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Management of rice sheath blight caused by *Rhizoctonia solani* Kuhn. using environmentally friendly method-an alternative to fungicides

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ABSTRACT

Paddy is the second crop where huge amounts of chemicals are used for production, majorly for disease control. To avoid excess use of chemicals in the rice fields and also to find the best alternative solution to fungicides for sheath blight disease caused by *Rhizoctonia solani*, research was carried out by using various bioagents, plant extracts and synthetic bio-polymers. Prior to the field experiments, *in vitro* studies were carried out to check the efficacy of various bio agents and plant extracts by using dual culture and poisoned food techniques, respectively. *Trichoderma harzianum* showed the highest mycelial growth inhibition (60.37%), followed by *Pseudomonas fluorescens* (54.4%), and the least effective was showed by *T. asperellum* (37.44%). The field experiments were carried out in *Kharif* 2019-20 and 2020-21, to test the fruitfulness of *in vitro* studies along with some biopolymers. In *Kharif* 2019-20 out of all the treatments, effective treatment with least mean disease severity was observed in chitosan (12.50%) and nano silicon (14.79%) application when compared to the fungicide hexaconazole 5 EC (13.89%) which was used as a positive control and the least effective treatment was observed in seaweed extract (18.81%), followed by *T. viride* (19.0%) whereas, the untreated control showed 28.73% of disease severity. In *Kharif* 2020-21 chitosan (18.77%) and nano silicon (23.95%) treatments were effective when compared to the hexaconazole 5 EC (23.21%) and highest disease severity was observed in seaweed extract (37.0 %), followed by *T. viride* (34.81%), whereas the untreated control showed 51.6 % of mean disease severity.

Key words: *Rice, Sheath blight, Disease severity, Bioagents, Biopolymers*

Introduction

For most people living in Asia, rice (*Oryza sativa* L.) is life. It has shaped the cultures, diets, and economies of thousands of millions of people (Gnanamanickam, 2009). Rice is anticipated to continue to be the major human staple food crop well into the 21st century. Plant diseases are one of the major constraints in achieving the potential yield of rice. Therefore, it is necessary to think about the rice security for the generations of the next decades. And, to meet the demand (Zeigler *et al.*, 1994). The annual losses due to rice diseases are estimated to be 10–15% on an average basis worldwide. Therefore,

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judicious management of rice diseases can result in improved productivity and additional grain harvested. Rice diseases are caused by a wide variety of pathogens, including fungus, bacteria, viruses, and nematodes (Ling, 1980).

Quantified annual yield losses based on surveys due to a combination of rice diseases ranged from 1 to 10% in Asia (Rice Diseases Workshop, 2014). The major rice diseases that often cause great economic losses are rice blast (*Magnoporthe grisea*), sheath blight (*Rhizoctonia solani*), bacterial blight (*Xanthomonas oryzae*) and *Tungro* virus disease, especially in South and South-east Asia (Ling, 1980).

Rhizoctonia solani causes *s*heath blight disease, which is responsible for up to 25% yield loss in rice when the disease is extended up to the flag leaves and in the range of 30–40% in the case of severe infection of the sheath and leaf blades (Annou *et al.*, 2005).The various methods used for managing this disease include the use of resistant varieties, cultural practices, and biological and chemical control. All these methods have varying degrees of success in managing rice diseases. The most important control tactics used worldwide include the use of resistant varieties and chemical control. But it is difficult to breed a completely resistant variety to this pathogen because of its polyphagous nature, so most of the management is done through chemicals (Huber and Thompson, 2007).

The volume of pesticides used to rice fields is substantial due to the vast area under rice cultivation around the world. The amount of pesticides applied to rice fields is extremely high, and the repeated use of chemicals is a known cause of disease resistance, residual toxicity, habitat pollution, human and animal health risks, as well as the rise in plant protection costs. As a result, other management practises should be prioritised. Management practices that address adverse effects of pesticide used in rice fields include increased adoption of integrated pest management principles and less toxic products (Lee and Rush, 1983).

Current research was conducted with the goal of developing new, safe, cost-effective, long-lasting, sustainable, and effective biological methods for the management of sheath blight disease in rice under field conditions, taking into account the issue of chemical dumping in rice and its hazardous effects on the environment.

