

Original Research Article

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Management of Root Rot Disease of Patchouli Caused by *Fusarium solani* (Mart) Sacc. Through Fungicide, Bioagent and Oilcake in Field

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ABSTRACT

Patchouli is an important aromatic plant from Lamiaceae family, well known for its essential oil properties. Root rot disease of patchouli is caused by *Fusarium solani* (Mart.) Sacc., a severe and wide spread disease in India and major constraint to patchouli cultivation in Assam. A roving survey was conducted on root rot incidence based on the occurrences of disease in major patchouli growing areas of Nagaon, Biswanath, Golaghat and Jorhat districts of Assam. Highest percentage of the disease incidence was recorded in the districts of Nagaon (69.01%). Field evaluations were conducted during monsoon season by combining fungicide, bio-agents and oilcake for the management of root rot disease. In field trial, all the treatments were found significantly superior over inoculated control in reducing disease incidence. Integration of seedling root dipping and soil drenching with Carbendazim @ 0.1% and soil application of *Biofor-pf-2* @ 100 g/plant and MOC @ 50 g/plant (T₇) recorded least percent disease incidence (5.58%) and maximum herbage yield as compared to inoculated control (88.88%). It was followed by soil application of *Biofor-pf-2* @ 100 g/plant and MOC @ 50 g/plant (T₆), seedling root dip and soil drenching with Carbendazim @ 0.1% and soil application of MOC @ 50 g/plant (T₅) recorded 8.33% and 11.11% PDI, respectively. The integration of *Biofor-pf-2* @ 100 g/plant with Carbendazim @ 0.1% (T₄) recorded maximum disease incidence (33.33%) in comparison to the inoculated control.

Keywords

Root rot disease,
Fusarium Solani,
Oilcake, Field,
Fungicide.

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Introduction

Patchouli (*Pogostemon cablin* (Blanco) Benth.), an important aromatic plant belonging to Lamiaceae family, is a native of Philippines (Arpana *et al.*, 2008). Patchouli oil is a dark orange coloured viscous liquid; it is one of the most important essential oils utilized in the perfumery and food industry. It also has therapeutic properties, namely antidepressant, anti-inflammatory, antiseptic, aphrodisiac, astringent, carminative, diuretic as well as febrifuge, fungicidal, insecticidal, sedative and tonic properties (Akhila *et al.*,

1984). Commercial cultivation of patchouli in India was first attempted by Tata oil mills in 1942. After the initial few attempts to grow the crop, its systematic cultivation was started in 1962 by Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow (Kumar *et al.*, 1986).

Patchouli is commercially cultivated in the Indian states of Assam, Goa, Gujarat, Kerala, Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu (Venugopal *et al.*, 2008).

In northeast, Patchouli cultivation is initiated in the first instance on about 1000 ha land by farmers and entrepreneurs and planned to go up to five times in future (Das, 2001). Patchouli was also cultivated in Itanagar of Arunacha Pradesh (Saha *et al.*, 1989 and 1992) but due to lack of follow up extension services and marketing support it could not gain popularity in the region. In Assam, The Rural and Development of Flower Agro-tech, an aromatic oil producing private company, cultivated the Indonesian type cultivar of patchouli plant successfully for a three year rotation in more than 10 acres of land in the hill slopes and foot hill areas of southern Hailakandi and Barak Valley in the year 1997-99 (Ahmed, 2002). The incidence of root rot disease of patchouli is common in Assam due to prevalence of high rainfall and humid condition. There is scanty of information on the management of the disease. It is difficult to control using single management practices in diseased prone area. Therefore, field experiment was conducted for the management of root rot of patchouli (*Fusarium solani*) by using fungicide (Carbendazim), commercial bio-formulation *Biofor-pf-2* (*Trichoderma harzianum* and *Pseudomonas fluroescens*) and oilcake (Mustard oil cake) alone and its combinations.

Materials and Methods

Disease survey

A roving survey was conducted in major patchouli growing areas of Assam, *viz.*, Jorhat, Golaghat, Nagaon, Biswanath districts. The survey was conducted during 2014-2016. Eight isolates of *F. solani* were collected from different districts of Assam, *viz.*, Herbal Garden (AAU) and Pokamua village in the district of Jorhat, Kaliabor nursery, Uluoni Gaon and Seuj Nagar in the district of Nagaon, Bijoypur and Madhupur in the district of Biswanath and Sugarcane

Research Station (SRS), Buralikson in the district of Golaghat were designated as JFS₁, JFS₂, NFS₃, NFS₄, NFS₅, BFS₆, BFS₇ and GFS₈. Per cent disease incidence was calculated by the formula given below (Vidhyasekaran and Muthamilan, 1995).

$$\text{Percent Disease Incidence (\%)} = \frac{\text{Number of Diseased Plants}}{\text{Total Number of Plants Observed}} \times 100$$

During the survey, the infected samples i.e. plants with root rot symptoms were collected from these areas and were kept in the plastic bags, which were brought to laboratory for the isolation of associated pathogens.

