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EFFICACY OF NATIVE BIO-AGENTS AGAINST *SCLEROTIUM ROLFSII* SACC. CAUSINGFOOT ROT OF FINGER MILLET

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ABSTRACT: Finger millet is one of the important millet crops widely cultivated across India. Although, it is known to be one of the hardiest crops, is affected by many diseases, of which foot rot caused by *Sclerotiumrolfsii* has been on the rise especially under irrigated and high rainfall situations.Nine Trichoderma spp. and 10 *Pseudomonas* spp. isolated from cultivated soils of Mandya, Karnataka, india were screened in *in-vitro* against *S.rolfsii*. Among the bio-agents Chandagaluisolate (CT) of Trichoderma and Kannahatty isolate (KP) of *Pseudomonas* were found to be very effective in suppressing the foot rot of finger millet in susceptible variety Indaf-5. These two potential bio-agents when tested in different delivery methods under greenhouse conditions, seedling root dip followed by soil application of *Trichoderma* (CT) and *Pseudomonas* (KP) were significantly superior over other methods in reducing foot rot incidence, besides enhancing seedling growth parameters and grain yield.

Key Words: *Sclerotiumrolfsii*, Finger Millet, Foot Rot, Bio-agents, isolation

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INTRODUCTION

Finger millet [*Eleusinecoracana*(L.)Gaertn.,] is an important staple food and one of the important millet cropsin India, Nepal and eastern Africa especially among the tribal folk. It is commonly referred to as *Ragi*, Chodi, Bird's foot, *Nagli, Mandua,Marua* in different regions of the country is nutritionally rich with high quality protein, plenty of minerals, dietary fiber, and phytochemicals. Grains have 8-10 times more calcium than rice and wheat and is recommended for diabetes and other life style diseases. It has a slightly higher water requirement than pearl millet and is grown on higher altitudes up to 2000 meters above sea level and on soils which are typically too poor to support any other crop. Further, it can be stored safely for many years without insect damage. This is particularly vulnerable in drought-prone areas where harvests frequently fail. The wide adaptability of the crop could be attributed to its C4 nature. Finger millet farmers face numerous challenges, including labour, credit, marketing, weeds, pests and diseases. With more research and an enabling policy environment, the crop has great potential for expansion. Although, it is found to be a hardy crop, it is also affected by many diseases, among them foot rot caused by *Sclerotiumrolfsii* has been an increasing problem especially in irrigated and heavy rainfall area (Nagarajaand Reddy, 2009). The disease has been reported to cause more than 50 per cent yield loss (Batsa and Tamang, 1983). *S. rolfsii* is a well known and most destructive soil borne fungus initially described by Rolfs (1892) on tomato. The fungus is widely distributed and causes severe damage to more than 500 crops (Aycock, 1966). Although there are several other Sclerotium producing fungi, the fungus characteristic of small tan to dark-brown or black spherical sclerotia with internally differentiated rind, cortex, and medulla were placed in the form genus Sclerotium (Punja and Rahe, 1992).

This pathogen causes a variety of symptoms on different hosts like collar rot in chickpea, southern blight of sugar beet, foot rot of finger millet, leaf spot in *Lotus meliloti*, bud rot of *Colocasiavariagata* and fruit rot in *Citrullus vulgaris* etc. Consequently the diseases caused by this fungus are more serious in tropical and subtropical regions than in temperate regions and this pathogen is of major importance throughout the world.

MATERIAL AND METHODS

Isolation and identification of the Fungus

Isolation

The collected specimen were cut into small bits and washed in running water. The bits were surface sterilized with one per cent sodium hypochlorite solution for one minute, washed thoroughly with three changes of sterilized distilled water to remove the traces of sodium hypochlorite transferred aseptically to Petri plates containing sterilized PDA medium and incubated at 27 ± 1^0C for three days. The fungal growth on fourth day, which arose through the infected tissue was taken by inoculation loop and transferred aseptically to the Petri plates containing PDA medium. Pure culture of the fungi was obtained by single hyphal tip method.

Identification

The pathogen *S.rolfsii* formed cottony white colonies on PDA containing Petri plates. The colonies appeared as dull white to pure white mycelial growth and formed sclerotial bodies after 6-7 days of incubation. Sclerotia were brown in colour and appeared mustard seed like. Based on these morphological characters the pathogen was identified as *S. rolfsii* (Agrawal and Kotasthane, 1971; Rangaswami, 1988).

