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RESEARCH ARTICLE

EFFICACY OF BIOAGENTS AND PLANT EXTRACTS FOR THE MANAGEMENT OF SHEATH ROT OF RICE CAUSED BY *SAROCLADIUM ORYZAE*

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ABSTRACT

Studies on the *in vitro* and *in vivo* antagonistic activity of native bioagents agents and plant extracts were evaluated against *Sarocladium oryzae*, which causes sheath rot of rice, were undertaken in the Department of Plant Pathology of the Annamalai University, Chidambaram. It was revealed that isolate- Pf S5 (*Pseudomonas fluorescens*-Sivapuri) recorded the maximum inhibition zone of 10.55 mm and a minimum of 23.40 mm mycelial growth of *S. oryzae* accounting for 74.00 per cent reduction in the mycelial growth over control and isolate- BsA1 (*Bacillus subtilis* Ambigapuaram) recorded the maximum inhibition zone of 9.92 mm and a minimum of 24.62 mm mycelial growth of *S. oryzae* accounting for 72.64 per cent reduction in the mycelial growth over control. Among the ten plant extracts tested (10% concentration) against the mycelial growth of *S.oryzae*, *E. globules* extract recorded the minimum mycelial growth (19.03 mm) and maximum per cent reduction of mycelial growth of pathogen (78.85%) over control. In the pot culture studies, seed treatment with *Fluorescent pseudomonads (PfS5*) @12g/kg plus *B. subtilis (BsA1)* @12g/kg plus *E.globule*s extract@10% followed by foliar application with *Fluorescent pseudomonads (PfS5*) and *B. subtilis (BsA1)* each @ 0.5% plus *E. globule*s extract@10%-T9 at 45 and 60 DAT recorded minimum sheath rot incidence(12.53), maximum plant growth (109.68 cm), number of tillers per hill (15.35), number of grains per panicle (145.90),weight of 1000 of seeds (24.22g) and yield (18.39g/plant).

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INTRODUCTION

Rice is a monocotyledonous annual grass and belongs to the family Graminae and the genus is *Oryza*. More than 90% of the world rice is produced and consumed in Asia, which is native for 60 per cent of the earth's population. Rice plays an important role in Indian agriculture, which is the staple food for more than 60% of the population. In India, it is grown in states like Uttar Pradesh, West Bengal, Andhra Pradesh, Tamil Nadu, Punjab, Orissa, Chhattisgarh and Bihar. These biggest rice producing states hold around 72% of India's absolute rice producing region and offer over 75% of the all-out rice production in the country. There is, however, great potential to increase the rice yield in India by improving crop management and expanding rice production. The major constraints are abiotic stresses (drought or excess of water, nutrient deficiencies and extreme temperatures) and biotic stresses (weeds, diseases and pests (1). The crop is constantly subjected to various fungal, bacterial and viral diseases. Among the fungal diseases, sheath rot is considered as one of the most important emerging diseases of rice, causing yield losses from 20–30% up to 85% (2) (3) (4).

Sarocladium oryzae was known to be the first major important pathogen of fungi that caused sheath rot disease of rice after been first isolated in 1922 in Taiwan (5) (6). *S. oryzae* also is known to produce antimicrobial secondary metabolites such as helvolic acid and cerulenin (7) (8). It develops well in rain-fed rice fields, and found in lowland and medium land environments (9) (10). This disease is mainly caused by the seed-borne fungus *S.oryzae* (6). Since its first description in 1922 in Taiwan as *Acrocylindrium oryzae*, the fungus has spread worldwide. Sheath rot occurs in both rainfed and irrigated ecosystems, affecting all rice varieties. Dwarf and high-yield Asian varieties are the most susceptible. *S. oryzae* infection becomes visible on the flag leaf sheath as greyish-brown necrotic lesions, enlarging as the disease progresses until the whole leaf sheath is necrotic. Enclosed panicles are affected, leading to sterile, empty or discoloured grains or during severe infection, only partial or no emergence of the panicle (6) (3). *S. oryzae* can survive in seeds, plant residues, weeds and soil and it is transmitted by seeds, wind and insects. Wounds or natural damages may favours the pathogen to enter into the host (2) (3).