Isolation, purification and identification of pathogen

Infected tissues of the root were cut into small pieces of 2-3 cm size and surface-sterilized with 0.1% sodium hypochlorite solution for one minute and washed repeatedly (thrice) in sterile distilled water and placed in Petriplates containing sterilized PDA, and incubated at 27±1°C. The purified culture of the pathogen was obtained by hyphal tip method and maintained on PDA slants throughout the present investigation. The pathogen isolates were mainly identified on the basis of cultural and morphological characters as *Fusarium solani* (Mart) Sacc. Booth (1971). Further, the identity of *Fusarium solani* was confirmed from National Center of Fungal Taxonomy (NCFT), New Delhi (I.D. No.-9080.17).

Pathogenicity test

In pathogenicity test, Thirty day-old patchouli seedlings were planted in 25 cm diameter plastic pots containing sterilized soil under net-house condition. The mass cultures of *F. solani* were applied to the pots @ 40 g and

kept for four days before transplanting of seedlings. Observations were made regularly for the appearance and development of symptoms. After symptom development, re-isolation was done from the artificially infected plants. The fungus was re-isolated from the artificially inoculated plants and resulting cultures were compared with the original ones.

Sources of fungicide, bioagents and oilcake

Fungicide

Carbendazim was tested in the present study.

Bioagents

Vermicompost based commercial bio-formulation (*Biofor-pf-2*) of *Trichoderma harzianum* and *Pseudomonas fluorescens* obtained from Department of Plant Pathology, AAU, Jorhat was used in the study.

Oilcake

Powdered mustard oil cake was evaluated in the present study.

Integrated management practices

The fungicide, bio-control agents and oilcake alone and in combination was evaluated in field for management of root rot patchouli. The field experiments were conducted in a randomized block design (RBD) with eight treatments and three replications during pre-monsoon season in artificially inoculated sick field at Instructional-cum-Research (ICR) Farm AAU, Jorhat. The individual plot size was 2x3 m and patchouli seedling was transplanted at spacing of 60 x 60 cm. The treatments combinations before transplanting were as follows: T₁: Root dipping & Soil drenching with Carbendazim @ 0.1%, T₂: *Biofor-pf-2* @ 100 g/plant, T₃: Mustard oil

cake 50 g/plant, T₄: T₁+T₂, T₅: T₁+T₃, T₆: T₂+T₃, T₇: T₁+T₂+T₃, and T₈: Inoculated control.

Method of treatment application

Treatment included root dipping of patchouli seedlings in Carbendazim solutions for 10 minutes and soil drenching with carbendazim was given seven before transplanting in plots.

Mustard oil cake powder (50 g/plant) was applied in plot at 10-15 cm soil depth before one month of transplanting for proper decomposition. Commercial bio-formulation of *Biofor-pf-2* @ 100 g/plant was applied in plot before one week of transplanting. Inoculums of *F. solani* multiplied on maize meal sand medium were added in the soil @ 50 g/plant. Observation of root rot disease incidence was recorded at the time of harvest.

Results and Discussion

Incidence of root rots disease of patchouli in different districts of Assam

Incidence of root rot of patchouli caused by *F. solani* was recorded during the cropping season of 2014-2016 in different patchouli growing areas of four districts of Assam (Table 2). Incidence of the disease was found to be serious in almost all the locations surveyed. Maximum disease incidence (74.54%) of the root rot was recorded at Kaliabor nursery in the district of Nagaon followed by Uluoni Gaon (70.00%), Seuj Nagar (62.50%) in the districts of Nagaon, Pokamua village (56.00%) in the district of Jorhat, Madhupur (54.54%), Bijoypur (53.84%) in the district of Biswanath, SRS, Buralikson (52.00%) in the district of Golaghat in contrast to lowest incidence (47.22%) of root rot at Herbal Garden, Department of Agronomy, AAU in the district of Jorhat. Sreedevi *et al.*, (2007) reported the

occurrence of fungal wilt of patchouli caused by *F. solani* from different districts of Karnataka and Singh and Angadi (1990) from IIHR, Bangalore (Table 1).