Proving pathogenicity

Sterilized soil was taken in earthen pots of size 45 x 30 cm^2. Thirty days old culture of *S. rolfsii*multiplied on sorghum grains was mixed thoroughly with soil. Surface sterilized finger millet seeds of foot rot susceptible variety Indaf-5 were sown; seeds sown in pots devoid of inoculum served as check. Moisture was maintained at 25 per cent moisture holding capacity of soil by adding water on weight basis throughout the period. Re-isolation was made from such affected portion of the plant tissue and compared with the original culture.

Evaluation of native bio-agents

Ninenative isolates of *Trichoderma* collected from different parts of theMandya districtwere designated as Chikkabyadarahalli isolate(CBT),Dudda isolate (DT), Kestur isolate (KT), Chandagalu isolate (CT), Madduru isolate (MT),Besagaralli isolate (BT), T.S.Chathra isolate (TST), Hulivana isolate (HT),Srirangapattana isolate (SPT) and ten *Pseudomonas* isolates *viz*., V.C.Farm isolate (VCP), Harakadalli isolate (HP), Kannahatti isolate (KP), Byadarahalli isolate (BHP), Nanjarayanapura isolate (NRP), Yedahalli isolate (YP), Ganadhalu isolate (GP), Basaralu isolate (BP)Nagamangala isolate (NP) and Arakere isolate (AP).To know the antagonistic effect of bio-agents,*in vitro* evaluation was carried out against *S.rolfsii.*For this study the bio-agents and test fungus were cultured on potato dextrose agar in order to get fresh and active growth of fungus.

Dual Culture Technique

Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petri plates and allowed to solidify. For evaluation of fungal bio-control agents, mycelial discs of test fungus were inoculated at one end of the Petri plate and antagonistic fungus was placed exactly opposite to it on the other end. In case of evaluation of bacterial antagonist, the bacterium was streaked one day earlier at one end of the Petri plate to the middle of the Petri plate and the active growing disc of the test fungus was placed at the other end. The plates were incubated at 27 ± 1^0C and the zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and the antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where, I = per cent inhibition
C = growth in control
T = growth in treatment

Efficacy of potential bio-agents against*S. Rolfsii* in different delivery methods

The most potential isolates of*Trichoderma* and *Pseudomonas* obtained from *in vitro* studies were tested under greenhouse condition against *S. rolfsii.* Complete Randomized Design (CRD) with three replications was followed for data compilation and analysis. The following treatments of different delivery methods were used.

T_1=Seed treatment with *Trichoderma* (CT) @10 g kg^{-1} of seed, T_2= Seed treatment with *Pseudomonas*(KP) @10 g kg^{-1} of seed, T_3=Seedling root dip in *Trichoderma* (CP)@ 10 g L^{-1} of water, T_4= Seedling root dip in *P*seudomonas (KP) @ 10 g L^{-1} of water, T_5= Soil application of *Trichoderma* (CP) @10 g $kg^{-1}$of soil ($2x10^6$ cfu ml^{-1}), T_6= Soil application of *Pseudomonas* (KP)@10 g kg^{-1} of soil ($2x10^8$cfu ml^{-1}), T_7= Soil application of *Trichoderma* (CP) and *Pseudomonas* (KP) each @ 5 g kg^{-1} of soil, T_8= Seedling root dip in *Trichoderma* (CP) and *Pseudomonas* (KP) each @ 5 g L^{-1} of water followed by soil application of *Trichoderma* (CP) and *Pseudomonas* (KP) each 5 g kg^{-1} of soil, T_9= Soil drenching of carboxin + thiram @ 0.02% , T_{10}= Control without inoculum and T_{11}= Control with inoculum. The bio-agents were mass multiplied by using sorghum grain and talc powder. Incorporated to the cement pots (45 cm x 30 cm) size containing sclerotium sick soil. Bio-agents were applied on weight per weight basis with the minimum spore load of $2x10^6$ for *Trichoderma* and $5x\ 10^8$ for *Pseudomonas*. Ten days old seedlings of foot rot susceptible variety (Indaf-5) at the rate of 20 seedlings per pot were transplanted, irrigated weekly once and observations on growth parameters *viz*., shoot height, shoot weight, root weight and root length was recorded after 30 and 70 DAP (Days After Planting).The per cent foot rot incidence and basal lesion length were recorded at tillering and maturity stage of the crop.After maturity five ear weight and grain weight were recorded at harvesting.