Chemical pesticides are exclusively used for the management of the disease but not considered as a long-term solution because it may lead to cause health and environment hazards, residue persistence and elimination of natural enemies and development of resistance. Increasing public concerns about the quality of food grains has accelerated the development of ecofriendly and economically feasible control methods. The use of microorganisms as biological control agents to control plant disease has emerged as powerful alternative method (11). Plant Growth Promoting Rhizobacteria, *Pseudomonas fluorescens* also plays an important role as biocontrol agent in management of several plant pathogens (12)(13). *Bacillus* species can produce various kinds of diffusible and volatile compounds with strong inhibitory activity against plant pathogens (14)(15). These diffusible compounds have low toxicity, high biodegradability, environmentally friendly characteristics (16). Plants provide abundant resources of antimicrobial compounds and have been used for centuries to inhibit microbial growth (17)(18). Flavanoids, triterpenoids, steroids and other phenolic compounds in plants have been reported to have antimicrobial activity (19)(20). However, no attempts have been made for the management of sheath rot disease by using the mixtures of plant extract, *Bacillus* strains and *Fluorescent pseudomonads* strain and to understand the mechanisms of disease resistance induced by these mixtures. Therefore, the present study was designed for testing the native bioagents against S. oryzae, antagonistic activity of plant extracts against S.oryzae, consortium of bioagents and plant extracts for the management of sheath rot of rice under pot culture.

MATERIALS AND METHODS

Isolation and identification of pathogen: The pathogen was isolated from the diseased rice sheaths showing the typical lesions of sheath rot. The edge of the lesions were cut into small pieces by means of a sterile knife. Then the pieces were surface sterilized in 0.1 per cent sodium hypochlorite solution for 30 seconds and washed in three repeated changes of sterile distilled water and then plated into sterile Petri dishes containing PDA medium. The plates were then incubated at room temperature 28±2°C. The tip of the hyphal growth radiating from the infected tissue was transferred into PDA slants (21). The fungus was purified again by single hyphal tip method and maintained on PDA slants for the further studies. A total of 10 isolates (So1 to So 10) were obtained from infected sheath region of rice plants collected from different districts of Tamil Nadu. Based on the pathogenicity test, the isolate- So5 was found to be more virulent when compared to other isolates tested in the present study. Hence, the isolate-So5 alone was taken for the subsequent experiments.

Isolation of bacterial antagonists from rice rhizosphere: Antagonistic bacteria (both *Pseudomonas* and *Bacillus*) were isolated from the rhizosphere soil collected from five different rice growing tracts of Tamil Nadu and they were isolated by using serial dilution plating technique. Colonies with characteristics of *Bacillus* spp, *Fluorescent pseudomonads* were isolated individually and purified by streak plate method (22) on nutrient agar medium and King's B medium, respectively. *Fluorescent pseudomonads* and *Bacilus* spp. were identified based on biochemical test. A list of bio-agents obtained from rice rhizosphere is given below,

S. No.	Location	Crop	Isolates code
a)	**Bio-control agents isolated from different locations**		
I.	***Fluorescent pseudomonads***		
1.	Ambigapuram (Cuddalore)	Rice	Pf A1
2.	Annamalainagar (Cuddalore)	Rice	Pf An2
3.	Kalambur (Tiruvannamalai)	Rice	Pf k3
4.	Krishnapuram (Tiruvannamalai)	Rice	Pf Kp4
5.	Sivapuri (Cuddalore)	Rice	Pf S5
II.	***Bacillus subtilis***		
1.	Ambigapuram (Cuddalore)	Rice	BsA1
2.	Annamalainagar (Cuddalore)	Rice	BsAn2
3.	Kalambur (Tiruvannamalai)	Rice	BsK3
4.	Krishnapuram (Tiruvannamalai)	Rice	BsKp4
5.	Sivapuri (Cuddalore)	Rice	BsS5

Screening of *Fluorescent pseudomonads* isolates against *S. oryzae* (So5): The antagonistic effect of five isolates of *P. fluorescens* and five isolates of *B. subtilis* were tested against *S. oryzae* by using dual culture technique (23). The mycelial growth of pathogen and inhibition zone was measured at the end of the incubation period and expressed in mm. Based on the dual culture technique, the most virulent isolate was selected and used for further study.

Collection and Preparation of aqueous extracts: The fresh, disease free leaves and bulbs of ten plant species were collected from locally available plant species and they were separately washed with tap water, then with distilled water to remove any epiphytes present and other wastes and then dried under shade for 3 weeks. The dried plant material was ground to fine powder (20 gm dry weight) and finally soaked overnight with sterile distilled water of 100ml. The extract was strained through two layers of muslin cloth, subsequently filtered through Whatman No: 1 filter paper and finally passed through Seitz filter to eliminate bacterial contamination. This formed the standard plant extract solution (100%) (24). This extract was further diluted to the desired concentration by adding requisite quantities of sterile distilled water. All the extracts were used at 100% concentration for screening antifungal activity against *S. oryzae* under *in vitro*. The plant species showed effectiveness in the preliminary screening alone were further diluted to different concentrations (5 and 10%) and tested against *S.oryzae*.

