patchouli is an aromatic crop which yields an essential oil containing various sesquterpenes and hydrocarbons such as; patchouli alcohol (patchoulol), patchoulene, bulnesene, guaiene, caryophyllene, elemene and copaene (Lawrence, 1981; Sugimura and Hasegawa ,1992).The plant prefers warm and humid climate condition.The plant is propagated vegetatively by stem cuttings (Vasantha Kumar and Narguda, 1987).

A commercially importat aromatic patchouli oil is widely used in perfumery, cosmetics,processed food and incense stick industries and also in Aromatherapy. The patchouli oil is one of the best fixatives for highly volatile aromatic oil ,which imparts strength, strong character, alluring notes and lasting qualities. Patchouli oil is blended with other essential oils like sandalwood, rose, geranium, vetiver, clove, lavender, mentha etc, which cannot be substituted by synthesis one.

Dry patchouli leaves are used for scenting wardrobes, the leaves and tops are used for bath (Angadi and Vasanth Kumar, 1995). In Chinese medicine decoction from the leaves is used with other drugs to treat nausea,vomiting, diarrhoea, cold and headaches (Leung,1980).Patchouli oil also posseses anti-insecticidal activities (Sharma *et al*.,1992), anti-fungal and bacteriostatic properties

(Kukreja *et al*.,1990).It has traditionally been used to treat acne,eczema, inflamed, cracked or mature skin, dandruff, athlete's foot, varicose veins, hemorrhoids and impetigo (Bown *et al*., 2001). Patchouli has been used for emotional disorders such as nervousness, depression,insomnia and has also been employed as an aphrodisiac (Keville *et al*.,1995).

Some soil-born fungus causing the root-rot diseases in patchouli as well as blight and leaf spots. The pathogen being soil-born in nature is very difficult to control. Fungicides provide certain degree of control against air and soil-born pathogens. The chemicals act by inhibiting the pathogen ability to synthesis cell wall membrane components and other metabolic activities. Protection of fungal diseases using chemical fungicides has been the regular practice. However, constant uses will lead development of resistant to fungicides in the pathogens and also may harm to the beneficial rhizosphere microbes. Some fungicides act as eradicates and kill fungi on leaf , stem and root etc. Beneficial microbes, including antagonistic fungi(*Trichoderma viride, Trichoderma harzianum*) and bacteria (*Pseudomonus flouroscences*) applied on the basal end of cuttings and soil treatment or both treatments provide opportunities and benefits for protection, especially for protection against soil-born fungal pathogens.

In view of the demanding situation, the present work was under taken to evaluate the efficacy of commercially available Bio- control agent under the trade name "Bio-product" in the controlling *Fusarium sp.* root rot diseases in patchouli. Successful reduction of diseases caused by *Macrophomina phaseolina* to the extent of 22 to 74 % has been reported in bean, corn and melon (Elad *et al*., 1986) by the application of the antagonistic fungi *Trichoderma viride*. Among the fungal antagonists, *T.viride* was reported to control a range of plant pathogen in various vegetable crops (Budge and Whipps, 1991; Manoranjitham *et al*., 2000) . Britto *et al*., (2007) reported the efficacy of a commercial formulation "Bio-cure F" based on antagonistic fungi (*T.viride* and *T.harzianum*). These antagonistic fungi were evaluated under greenhouse condition against *Macrophomina phaseolina*, causing root rot disease in Black Gram.

MATERIAL AND METHODS

Stem cuttings of patchouli were randomly selected for the experimental work from NEDFi R & D Centre for MAPs,Khetri,Kamrup,Assam.