Experimental design and data analysis

The experiments were conducted following Completely Random Design (CRD) with three replications. Data were analyzed by using MSTAT-C program andthe data were transformed wherever necessary.

RESULT AND DISCUSSION

Evaluation of native Trichoderma isolates.

Nine *Trichoderma* andten *Pseudomonas* isolates were evaluated by dual culture technique for their antagonistic effect against *S.rolfsii* under *in vitro* condition. The inhibition zone in mm was recorded and the per cent inhibition was calculated.

Table 1. Antagonistic effect of native *Trichoderma* isolates against *S.rolfsii*

S. No.	Isolates	Per cent inhibition of mycelial growth at 7 days of inoculation
1	Chikkabyadarahally (CBT)	38.89 (38.58)
2	Dudda (DT)	48.89 (44.36)
3	Kestur (KT)	39.07 (38.69)
4	Chandagalu (DT)	72.22 (58.19)
5	Maddur (MT)	44.44 (41.81)
6	Besagarahally(BT)	41.11 (39.88)
7	T.S.Chathra (TCT)	44.44 (41.81)
8	Hulivana (HT)	33.15 (35.15)
9	Srirangapatana (SPT)	27.22 (31.45)
10	Control	0.00 (0.00)
S. Em±		0.60
CD (P=0.01)		2.45

*Figures in parentheses are *arc sin* transformed values

In vitro studies on antagonistic activities of *Trichoderma* isolates against *S. rolfsii* after 7 days of incubation revealed significant reduction in per cent inhibition of mycelial growth of *S. rolfsii*. Among the nine isolates evaluated against *S. rolfsii,* Chandagalu isolate (CT) was significantly superior over all the isolates by recording 72.22 per cent inhibition of mycelial growth. In other isolates, inhibition of mycelial growth ranged from 27.22 to 48.89 per cent (Table 1, Fig. 1).

These results are in accordance with the observations of Bhuiyan *et al*. (2012) who showed significantly variable antagonism ranging from 65.01 to 83.06 per cent reduction of radial growth in dual culturing of *S. rolfsi* with 20 *T. harzianum* isolates collected from *rhizosphere* and *rhizoplane* of different crops. Kulkarni (2007) also reported variable inhibition of mycelial growth by different *T. harzianum* isolates. Likewise Manu*et al*. (2012), Parmar *et al.* (2015), Pandav *et al*. (2013) and Patro and Madhuri (2013), Chanutsa *et al.* (2014) and Jabbar Sab *et al.* (2014), Yasmin *et al.* (2014) and Aly *et al*. (2015) have all reported that *Trichoderma* spp. were an important antagonist to inhibiting the growth of *S. rolfsii.*Mycoparasitism is the mechanism of inhibition of *Sclerotium* by *Trichoderma* which invades and parasites the mycelia (Elad *et al.*, 1980). It may also be due to the production of cell wall degrading enzymes (CWDE) which has high endochitinase activity that can break down the cell wall of the fungus.