Pathogenicity of *Fusarium solani*

The result revealed that the inoculated plants were successfully infected by all the isolates, whereas control plants (without inoculation) remained healthy. The characteristic symptoms started as yellowing of lower leaves 25-35 days after inoculation which extended upwards and whole leaves gradually turned brown coloured after 40-45 days of inoculation. Isolate NFS₄ (Uluoni Gaon) showing the symptoms at 25-35 days after transplanting was graded (+++) as highly pathogenic isolates. When the infected plant was uprooted, dark brown discolouration of vascular tissue was observed. Rotting of roots was prominent. The similar result was observed by Nikam *et al.*, (2007) who reported that pathogenicity of the chickpea wilt caused *Fusarium oxysporum* f.sp. *ciceris* by sick soil inoculation technique in earthen pots under greenhouse conditions (Table 2).

Evaluation of fungicide, bio-formulation and oilcake in field condition against root rot of patchouli caused by *F. solani*

In the field experiment, typical symptoms of root rot and wilting of patchouli incidence started from 30-35 days after transplanting and continued to 120 days after transplanting in all the plots. Integrated treatments of seedling root dip and soil drenching @ 0.1% along with soil application of *Biofor-pf-2* @ 100 g/plants (Bio-formulation) and organic amendment with MOC @ 50 g/plants (T₇) recorded minimum percent disease incidence (5.55%) as compared to inoculated control (88.88%). It was followed by soil application of *Biofor-pf-2* @ 100 g/plants and MOC @ 50 g/plants (T₆), Root dip and soil drenching with Carbendazim @ 0.1% and soil

application of MOC (T₅) recording 8.33 and 11.11 per cent PDI, respectively. The present studies are supported by earlier works where integration of fungicide with bio-control agents gave maximum disease control in several crops (Sawant and Mukhopadhyay, 1990; Dubey, 1997). Singh (2009) reported that the application of two bioagents *viz.*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and two fungicides Carbendazim (0.1%) and mancozeb (0.25%) at 45 day after sowing was found effective for the control of coriander wilt caused by *Fusarium oxysporum* f.sp. *coriandrii*. Integration of chilli seedling root dip with Carbendazim + Mancozeb, addition of vermicompost, drenching of fungicide Carbendazim + Diathane M-45 and soil application of *T. viride* was found to be effective in reducing mortality of chilli plants (5.83 %) caused by *F. solani* (Madhavi and Bhattiprolu, 2011). The seed and seedling treatment with fungicide *viz.*, Captan, Metalaxyl and Carboxin may eradicate the wilt causing pathogens or other microflora there by less competition for bio-control agents to colonize the seed and root surface and proliferate (Chet *et al.*, 1982; Ram *et al.*, 2000). Combined soil application of *T. viride* and ground nut cake followed by neem cake had given good control against chickpea wilt caused by *Fusarium oxysporum* f.sp. *ciceris* (Nikam *et al.*, 2007) (Table 3).

Biocontrol agents also act indirectly by inducing systemic resistance in plants by increased nutrient uptake and make them unavailable to plant pathogens and by inactivating the pathogen enzymes (Chaube *et al.*, 2001). Some of these oil cakes are found to increase the nitrogen uptake of the plant and protect the plants from soil nematodes, insects, and parasites (Ramachandran *et al.*, 2007). Literature shows that several antimicrobial by-products (e.g. organic acids, hydrogen sulfide, phenols, tannins and nitrogenous compounds) are released during the decomposition of organic amendments.

Chauhan (1963) also reported that *Fusarium* wilt of gram observed that the soil treatment with groundnut, sesame and mustard oil cake significantly reduced the population of wilt pathogen. The combination of neem cake with *T.viride* reduced *Fusarium* population and increased *Trichoderma* population. Integrated

treatments were more effective than their individual treatments. It was observed that there is good compatibility of fungicides Carbendazim, Neem products and bio-control agents (*T. harzianum* and *T. viride*), for control of soybean root-rot (Gyanendra and Verma, 2005) (Table 4).

Table.1 Survey on the incidence of root rot of patchouli in different districts of Assam during 2015-2016

Sl. No	Isolates No	Name of the Districts	Location	Plants Examined	Plants affected	Disease incidence (%)	Average (%)
1	JFS ₁	Jorhat	Herbal Garden, AAU	36	17	47.22	51.61
2	JFS ₂	Jorhat	Pokamua village	25	14	56.00	
3	NFS ₃	Nagaon	Kaliabor Nursery	110	82	74.54	69.01
4	NFS ₄	Nagaon	Uluoni Gaon	70	49	70.00	
5	NFS ₅	Nagaon	Seuj Nagar	40	25	62.50	
6	BFS ₆	Biswanath	Bijoypur	39	21	53.84	54.19
7	BFS ₇	Biswanath	Madhupur	44	24	54.54	
8	GFS ₈	Golaghat	SRS, Buralikson	50	26	52.00	52.00