S.No.	Bio-agent	Delivery system
T1	T.v (1 gm)	Cuttings
T2	T.v. (2 gm)	Cuttings + soil
T3	T.h. (1 gm)	Cuttings
T4	T.h. (2 gm)	Cuttings + soil
T5	P.fl. (1 gm)	Cuttings
T6	P.fl. (2 gm)	Cuttings + soil
T7	T.v. + T.h. (1 gm + 1 gm)	Cuttings
T8	T.v. + T.h. (2 gm + 2 gm)	Cuttings + soil
T9	T.v. + P.fl. (1 gm + 1 gm)	Cuttings
T 10	T.v. + P.fl. (2 gm + 2 gm)	Cuttings + soil
T 11	T.h. + P.fl. (1 gm + 1 gm)	Cuttings
T 12	T.h. + P.fl. (2 gm + 2 gm)	Cuttings + soil
T 13	T.v.+ T.h. + P.fl.(1gm + 1gm + 1gm)	Cuttings
T14	T.v. + T.h. + P.fl. .(2gm + 2gm + 2gm)	Cuttings + soil
T15	Pathogen inoculated	Control I
T 16	Pathogen un-inoculated	Control II

Here- T.v.- *Trichoderma viride*, T.h.- *Trichoderma harzianum* , P. fl.- *Pseudomonas fluorescences* , Pathogen inoculated- *Fusarium* sp.

The sandy soil mixed with dry FYM and filled in polybags (4cm X 5 cm) and used for polybag culture studies. The polybags were arranged in wooden frame with boundaries for all treatments separately. Commercial formulations "Bio-product" (*Trichoderma viride, Trichoderma harzianum and Pseudomonus flouroscences*) a bio-control agent produced by Bio-control Laboratory, State Agri Research Centre, Tripura was used in the present study. The pathogenic fungi (*Fusarium sp.*) were cultured in Potato Dextrose Agar (PDA) medium by single spore isolation method.It was identified by studying under light microscope and consulting standard literatures. Fifteen treatments and a control were designed in the present investigation. They are,

Patchouli cuttings were treated with different dosages of carrier based of "Bio-product" by making paste (1 gm in 10 ml sterilized water). For soil application, Bio-product was applied at the specified dosage (1 gm)/ 5 kg of soil and mixed well and filled in polythene bags (4 X 5 cm). Field isolated, *Fusarium sp.* was made pure culture and used as a pathogen. 1 gm (mycelia + medium) of fully grown mycelia of pathogen on the medium dissolved in 100 ml sterilized distilled water and made into solution. 2.5 ml of this solution is added to each poly bag (except treatment T16) with the help of sterilized syringe , 7 days after putting of cuttings in polybags.

All the selected cuttings were having four nodes with apical portion and of same length. In each treatment there were total 60 numbers of cuttings with three replicates. The cuttings were watered everyday. The cuttings were cultured for 30 days. The observation for the following morphometric parameters were measured 20 and 30 days after plantation.

1. Height of cuttings (cm)
2. Number of nodes / cuttings
3. No. of developed axillry buds / cuttings
4. No. of leaves / cuttings
5. Maximum length of leaves (cm)
6. Maximum breadth of leaves (cm)
7. No. of roots / cuttings
8. Maximum length of roots (cm)

The following antagonists, viz- *Trichoderma viride, Trichoderma harzianum and Pseudomonus flouroscences* were collected from Bio-control Laboratory, State Agri Research Centre, Tripura. Their antagonism were tested by dual-culture technique,(Dennis and Webster, 1971) on PDA medium. A 5mm diameter mycelial disc of the pathogen (*Fusarium sp.* from the pure culture) was placed at one end of the petridish containing PDA medium. A similar disc of mycelial growth of antagonistic fungi was placed at the opposite end. The plates were incubated at 28 ± 2ºC. Replicates were maintained. The diameter of mycelial growth of antagonists and pathogen colonies were measured for 24 and 48 hrs after inoculation.