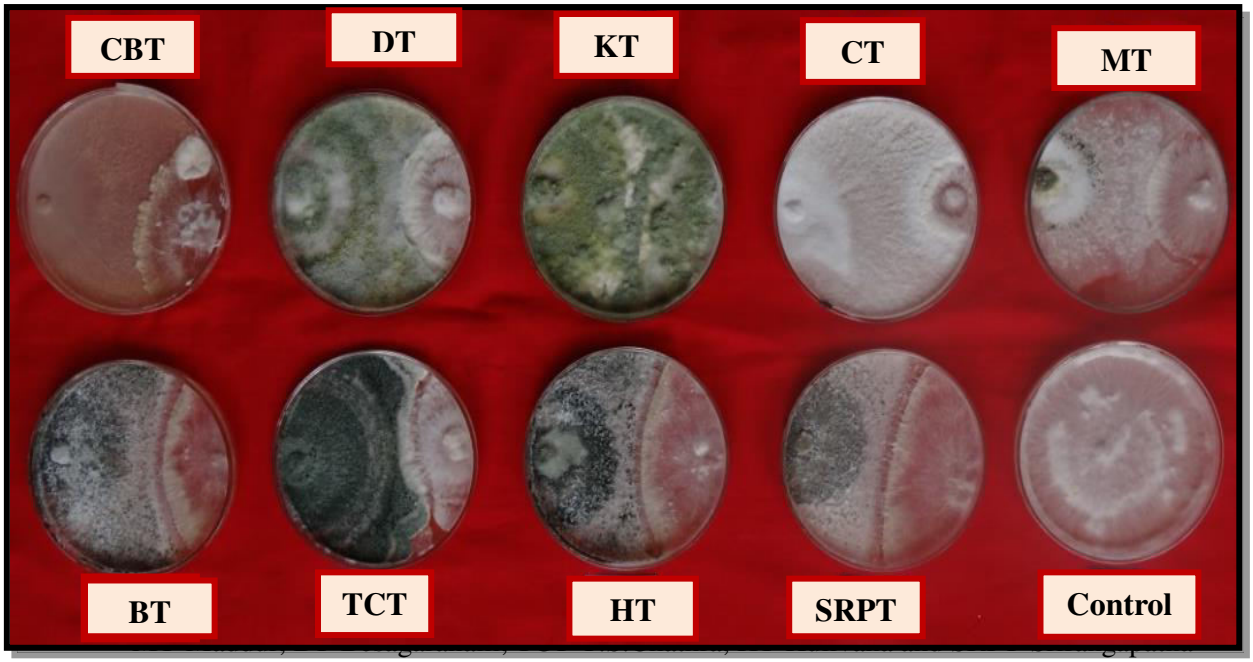

Fig.1: *In vitro* evaluation of *Trichoderma* spp.against *S.rolfsii*

Table 2. Antagonistic effect of native *Pseudomonas* isolates against *S.rolfsii*

S. No.	Isolates	Per cent inhibition of mycelial growth
1	VCP	32.78 (34.92)
2	HP	30.74 (33.66)
3	KP	57.04 (49.05)
4	BHP	25.74 (30.45)
5	NRP	25.56 (30.36)
6	YP	20.74 (27.08)
7	GP	21.11 (27.32)
8	BP	40.07 (39.18)
9	NP	20.19 (26.68)
10	AP	31.85 (34.35)
11	Control	0.00 (0.00)
S. Em±		2.44
CD (P=0.01)		7.20

VCP-V.C.Farm, HP-Harakadalli, KP-Kannahatti, BHP-Byadarahalli, NRP-Nanjarayanapura, YP-Yedahalli, GP-Ganadalu, BP-Basaralu, NP-Nagamangala& AP-Arakere