Table.2 Pathogenic variability of different isolates of *F. solani*

Sl. No	Isolates	Name of the Districts	Locality	Pathogenicity	Reaction
1	JFS ₁	Jorhat	Herbal Garden, AAU	++	Pathogenic
2	JFS ₂	Jorhat	Pokamua village	+	Pathogenic
3	NFS ₃	Nagaon	Kaliabor Nursery	++	Pathogenic
4	NFS ₄	Nagaon	Uluoni Gaon	+++	Highly pathogenic
5	NFS ₅	Nagaon	Seuj Nagar	++	Pathogenic
6	BFS ₆	Biswanath	Bijoypur	+	Pathogenic
7	BFS ₇	Biswanath	Madhupur	++	Pathogenic
8	GFS ₈	Golaghat	SRS, Buralikson	++	Pathogenic

(+++) Initial wilting symptoms appear 25-35 DAT

(++) Initial wilting symptoms appear 35-45 DAT

(+) Initial wilting symptoms appear 45-55 DAT

Table.3 Field evaluation of fungicide, bio-formulation and oilcake in the management of root rot of Patchouli caused by *F. solani*

Treatments		Percent Disease Incidence (%)**	Percent disease reduction over control
T ₁	Root dipping and soil drenching with Carbendazim @ 0.1%	30.55 (33.52) ^{c*}	69.45
T ₂	<i>Biofor-pf-2</i> @ 100 g/pot	19.44 (26.13) ^{de}	80.56
T ₃	Mustard oil cake (MOC) @ 50 g/pot	16.66 (24.04) ^{ef}	83.34
T ₄	Root dipping and soil drenching with Carbendazim @ 0.1% + <i>Biofor-pf-2</i> @ 100 g/pot	33.33 (35.24) ^{bc}	66.67
T ₅	Root dipping and soil drenching with Carbendazim @ 0.1% + MOC @ 50 g/pot	11.11 (19.46) ^{fgh}	88.89
T ₆	<i>Biofor-pf-2</i> @ 100 g/pot + MOC @ 50 g/pot	8.33 (16.74) ^{gh}	91.67
T ₇	T ₁ + T ₂ + T ₃	5.55 (13.56) ^h	94.45
T ₈	Inoculated control	88.88 (70.45) ^a	11.12
SEm (±)		6.35	
CD (P = 0.05)		6.73	

Means within columns separated by Duncan's multiple range test P = 0.05

Figures followed by same letters are statistically not different

*Figures with in parentheses are angular transformed values

** Values are mean of three replications

Table.4 Effect of different treatments on shoot and root length and number of branches of Patchouli in field condition

Treatments	**Shoot length (cm)				**Root length (cm)	**No of Branches
	30 DAT	60 DAT	90 DAT	120 DAT		
T ₁ : Root dipping & soil drenching with Carbendazim @ 0.1%	20.66 ^{gdc}	30.65 ^{ged}	41.86 ^g	43.56 ^{gf}	17.72 ^f	15.33 ^{gf}
T ₂ : <i>Biofor-pf-2</i> @ 100 g/plant	22.91 ^{df}	30.66 ^{fg}	46.66 ^{ef}	50.37 ^{ef}	24.13 ^e	22.76 ^e
T ₃ : Mustard oil cake @ 50 g/plant	22.16 ^{ed}	30.92 ^{ef}	49.06 ^d	50.56 ^{de}	25.03 ^{de}	23.00 ^{de}
T ₄ : T ₁ + T ₂	21.58 ^{fd}	32.30 ^{de}	45.46 ^f	47.46 ^f	17.16 ^{gf}	18.46 ^f
T ₅ : T ₁ + T ₃	25.38 ^{ca}	37.36 ^{ab}	65.43 ^b	67.70 ^{bc}	25.13 ^{cde}	33.40 ^c
T ₆ : T ₂ + T ₃	25.75 ^{bc}	35.55 ^c	64.70 ^{cb}	67.43 ^c	27.57 ^b	34.33 ^{bc}
T ₇ : T ₁ + T ₂ + T ₃	26.41 ^{ab}	36.16 ^{bc}	68.06 ^a	71.43 ^{abc}	29.68 ^a	40.56 ^a
T ₈ : Inoculated control	20 ^{hg}	20.86 ^h	22.33 ^h	14.66 ^h	7.36 ^h	4.66 ^h
SEm (±)	2.48	2.25	3.00	6.47	1.96	3.90
CD (P = 0.05)	2.63	2.12	3.18	6.86	2.08	4.13

Means within columns separated by Duncan's multiple range test P = 0.05

Figures followed by same letters are statistically not different

** Values are mean of three replications

Table.5 Effect of fungicide, bio-formulation and oilcake alone and combination on fresh and dry weight of shoot and root of patchouli after 120 days of transplanting