RESULTS AND DISCUSSION

Twenty days after planting, a significant increase in height of cuttings was observed in the treatment T14 (*T.viride* + *T. harzianum* + *Pseudomonus flouroscences* – cuttings + soil application) followed by treatment T12 (*T. harzianum* + *Pseudomonus flouroscences* – cuttings + soil application) & T10 (*T.viride* + *Pseudomonus flouroscences* – cuttings + soil application) respectively and against significantly low height of cuttings in treatment T15 (control-I). The root length was observed to be maximum in treatment T14, while it was minimum in treatment T15 (control-I). The number of developed axillary buds and roots per plant was observed to be more in treatment T14, while low number was seen in treatment T15 (Table : 1 & fig: 1).

The Highest height of cuttings was observed in treatment T14 and lowest height of cuttings in treatment T15, thirty days after planting. The root length was observed to be maximum in treatment T14, while it was minimum in T15. In treatment T14, the highest length of the root was found to be 14.2 cm. The number of developed axillary buds & roots per plant was observed to be more in treatment T14, while low number was seen in treatment T15 (Table : 2 & fig.: 2).

The effect of antagonists on the number of nodes was also observed. It was more in treatment T14 and T13, when compared to other treatments. However a low number of nodes were observed in treatment T15. The efficacy of

antagonists on rooting and mortality of patchouli was also measured. Under nursery shade house , the rooting percentage of patchouli cuttings was found to be significantly 100% in treatment T14, low percentage of rooting was recorded in the treatment T15 (71%). The percentage of mortality was found to be (0%) in T14, while highest in percentage of mortality was seen in T15 (8%) (Table: 2).

T. viride , *T. harzianum* and *Pseudomonus flouroscences* used in the present study have shown an inhibitory effect on *Fusarium sp.* (Fig. 3). The radial mycelial growth in in-vitro study against *Fusarium sp.* was maximum in *T.viride* (43.0 mm) as compared to *T.harzianum* (42.0 mm) and *Pseudomonus flouroscences* (33.5 mm), (Table 3). However, the radial growth of Fusarium sp. against *T.viride* was observed to be 40.5 mm, while it was seen 36.0 mm in *T.harzianum* and 8.0 mm in *Pseudomonus flouroscences* in dual plate experiment. It was observed that the radial mycelial growth of Fussarium sp. (8.0 mm) was highest inhibited by *Pseudomonus flouroscences* (fig. 4). The inhibitory effect of *P. fluorescens* may be due to biosynthesis of antibiotics (Howell and Stipnovic, 1980) .

From the study it may be concluded that *T. viride*, *T. harzianum* and *Pseudomonus flouroscences* control the root rot disease of Patchouli and accelerate growth. *In-vitro* experiment (dual culture technique) also exhibit significant inhibition of growth of *Fusarium sp.* by the above mentioned microbes.

Table 1: Effect of Bio-product treatments on the following morphometric parameters in Patchouli (after 20 days)

	Mean of three replications									
Treatments	**Height of cuttings (cm)**	**No. of Nodes**	**No. of develo-pedd axillry buds/ plant**	**No. of leaves**	**Maximum length of leaves**	**Max. bread the of leaves (cm)**	**No. of Roots / plant (cm)**	**Max. length of roots**	**% of mortality**	**% of rooting**
T1	12.66	1.80	2.80	6.00	3.55	2.70	15.60	3.97	4	66.66
T2	13.14	2.00	3.00	6.80	4.05	2.97	18.00	5.03	0	71.43
T3	13.18	2.00	3.00	6.00	3.77	2.82	16.25	5.75	0	85.71
T4	13.32	2.20	3.20	7.20	4.25	2.90	18.60	6.73	0	71.43
T5	14.58	2.20	3.20	6.80	4.03	3.00	21.75	6.25	0	85.71
T6	13.00	2.20	3.20	7.40	4.08	3.10	26.25	7.57	0	100
T7	14.40	2.40	4.00	7.00	4.43	3.33	25.50	7.45	4	85.71
T8	14.48	2.40	4.20	8.50	4.93	3.55	36.00	8.93	0	100
T9	14.80	2.40	4.20	7.75	4.33	3.60	26.25	7.85	8	100
T10	16.22	2.60	4.20	9.20	5.23	4.06	36.75	8.20	0	100
T11	14.65	2.80	4.00	7.75	4.10	2.90	32.50	7.88	4	100
T12	16.28	2.80	4.60	9.40	4.77	3.70	38.00	8.68	0	100
T13	15.86	3.00	4.80	8.00	4.93	3.83	36.00	8.67	0	100
T14	16.62	3.00	5.20	10.5	5.10	3.87	38.75	9.05	0	100
T15	11.60	1.60	2.60	6.20	3.40	2.46	14.50	3.05	12	57.14
T16	13.36	1.80	3.00	6.60	3.73	2.77	17.50	5.90	8.00	66.67
SEm±	0.29	0.09	0.17	0.27	0.13	0.09	1.85	0.38	0.79	3.25
CV%	9.39	13.57	14.6	15.91	13.96	13.81	17.82	8.18	NA	NA