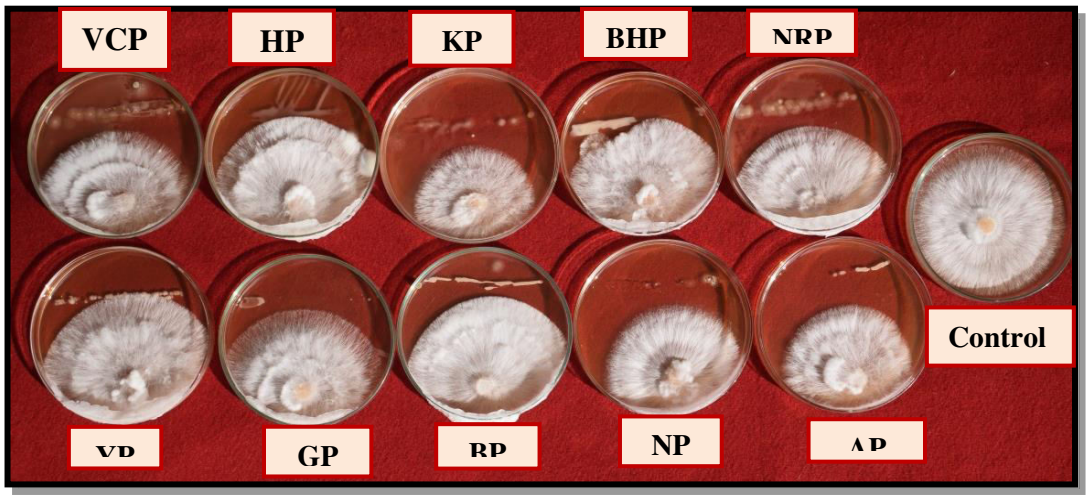

Fig 2: *In vitro* evaluation of *Pseudomonas*spp.against *S. rolfsi*

At 7 days of incubation,significantly highest inhibition of mycelial growth of the test fungus (57.04%) was recorded in Kannahatty isolate (KP), but in other isolates it ranged from 20.19 to40.07 per cent (Table 2, Fig. 2). Pushpavathi and Chandrasekhararao (1998) found that, *Bacillussubtilis* and *Pseudomonas fluorescens* are able to reduce the mycelial growth of the pathogen significantly. Most of the PGPR isolates showed antifungal activity against *F. oxysporum*, and *R. solani*, and only one against *S. rolfsii*(Manivannan *et al.*, 2012).Rakh *et al.* (2011) found that highest antagonistic activity by the *Pseudomonas* spp. was up to 94 per cent of the growth of the test fungus in terms of dry weight, further; Chanutsa *et al.* (2014) recorded 100 per cent inhibition of*S. Rolfsii* by culture filtrates of *P. fluorescens.*

Table 3. Effect of different delivery methods of native bio-agents on foot rot incidence and grain yield in finger millet

Treatments	Foot rot (%) at tillering stage	Basal lesion length (cm)	Foot rot (%) at crop maturity	Basal lesion length (cm)
T1	3.33 (10.37)	1.67	17.17 (24.46)	9.70
T2	5.00 (12.64)	2.03	20.00 (26.54)	10.33
T3	0.00 (0.00)	0.00	9.17 (17.62)	5.73
T4	0.83 (3.03)	0.50	10.33 (18.73)	7.77
T5	0.00 (0.00)	0.00	12.67 (20.85)	5.47
T6	0.83 (3.03)	0.50	15.60 (23.25)	8.63
T7	0.00 (0.00)	0.00	7.03 (15.38)	4.73
T8	**0.00 (0.00)**	**0.00**	**2.20 (8.51)**	**2.93**
T9	0.00 (0.00)	0.00	8.97 (17.39)	6.53
T10	0.00 (19.79)	0.00	0.00 (0.00)	0.00
T11	11.67 (48.86)	8.10	36.67 (37.23)	25.07
S. Em ±	1.19	0.30	1.06	0.93
CD (P= 0.01)	3.42	1.20	3.11	3.65

*Figures in parentheses are arc sin transformed values

Table 4: Effect of different delivery methods of native bio-agents on growth in finger millet in greenhouse

Treatments	30 DAP				70 DAP				Five ear weight (g)	Grain yield/five ears (g)
	Plant height (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Plant height (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)		
T1	12.33	2.94	12.84	0.22	42.37	21.97	21.03	12.23	43.01	33.98
T2	11.73	2.79	12.89	0.37	42.40	19.97	19.60	11.67	45.19	35.70
T3	12.14	2.92	13.24	0.28	50.63	23.67	24.47	12.87	47.03	37.15
T4	11.97	3.00	14.07	0.51	49.47	18.60	19.80	11.20	45.63	36.05
T5	14.21	5.18	13.33	0.54	55.27	26.30	22.31	13.10	53.42	42.20
T6	13.81	3.92	13.99	0.54	51.57	20.87	21.45	11.57	49.55	39.14
T7	17.01	7.11	15.01	0.76	59.40	31.50	24.17	15.27	61.98	48.97
T8	**20.97**	**10.77**	**22.03**	**1.34**	**65.67**	**33.33**	**27.53**	**17.00**	**69.54**	**54.94**
T9	15.77	2.20	17.15	0.82	42.50	24.27	20.17	12.17	44.21	34.93
T10	10.83	2.03	12.10	0.21	41.60	21.20	19.23	10.47	40.82	32.25
T11	6.76	1.86	7.87	0.15	27.57	4.63	10.03	0.80	30.42	24.03
S. Em ±	**0.23**	**0.14**	**0.23**	**0.01**	**2.83**	**1.04**	**0.65**	**0.39**	1.11	1.31
CD (P= 0.01)	**0.95**	**0.59**	**0.94**	**0.07**	**11.41**	**4.19**	**2.65**	**1.57**	**4.50**	**5.27**

DAP= Days after planting

Effect of potential *Trichoderma* and *Pseudomonas* isolates against *S. rolfsii*.