Efficacy of plant extracts on radial growth of *S. oryzae* (Poisoned food technique): Efficacy of ten plant extracts were tested on the growth of *S. oryzae* by using poisoned food technique (25). The mycelial growth of the fungus was measured after 15 days. The per cent inhibition of mycelial growth was calculated.

Compatibility between effective isolates of *Fluorescent pseudomonads* (PfS5) and *Bacillus* (BsA1): The effective isolates of *Fluorescent pseudomonads* and *Bacillus* were tested for their compatibility among each other following the method of Fukui *et al*., 1994. The compatibility was determined for *Fluorescent pseudomonads* and *B.subtilis* isolates using NA medium. The bacterial strains were streaked horizontally and vertically to each other. The plates were incubated at room temperature (28±2°C) for 72 h and observed for the inhibition zone. Absence of inhibition zone indicates the compatibility with respective bacterial strains and the presence of inhibition zone indicated the incompatibility.

Compatibility between effective *Fluorescent pseudomonads* (PfS5), *Bacillus* (BsA1) and *Eucalyptus* leaf extract: The compatibility test was performed by using the paper disk diffusion method. Each bacteria were grown on Tryptic Soy Broth (TSB, HiMedia, India) medium for 24 h at 30°C. A total of 200 µl of bacterial suspension was then grown on Tryptic Soy Agar (TSA, HiMedia, India) medium in a Petri dish with a diameter of 9 cm. After that, 300 µl of the *Eucalyptus* leaf extract suspension was dropped onto a sterile filter paper with a diameter of 5 mm. Combinations are compatible if no clear zone is formed. The concentration of *Eucalyptus* leaf extract used was 10%, sterile distilled water was used as a negative control, and 70% ethanol was used as a positive control. Each treatment was replicated four times (27).

Consortia of selected bio agents and plant extracts on the incidence of rice sheath rot disease

A pot culture experiment was conducted during Rabi (Oct-March) 2021-2022 at Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalainagar to test the efficacy of consortium of *Fluorescent pseudomonads* (PfS5), *B. subtilis* (BsA1) and *Eucalyptus* leaf extract for assessing their influence on the incidence of sheath rot of rice. Seed treatment with *P. fluorescens* (PfS5) and *B. subtilis* (BsA1) were described in earlier methods. Whereas, two gram of *Eucalyptus* powder was suspended in 20 ml sterile distilled water in a 50 ml conical flask to obtain a 10% (w/v) concentration (28). Treated seed were sown in nursery beds as per the treatment given below,

Treatment schedule

T1- *Fluorescent pseudomonads* (PfS5ST)
T2- *B.subtilis* (BsA1-ST)
T3- *E.globules extract* (ST)
T4- *Fluorescent pseudomonads* (PfS5-FA)
T5- *B.subtilis* (BsA1-FA)
T6- *E.globules extract* (FA)
T7- T1+T2+T3
T8- T4+T5+T6
T9- T7+T8
T10- Carbendazim 50%, WP@2g/kg of seed (ST); 0.1%(FA)
T11- Control

Rice seeds (variety RNR15048) treated with consortium of *Fluorescent pseudomonads* (PfS5) and *B. subtilis* (BsA1) along with *Eucalyptus* powder on 30 days old rice seedlings were transplanted in cement pots @ six hills per pot. The upper most flag leaves of tillers at the booting and the panicle emerging stages were inoculated with *S. oryzae* isolates by the standard grain inoculum technique (12). The crop was maintained in a poly house with frequent spraying of water to provide adequate moisture and relative humidity to enable successful infection by the pathogen. The experiment was conducted in a Randomized Block Design with three replications for each treatment and a suitable control. The seeds treated with fungicide Carbendazim 50% WP @ 2g/kg of seed; 0.1 per cent for foliar application was used for comparison and the standard agronomic practices as recommended by the TNAU were followed. Treatments were given as per the schedule and observations on lesion size, number of infected/discolored grains, sheath rot disease incidence (PDI) and biometrics viz., plant height at 30, 60 and 90 DAT, number of tillers per hill, grains per panicle and yield (g) per pot were recorded at the time of harvest following standard procedures. The incidence of sheath rot disease was recorded at 14 days after inoculation using of 0-9 scale proposed by Vivekananthan *et al*., 2005.