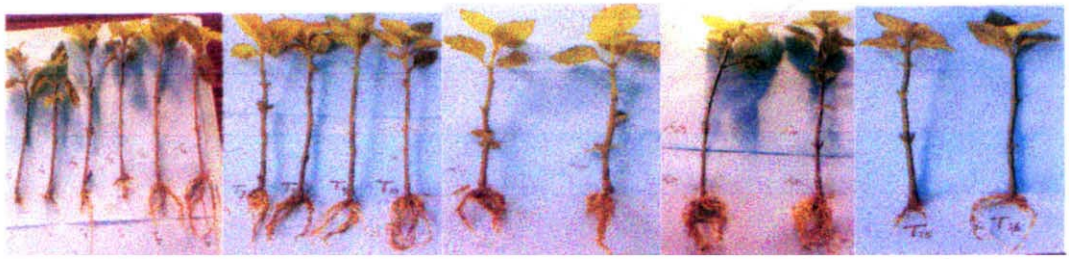


Fig. 1a **Fig. 1b** **Fig. 1c** **Fig. 1d** **Fig. 1e**

Fig. 1(a-e): Effect of bio-product treatments on growth in Patchouli (After 20 days)

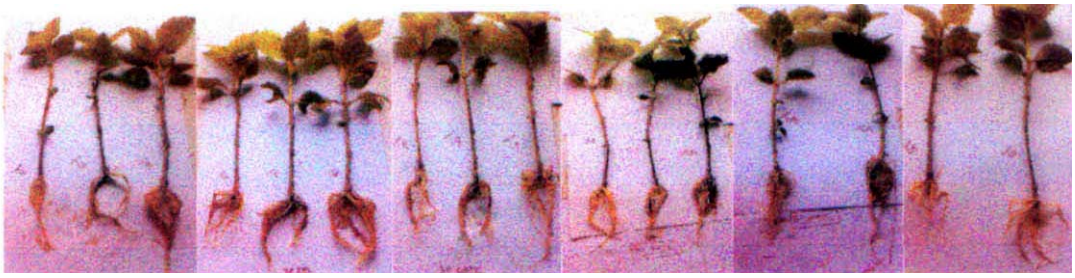


Fig. 2a **Fig. 2b** **Fig. 2c** **Fig. 2d** **Fig. 2e** **Fig. 2f**

Fig. 2(a-f): Effect of bio-product treatments on growth in Patchouli (After 30 days)