The integrated management approaches for foot rot of finger millet were designed in a pot culture experimentusingpotential bio-agents *viz.*, Chandagalu isolate of *Trichoderma* (CT) and Kannahatty isolate of *Pseudomonas* (KP).

Effect of different delivery methods on foot rot incidence and basal lesion length

Among the different delivery methods tried, T_8 (Seedling root dip with *Trichoderma* (CT) 5 g L^{-1} + *Pseudomonas* (KP) 5 g $L^{-1}$of water followed by soil application of *Trichoderma* (CT)+ *Pseudomonas* (KP)Each 5 g kg^{-1} of soil along with 300-500 g enriched compost incubated for 15 days showed least foot rot incidence (0.00, 2.20 %) in comparison to untreated check (11.67, 36.67%) both at tillering as well as maturity stages respectively.

Similar trend was observed in case of basal lesion length of the infected plants.Least lesion length was recorded in T_8 (0.00, 2.93 cm) followed by T_7 (0.00, 4.73 cm) and T_5 (0.00, 5.47 cm)in comparison to untreated check (8.10, 25.07 cm) at both the stages. Thus as the basal lesion length progressed the disease incidence also increased from tillering to maturity stages**.**

Among the several bio-control methods, soil amendments and effective bio-control agents, act as viable and potent alternatives to conventional chemicals for the management of soil borne plant diseases (Jeyarajan and Angappan, 1998; Naik and Sen, 1994).

Effect of native bio-agents on plant growth and yield of finger millet

Among the treatments, T_8 (seedling root dip + soil application of *Trichoderma* (CT) + *Pseudomonas* (KP) each 5 g kg^{-1} of soil along with 300-500 g enriched compost incubated for 15 days) showed significantly higher plant height (20.97 cm), shoot weight (10.77 g), root length (22.03 cm) and root weight (1.34 g) at 30 days after planting (DAP). Though, T_8 recorded higher plant height (65.67 cm), shoot weight (33.33 g), root length (27.53 cm) and root weight (17.00 g) even at 70 DAP it was comparable to few other treatments also. Contrarily, plants in control showed less height (6.76, 27.57 cm), shoot weight (1.86, 4.63 g), root length (7.87, 10.03 cm) and root weight (0.15, 0.80 g) both at 30 and 70 DAP respectively (Table 4).

Soil application of *Trichoderma* spp. amended with FYM helped in population build up besides plant growth promotion and might have Induced Systemic Resistance (ISR) as reported by Ganesan (2004). Almeida and Londim (1981) found that the *Trichoderma* spp. significantly reduced the sclerotial wilt of cowpea. Similarly, Thiribhuvanamala *et al.* (1999) showed that, soil amendment with *T. harzianum* resulted in better plant stand of up to 80 per cent at 60 days after sowing followed by *T. viride* and *P. fluorescens* in pot culture studies. Further, according to Vikram and Hamzehzarghni (2011) although individual applications of either *T. harzianum* or neem cake or captan did not give similar results as single inoculations of either FPD-10 or FPD-15, it did significantly reduce the pod infection caused by *S. rolfsii* and improved pod yield. Several other workers (Biswas *et al*., 2000; Rakh*et al.*, 2011; Chakravarty and Kalita, 2012) have reported not only the control *S. rolfsii* with *Trichoderma*spp. but also improvement in growth and overall health of the plants.

CONCLUSION

Among the different native isolates evaluated, Chandagalu isolate (CT) of *Trichoderma* and Kannahatty isolate (KP) of *Pseudomonas* showed maximum inhibition of *S.rolfsii* the incitant of foot rot in finger millet, these two potential bio-agents have been tested by different delivery methods under greenhouse conditions to confirm their efficacy, seedling root dip followed by soil application of *Trichoderma* (CP) +*Pseudomonas* (KP) was significantly superior over other methods of application. This treatment may be validated at farmers' field to re-confirm their efficacy.

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