RESULTS AND DISCUSSION

***In vitro* inhibition of mycelial growth of *S. oryzae* (So5) by native *Fluorescent pseudomonads* isolates:** The results presented in the table 1 revealed varying degree of antagonism by the bacterial isolates against *S. oryzae*. Among the isolates tested, isolate- Pf S5 recorded the maximum inhibition zone of 10.55 mm and a minimum of 23.40 mm mycelial growth of *S. oryzae* accounting for 74.00 per cent reduction in the mycelial growth over control. This was followed by isolate-Pf An2 (26.07 mm). The least mycelial growth inhibition was observed with the isolate- Pf K3. Similar observations were made by Sampada *et al*., 2017 reported that *P. fluorescens* (Pf8) resulted maximum growth reduction of *S. oryzae* by 80 per cent over control which was followed by *P. fluorescens* (Pf5) which recorded the growth reduction of 76.58 per cent over control. Bora and Ali 2019 reported that all the microbial antagonists were found to be effective in inhibiting the radial growth of *S.oryzae.* Out of all the tested antagonists, *P. fluorescence* showed highest (82.06%) inhibition of mycelial growth of the pathogen. The present study clearly indicated that the mycelial inhibition occurred due to production of antifungal metabolites produced by isolate- Pf S5 which caused inhibition on the growth of *S. oryzae.* .Moreover, *Pseudomonas* can also synthesize enzymes which may modulate the plant hormone levels, limit the available iron by production of siderophores and can also kill the pathogen by producing antibiotics such as Phenazine, 2,4-diacetylphloroglucinol (DAPG), Pyrrolnitrin and Pyoluteorin Pyrrolnitrin (31)(32); volatile compounds such as hydrogen cyanide (HCN)(33)(34); siderophores and biofilms (35)(36). The above results lend support to the present findings.

***In vitro* inhibition of mycelial growth of *S. oryzae* (So5) by native *Bacillus* isolates:** The results presented in the table 2 revealed varying degree of antagonism by the bacterial isolates against *S. oryzae*. Among the isolates tested, isolate- BsA1 recorded the maximum inhibition zone of 9.92 mm and a minimum of 24.62 mm mycelial growth of *S. oryzae* accounting for 72.64 per cent reduction in the mycelial growth over control. This was followed by isolate-BsS5 (08.25, 27.70 mm). The least mycelial growth inhibition was observed with the isolate- BsAn2. Similarly, Gopalakrishnan and Valluvaparidasan 2006 (37) suggested that, *B. subtilis* isolate 9 was found to be highly effective in inhibiting the mycelial growth of *S. oryzae* by 82.18 per cent. *B. subtilis* strain BTK1 was confirmed by 16s rRNA and it showed effective in inhibiting mycelial growth of pathogen (75%). Moreover, volatile compounds emitted from the *Bacillus* spp. were found to inhibit the mycelial growth of *S. oryzae*. (15). *B. subtilis* strains are known to synthesize antibiotic lipopeptides, including fengycin, surfactin, and iturin (16)(38). Surfactants and antimicrobial compounds produced by *B. subtilis* are receiving more attention. Lipopeptide genes occur in many species and strains of bio-control agents and some with enhanced capacity to produce antibiotics and limit fungal root pathogens have been commercialized (39).

Table 1. In vitro efficacy of *Fluorescent pseudomonads* isolates against *S.oryzae* (So5)

Nativeisolates	Place of collection	*S. oryzae*		
		Linear growthof pathogen (mm)	Per cent inhibition over control	Inhibitionzone (mm)
Pf A1	Ambigapuram	29.60[bc]	67.11	8.98
Pf An2	Annamalainagar	26.07[b]	71.03	9.25
PfK3	Kalambur	32.00[d]	64.44	7.55
Pf Kp4	Krishnapuram	31.00[c]	65.55	8.09
Pf S5	Sivapuri	23.40[a]	74.00	10.55
Control		90.00[e]	0.00	0.00
SE(d)	-	0.799	-	-
C.D(0.05)		2.013	-	-

Values are mean of three replications; Values in the column followed by same letters not differ significantly by DMRT(p=0.05)

Table 2. In vitro efficacy of *B. subtilis* isolates against *S.oryzae* (So5)