Materials and Methods

The study was conducted in the Department of Plant Pathology, College of Agriculture, V.C. Farm, Mandya, University of Agricultural Sciences, Bangalore during *Kharif* 2020 and 2021. The *Rhizoctonia solani* and different bioagents maintained as pure culture in the Dept. of Plant Pathology, V.C. Farm, Mandya, listed in Table 1, were collected and used for the study. The bioagents were tested *In vitro* against *R. solani* using the dual culture procedure. The Poisoned Food Technique was used to test phyto-extracts. Fresh, healthy plant components from eleven plant species of various families were collected from the college premises as listed in Table 2. In both studies, each treatment was repeated three times, and the Petri plate without addition of plant extracts served as a control. Radial mycelial growth was recorded which was measured every 48 hours until the colony in the control plate was completely covered with pathogen mycelium. In both studies, percent growth inhibition (PGI) was calculated by using the formula given by Vincent (1947).

Table 1. List of bioagents used for *in vitro* evaluation against *R. solani*

Sl. No.	Bioagents
1	*Trichoderma viride*
2	*Trichoderma harzianum*
3	*Trichoderma asperellum*
4	*Bacillus subtilis*
5	*Bacillus pumilis*
6	*Pseudomonas fluorescens*

The field experiments (*Kharif* 2019-20 and *Kharif* 2020-21) were laid out in a randomised complete block design (RCBD) with ten treatments, replicated three times. The inoculum of *R. solani* was applied to each plot uniformly. The culture was mass multiplied on sorghum grains (Kumar *et al.*, 2018). Two weeks after amending the plots, 25-day old KMP 101 (Thanu) seedlings were transplanted at a spacing of 20×10 cm. Except for treatments; all other recommended packages of practises were followed for growing the crop. All treatments were applied through foliar application at 25 and 50 DAT. Fungicide Hexaconazole 5 EC, known to control sheath blight disease, was included as a positive control. Eight plants were randomly selected from the net plot and per cent disease severity was recorded at 30, 60 and 90 DAT by using a field key 0-9 scale by following the standard evaluation system (SES) as per IRRI (1996) for sheath blight of rice. These scales were converted to per cent disease index (PDI) by using the formula given by Wheeler (1969). Observation on growth and yield parameters were recorded at harvest then yield and benefit cost ratio was expressed.

Results and Discussion

Dual Culture

T. harzianum containing plates had the highest mycelial inhibition (60.37 percent) in the dual culture technique, followed by *B. pumilis* (54.4 percent), *T. viride* (53.70 percent), and *P. fluorescens* (51.49 percent). The least inhibition was showed by*T. asperellum* (39.44 percent) and *B. subtilis* (42.59 percent)(Figure 1). Singh *et al.* (2015) published similar findings, stating that several *Trichoderma*, *Bacillus* and *Pseudomonas species* inhibited *R. solani in vitro*, mostly through antifungal activity when they come into contact with the pathogen or by the generation of persistent secondary metabolites.

Poisoned food technique

After four days of incubation, the results of the poisoned food technique indicated that all of the botanicals studied at four concentrations (1, 5, 10 and 20%) significantly reduced *R. solani* mycelial development when compared to the control. At a concentration of 1%, garlic extract showed highest inhibition (21.1%), followed by brahmi (18.8%), seaweed (16.67%), and fenugreek (4.7%) and *Phyllanthus niruri* (5.3 percent). At a concentration of 5 per cent, seaweed extract showed maximum inhibition (93.5%), followed by garlic (45 %) and fenugreek (19.74 %) and lowest inhibition was showed by ginger (5.3 %), followed by aloevera (6.6 %) and cyprus (6.8 %). At 10 per cent concentration, highest mycelium inhibition was observed in garlic extract (94.44 %), followed by seaweed (91.9 %), fenugreek (27.0 %) and pudina (21.1 %) and least inhibition was showed by ginger (5.29 %) andbrahmi (8.14%).At 20 per cent concentration seaweed and garlic extracts totally suppressed mycelial development (100 %), followed by fenugreek (45 %), drumstick (44.4 %) and periwinkle (32.2 %) and least inhibition was observed in ginger (6.2 %), followed by *Phyllanthus niruri* (9.7 %)(Figure 2).

Field Experiments

Field experiment was conducted during *Kharif* 2019-20 to test the efficacy of the treatments which showed the best results under *in vitro* studies, *viz.*, *T. harzianum*, *T. viride*, *B. subtilis*, *B. pumilis*, *P. fluorescens* and seaweed extract (*Kappaphycus alverezii*), along with biopolymers chitosan and nano silicon and as a positive control one synthetic fungicide (hexaconazole 5 EC) was used. Each treatment's foliar spray was taken at 25, 50 and 90 DAT and disease severity was observed at 30, 60 and 90 DAT the same experiment was repeated in *Kharif* 2020-21 for the confirmation of the results. Details of the treatments and sheath blight severity observed are given in the Table 3.