Treatments	Shoot weight (g)**		Root weight (g)**	
	Fresh	Dry	Fresh	Dry
T ₁ : Root dipping and soil drenching with Carbendazim @ 0.1%	592.66 ^f	282.16 ^f	56.36 ^g	33.07 ^g
T ₂ : <i>Biofor-pf-2</i> @ 100 g/plant	992.21 ^c	302.46 ^c	96.93 ^e	54.08 ^e
T ₃ : Mustard oil cake @ 50 g/plant	1060.95 ^d	420.03 ^d	98.41 ^d	60.85 ^d
T ₄ : T ₁ + T ₂	584.25 ^g	203.30 ^g	60.69 ^f	41.03 ^f
T ₅ : T ₁ + T ₃	1451.58 ^c	520.96 ^c	146.64 ^b	83.72 ^b
T ₆ : T ₂ + T ₃	1555.99 ^b	564.06 ^b	138.96 ^c	79.84 ^c
T ₇ : T ₁ +T ₂ +T ₃	1675.96 ^a	652.56 ^a	171.30 ^a	95.40 ^a
T ₈ : Inoculated control	78.66 ^h	30.86 ^h	23.66 ^h	8.07 ^h
SEm(±)	7.06	2.55	1.82	1.85
CD (0.05)	7.48	2.77	1.93	1.96

Means within columns separated by Duncan's multiple range test P = 0.05

Figures followed by same letters are statistically not different

**Values are mean of three replications



Plate.1 A view of experimental field at ICR farm, AAU, Jorhat

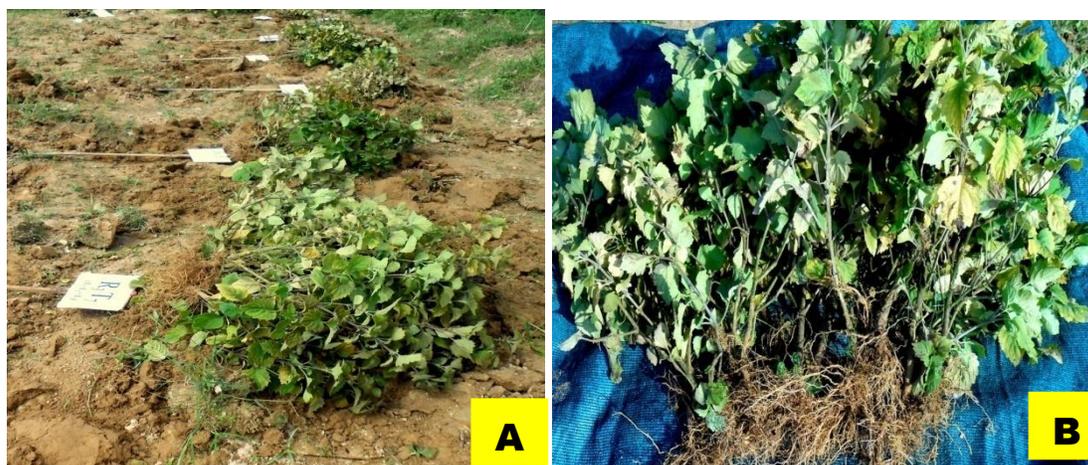


Plate.2 (A-B) A view of harvesting of Patchouli plant in field



Plate.3 Measurement of root length (cm) by using scale



Plate.4 Effect of fungicide, bio-formulation and oilcake on mortality of Patchouli