Nativeisolates	Place of collection	*S. oryzae*		
		Linear growthof pathogen (mm)	Per cent inhibition over control	Inhibitionzone(mm)
BsA1	Ambigapuram	24.62[a]	72.64	9.92[a]
BsAn2	Annamalainagar	34.01[e]	62.21	5.35[e]
BsK3	Kalambur	32.00[d]	64.41	6.51[d]
BsKp4	Krishnapuram	29.33[c]	67.41	7.02[c]
BsS5	Sivapuri	27.70[b]	69.22	8.25[b]
Control		90.00[f]	0.0	0.0[f]
SE(d)	-	0.473	-	0.165
CD(0.05)	-	1.418	-	0.179

Values are mean of three replications; Values in the column followed by same letters not differ significantly by DMRT(p=0.05)

Table 3. Antifungal activity of plant extracts against *S.oryzae* (So5)

S.No	Plant extracts	Vernacular name	Parts used	Linear growth of pathogen(mm)	Inhibition (%)
1	*Abutilon indicum*	Thuthi	Leaves	37.77[d] (37.92)	58.03
2	*Acalipha indica*	Kuppaimeni	Leaves	40.85[f] (39.72)	54.61
3	*Allium cepae*	Vengayam	Bulb	27.24[b] (31.46)	69.73
4	*Aloe vera*	Katralai	Leaves	48.57[i] (44.18)	46.03
5	*Azadiracta indica*	Vembu	Leaves	43.98[g] (41.54)	51.13
6	*Carica papaya*	Papaya	Leaves	39.62[e] (39.00)	55.97
7	*Eucalyptus globules*	Eucalyptus	Leaves	19.03[a] (25.86)	78.85
8	*Lantana camera*	Road side weed	Leaves	30.57[c] (33.56)	66.03
9	*Pongamia glabra*	Pungam	Leaves	46.14[h] (42.78)	48.73
10	*Solanum nigrum*	Blackberry nightshade	Leaves	49.95[j] (44.97)	44.50
Control	-	-	-	90.00[k] (71.56)	0.00
SE(d)	-	-	-	0.411	-
CD(0.05)	-	-	-	1.62 9	

Values are mean of three replications; Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

Table 4. Efficacy of antagonistic formulation and plant extract against sheath rot disease and growthpromotion in rice seedling under pot culture conditions

T. No	Treatments	PDI (%)	Reductionin PDI	Growth promotion					Per cent increase in yield (%)
				Plant height at harvest (cm)	No. of tillers/hill	No. of grains /panicle	1000 gram weight (g)	Grain yield (g/plant)	
T1	*Fluorescent pseudomonads (PfS5*ST)	30.65[f](37.35)	47.31	84.25[de]	11.03[f]	116.32[d]	20.84[f]	11.22[ef]	70.65
T2	*B. subtilis (BsA1-ST)*	32.35[g](38.51)	43.97	83.89[f]	10.79[g]	99.39[e]	20.21[g]	10.83[g]	68.67
T3	*E. globules* extract (ST)	37.32[i](41.83)	32.34	76.34[g]	9.39[i]	81.30[g]	19.59[h]	9.75[g]	61.56
T4	*Fluorescent pseudomonads (PfS5*-FA)	24.50[d](32.96)	57.56	86.23[d]	12.89[d]	121.09[d]	21.98[d]	12.05[d]	71.94
T5	*B.subtilis (BsA1-FA)*	28.22[e](35.65)	51.98	85.78[d]	11.65[e]	119.67[d]	21.01[e]	11.56[de]	71.23
T6	*E. globules* extract (FA)	34.88[h](40.22)	38.76	81.87[e]	10.18[h]	89.80[f]	19.98[gh]	10.13[g]	66.67
T7	T1+ T2 +T3	20.52[c](29.92)	61.34	97.12[c]	13.29[c]	130.97[c]	22.52[c]	13.76[c]	72.29
T8	T4+ T5+ T6	18.48[b](28.28)	65.86	101.98[b]	14.55[b]	139.30[b]	23.59[bc]	15.98[b]	74.56
T9	T7+T8	12.53[a](23.03)	70.38	109.68[a]	15.35[a]	145.90[b]	24.22[b]	18.39[a]	78.30
T10	Carbendazim @0.1% (FA), 2g/kg (ST)	12.41[a](22.91)	70.35	109.63[a]	15.29[a]	145.81[a]	24.20[a]	18.37[a]	78.28
T11	Control	46.25[j](47.61)	0.00	71.33[h]	8.55[j]	58.11[h]	19.35[i]	8.26[h]	0.00
	SE(d)	0.976		0.994	1.876	0.854	0.356	0.299	
	CD(0.05)	1.421	-	1.721	4.902	1.078	0.640	0.549	-

ST- Seed Treatment, FA- Foliar application, PDI- Percent Disease Index; Values are mean of three replications; Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

B. subtilis strains PCL1608 and PCL1612 produce a high level of antibiotics, especially iturin A which serves as the principal mechanism underlying the control of soil-borne pathogens (40). These earlier reports corroborate with the present observations.