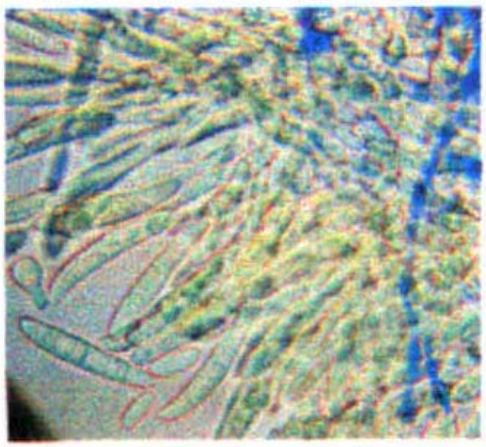
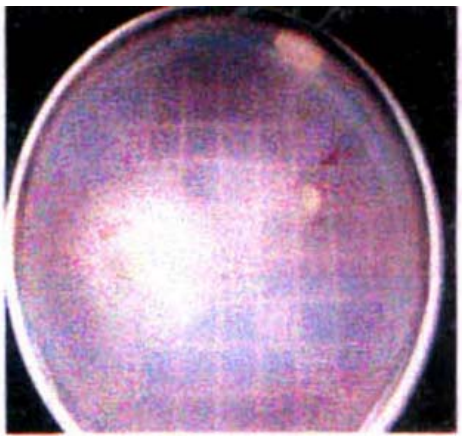


Fig. 3: *Fusarium* sp. (Pathogenic fungi)



Ps **Fu**

Fig. 4: Inhibitory effect on *Fusarium* sp. by *Pseudomonus flouroscences*

Table 2: Effect of Bio-product treatments on the following morphometric parameters in Patchouli (after 30 days)

	Mean of three replications									
Treatments	**Height of cuttings (cm)**	**No. of Nodes**	**No. of develo-pedd axillry buds/ plant**	**No. of leaves**	**Maximum length of leaves**	**Max. breadth of leaves (cm)**	**No. of Roots / plant (cm)**	**Max. length of roots**	**% of mortality**	**% of rooting**
T1	16.80	3.00	4.75	10.40	4.97	4.03	37.4	9.84	0	100
T2	18.00	3.17	4.80	9.60	5.27	4.10	38.2	10.77	0	100
T3	17.80	3.00	4.25	10.60	5.17	4.10	37.00	10.00	4	100
T4	18.25	3.25	5.20	10.80	5.50	4.60	37.25	10.84	0	100
T5	17.74	3.20	4.60	8.80	5.60	4.45	43.75	11.05	0	100
T6	18.38	3.25	4.60	11.20	5.97	4.60	51.00	11.68	0	100
T7	17.92	3.20	5.57	10.50	4.67	3.45	35.50	9.60	4	100
T8	19.45	3.00	5.00	11.20	5.87	4.73	48.67	10.67	0	100
T9	19.32	3.40	5.50	12.50	5.83	4.73	48.30	11.93	0	100
T10	20.40	3.50	5.50	13.25	6.07	4.80	50.00	12.05	0	100
T11	18.43	3.20	5.00	11.00	5.76	3.80	41.67	10.68	0	100
T12	20.73	3.25	5.75	12.50	5.80	4.50	52.50	11.77	0	100
T13	20.64	3.50	6.00	12.00	6.07	4.83	53.00	12.17	0	100
T14	21.23	3.60	6.25	13.75	6.33	5.06	55.33	13.57	0	100
T15	15.88	2.80	3.80	10.80	4.37	3.25	21.60	7.04	8	71
T16	18.57	3.25	5.20	11.60	5.55	4.25	44.80	10.72	0	100
SEm±	0.32	0.05	0.14	0.28	0.13	0.11	1.80	0.32	0.49	2.17
CV%	7.56	6.49	11.96	11.25	10.61	10.95	18.57	14.18	NA	NA

Table 3 : *In vitro* antagonism assay

S. No.	**Mean Radial Mycelial Growth (mm)**							
	T.viride		***T.harzianum***		***P.flouroscences***		***Fusarium sp.***	
	24 hrs	**48 hrs**	**24 hrs**	**48 hrs**	**24 hrs**	**48 hrs**	**24 hrs**	**48 hrs**
1	29.50	43.00					25.50	40.50
2			19.00	42.00			17.00	36.00
3					29.50	33.50	2.50	8.00s
4	14.00	33.00	2.00	9.50				
5	16.00	32.20			15.50	32.40		
6			24.50	36.00	18.00	30.50		
SEm±	2.22	2.16	3.48	5.13	2.76	0.48	3.90	5.02

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