Plants at 120 Days After Transplanting

A. R_1T_8 : Inoculated control

B. R_2T_8 : Inoculated control

C. R_3T_8 : Inoculated control

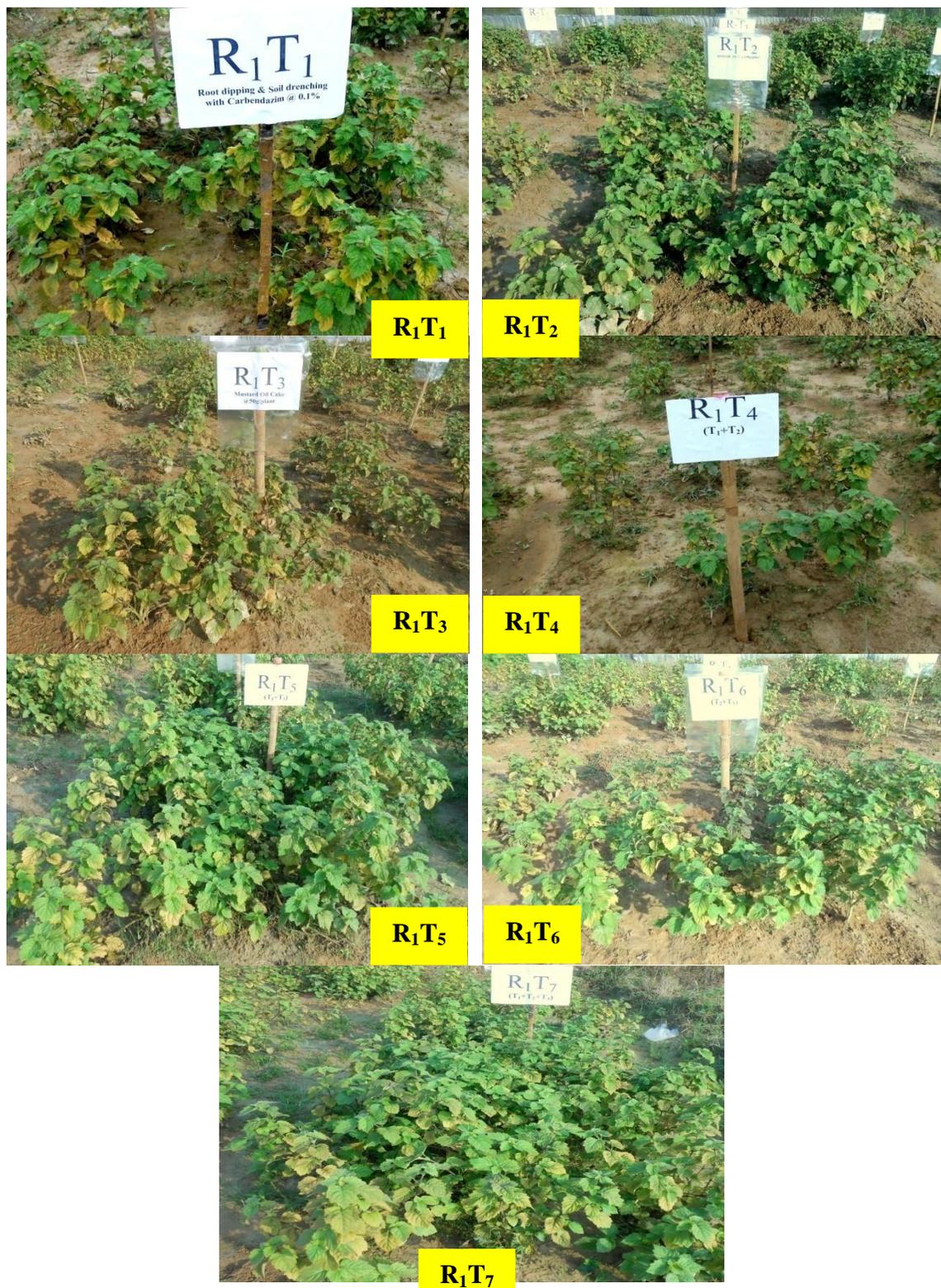


Plate.5 Effect of different treatments on plant growth at 120 Days After Transplanting