Antifungal activity of plant extracts against *S.oryzae* (So5)
In general, all the plant extracts were recorded appreciable amount of reduction in mycelial growth of pathogen. Among the ten plant extracts tested (10% concentration) against the mycelial growth of *S.oryzae*, *E. globules* extract recorded the minimum mycelial growth (19.03 mm) and maximum per cent reduction of mycelial growth of pathogen (78.85%) over control. This was followed by *Allium cepae* and *Lantana camera* extract (27.24 mm, 69.73%; 30.57 mm, 66.03%). The lowest per cent inhibition of pathogen was observed by *Solanum nigrum* extract (49.95mm, 44.50%). Similarly, Meera and Balabaskar 2012 reported that *Eugenia caryophyllata* and *Eucalyptus globules* at 10% concentration were observed to be the most effective in inhibitory the mycelial growth, biomass production, spore germination and germ tube length of *S. oryzae*. Antifungal activity of *E. globules* may be due to the presence of eucalyptin, eucalyptol and elligatannin componds (41). Besides several workers have studied the efficacy of various plant extracts against *S. oryzae* (42)(43)(44)(18). All these earlier reports lend support to the present investigations (Table 3).

Efficacy of antagonistic formulations and plant extract against sheath rot disease and growth promotion in rice seedling under pot culture conditions: In the pot culture studies, seed treatment with *Fluorescent pseudomonads (PfS5*) @12g/kg plus *B. subtilis (BsA1)* @12g/kg plus *E.globule*s extract@10% followed by foliar application with *Fluorescent pseudomonads (PfS5*) and *B. subtilis (BsA1)* each @ 0.5% plus *E. globule*s extract@10%-T9 at 45 and 60 DAT recorded minimum sheath rot incidence(12.53), maximum plant growth (109.68 cm), number of tillers per hill (15.35), number of grains per panicle (145.90),weight of 1000 of seeds (24.22g) and yield (18.39g/plant) and it was on bar with chemical treatment with sheath rot incidence (12.41),plant growth (109.63cm), number of tillers per hill(15.29), number of grains per panicle (145.81), weight of 1000 of seeds (24.20g) and yield (18.37g/plant) .This was followed by T8 –foliar application with *Fluorescent pseudomonas (PfS5*) and *B. subtilis (BsA1)* each @ 0.5% plus *E. globule*s leaf extract@10%-T8 at 45 and 60 DAT recorded sheath rot incidence (18.48), plant growth (101.98 cm),number of tillers per hill (14.55),number of grains per panicle (139.30),weight of 1000 of seeds (23.59g) and yield (15.98g/plant)).In control plants recorded maximum sheath rot incidence (46.25) with minimum plant growth parameters of height (71.33 cm),number of tillers per hill (8.55),number of grains per panicle (58.11),weight of 1000 seeds (19.35g) and yield (8.26g/plant) (Table 4). The combination of *Pseudomonas* strains Pf1, TDK1 and PY15 reduced sheath rot disease in rice by 83% (45). Sundaramoorthy *et al*., 2013 reported that foliar application of *P.fluorescens and B.subtilis* effectively reduced sheath rot disease incidence. All these earlier reports collaborates with the present findings. Similar to present study Prakash *et al*., 2005 reported that Eugenol (1- hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *Ocimum Sanctum*, as largely responsible for the therapeutic potentials of Tulsi. Likewise, chemical composition of essential oil from *O. gratissimum* had been studied by Nguefack *et al*., 2007, who attributed the high content of thymol for its effectiveness. The ability of *P.fluorescens* strains to increase plant growth and yield in various crops has been well established(49)(50). Meera *et al*., 2013 reported that *P.fluorescens* as seed treatment plus foliar spray of *E. globules* @12% conc. recorded maximum disease suppression and enhanced plant growth and yield. Several studies also confirm that plant extracts could be used for managing plant diseases with success. Chaliganjewar *et al*., 2010 reported that extract of *Kalanchoe pinnata* effectively reduced sheath rot disease incidence with highest healthy spikelets and grains.

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