R₁T₁: Root dipping and soil drenching with Carbendazim @ 0.1%

R₁T₂: *Biofor-pf-2* @ 100 g/plants R₁T₃: MOC @ 50 g/plants

R₁T₄: T₁ + T₂ R₁T₅: T₁ + T₃

R₁T₆: T₂ + T₃ R₁T₇: T₁ + T₂ + T₃

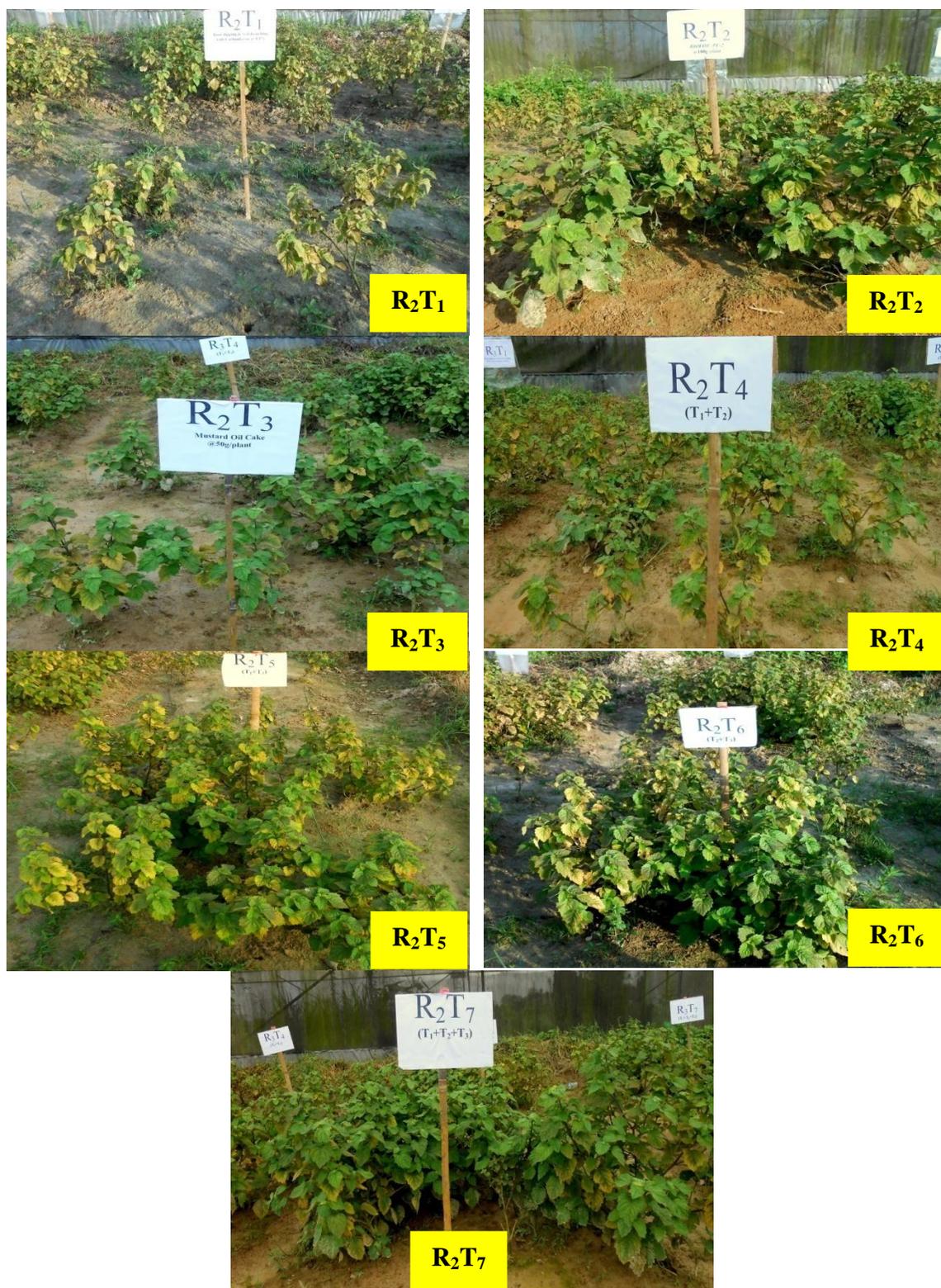


Plate.6 Effect of different treatments on plant growth at 120 Days After Transplanting

R₂T₁: Root dipping and soil drenching with Carbendazim @ 0.1%

R₂T₂: *Biofor-pf-2* @ 100 g/plants R₂T₃: MOC @ 50 g/plants

R₂T₄: T₁ + T₂ R₂T₅: T₁ + T₃

R₂T₆: T₂ + T₃ R₂T₇: T₁ + T₂ + T₃

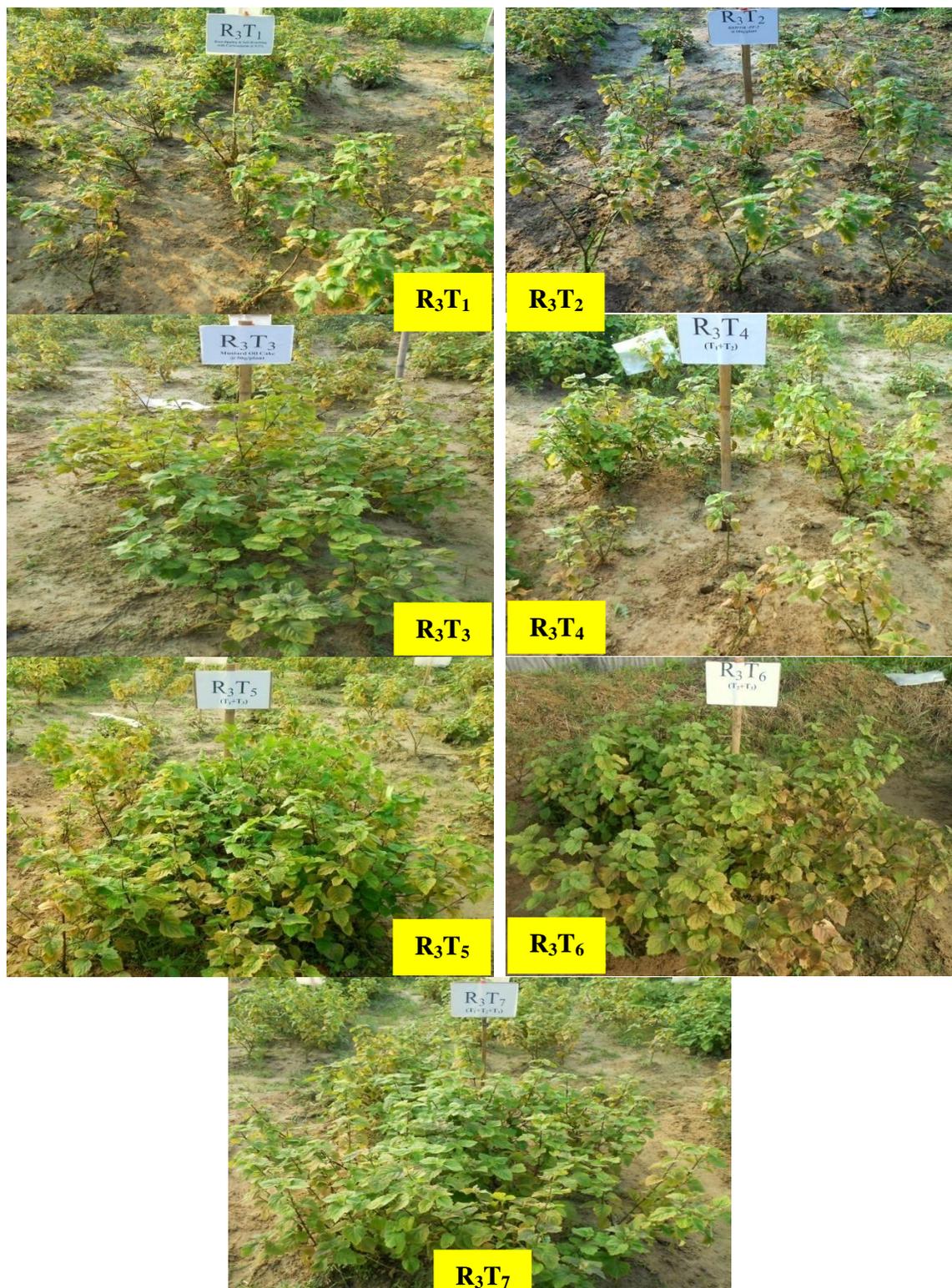


Plate.7 Effect of different treatments on plant growth at 120 Days After Transplanting

R₃T₁ : Root dipping and soil drenching with Carbendazim @ 0.1%

R₃T₂ : *Biofor-pf-2* @ 100 g/plants R₃T₃ : MOC @ 50 g/plants

R₃T₄ : T₁ + T₂ R₃T₅ : T₁ + T₃

R₃T₆ : T₂ + T₃ R₃T₇ : T₁ + T₂ + T₃

In the present investigation, MOC (T₃) @ 50 g/plants, *Biofor-pf-2* (T₂) @ 100 g/plants and Root dipping and soil drenching with Carbendazim @ 0.1% (T₁) were significantly reducing the disease incidence by recording a PDI 16.66, 19.44, 30.55 per cent respectively over the inoculated control (88.88%). Mujeebur *et al.*, (2004) found that chickpea seeds treated with commercial formulations of *T. harzianum* and *P. fluorescens* individual and in combination found to control wilt caused by *F. oxysporum* f.sp. *ciceri* under field condition. Soil application of *T. harzianum* and *Pseudomonas* effectively controlled the rhizome rot of ginger caused by *F. solani* and *P. myriotylum* (Ram *et al.*, 1997). Baily and Lazarovits (2003) reported that organic amendments, manures and composts with high nitrogen contents may suppress soil borne diseases by releasing allelochemicals during microbial decomposition. Antagonistic activity of *Pseudomonas* spp. against *Fusarium oxysporum* is mainly due to the antibiotics, Fe chelating siderophores and hydrogen cyanide (Rajeshwari and Kannabiran, 2011). Seedling root dipping and soil drenching with Carbendazim @ 0.1% + *Biofor-pf-2* (T₄) @ 100 g/plants was observed less effective by recording a PDI of 33.33% over inoculated control. Earlier, Sreedevi *et al.*, (2009) reported that maximum control of vascular wilt of patchouli caused by *F. solani* by treating seedlings with Carbendazim or mancozeb (Table 5).

However, the results comprehensively proved that combined application of Carbendazim, bioagent and oilcake could be employed in the management of root rot of patchouli in field. The present investigation also showed that the disease incidence reduced when MOC was used as soil amendment. This might be due to the fact that MOC contributed a food base to be used by *T. harzianum* for rapid multiplication leading to its enhanced

antagonistic activity. At the same time the test pathogen *F. solani* was highly sensitive to Carbendazim and mustard oil cake. This seems to be the main reason for getting excellent control of root rot disease of patchouli when Carbendazim, bioagent and oilcake were used for soil application in combination. Hence, Integration of Carbendazim, bio-formulation and mustard oilcake can be recommended to the farmers for effective management of root rot disease of patchouli.

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