Sheath blight severity

In *Kharif* 2019-20, all the treatments were shown to be more successful than the untreated control in reducing the severity of sheath blight. On the basis of mean disease severity and per cent reduction over control, treatment foliar application of 0.1 per cent

Table 2. List of plant extracts used for *in vitro* evaluation against *R. solani* of rice

Sl. No	Botanical name	Common Name	Family	Plant parts used
1	*Catheranthus roseus*	Periwinckle	*Apocynaceae*	Leaves
2	*Mentha arvensis*	Pudina	*Lamiaceae*	Leaves
3	*Trigonella foenumgraecum*	Fenugreek	*Fabaceae*	Leaves
4	*Zingiber officinale*	Ginger	*Zingiberaceae*	Rhizome
5	*Centella asiatica*	Brahmi	*Apiaceae*	Leaves
6	*Moringa oleifera*	Drumstick	*Moringaceae*	Leaves
7	*Cyprus rotundus*	Cyprus	*Cyperaceae*	Tuber
8	*Aloe vera*	Aloevera	*Asphodelaceae*	Leaves
9	*Allium sativum*	Garlic	*Amaryllidaceae*	Bulbs
10	*Phyllanthus niruri*	Nela nalli	*Phyllanthaceae*	Leaves
11	*Kappaphycus alverezii*	Sea weed (Red algae)	-	-

chitosan (T_8) was found to be most effective (12.50 % and 56.49 %) than 0.1 per cent hexaconazole 5 EC (T_9) (13.89 % and 51.65 %), followed by nano silicon at 0.1 % (T_7) (14.79 % and 48.52 %) and *Trichoderma harzianum* (T_4) (15.71 % and 45.31 %) and the least effective treatment was seaweed extract @ 0.1 % (T_6) (18.81% and 34.52 %), followed by *Trichoderma viride* (T_5) (19.0 % and 33.86 %) and *Pseudomonas fluorescens* (T_2) (18.95 % and 34.04 %) whereas (T_{10}) untreated control showed 28.73 per cent mean severity.

In *Kharif* 2020-21, all the treatments showed the same trend with respect to disease severity. Foliar application of 0.1 per cent chitosan (T_8) was found to be most effctive (18.77 % and 63.64%) than 0.1 per cent hexaconazole 5 EC (T_9) (23.21 % and 55.02 %), followed by nano silicon at 0.1 % (T_7) (23.95 % and 53.59 %) and *Trichoderma harzianum* (T_4) (24.44 % and 52.63%) and the least effective treatment was seaweed extract @ 0.1 % (T_6) (37.00 % and 28.35%), followed by *Trichoderma viride* (T_5) (34.81% and 32.54 %) and *Pseudomonas fluorescens* (T_2) (44.2% and 14.35 %) whereas (T_{10}) untreated control showed 51.60 per cent mean severity.

Divya *et al.* (2020) employed chitosan nanoparticles as a foliar spray on rice in greenhouse environments, they had a similar result. Sheath blight disease could be reduced by as much as 75%. To suppress the *Rhizoctonia* disease complex in beets under greenhouse conditions, Gooday (1990) used silicon nanoparticles for seed priming and as a foliar spray at two concentrations (100 and 200 mg/l), with the 200 mg/l concentration demonstrating the greatest disease reduction.

The disease-suppressing characteristics of the chitosan polymer may be linked to the activation of chitinase enzymes in plants, which causes the breakdown of the primary fungal component chitin (Han *et al.*, 2004). It boosts the activity of phytoalexin enzymes in plants, such as PAL and chitinase, which helps with microbial disease prevention and plant growth (Luan *et al.*, 2006). Rice plants have been demonstrated to develop systemic resistance to *R. solani* and other diseases when exposed to chitosan particles. Chitosan had control over a number of defense-related genes and was responsible for activating defence responses, which could explain why the hexaconazole 5EC had a greater control on sheath blight severity.

Influence of treatments on plant height

In *Kharif* 2019-20, there was a significant difference between the treatments. Foliar application of 0.1 per cent chitosan (T_8) treated plants had the highest plant height and per cent increase over control (110.23 cm and 9.34 %), which was higher than the chemical hexaconazole 5 EC (T_9) treated plot (108.03 cm and 8.02 %) followed by the nano silicon (T_7) (108.17 cm and 7.78 %) and the least was recorded in *Pseudomonas fluorescens* (T_2) (101.56 cm and 1.74 %), *B. pumilis*(T_3) (102.79 and 2.91 %) and seaweed (T_6) (105.27 and 5.41 %) as depicted in Figure 3.

The same trend was observed in *Kharif* 2020-21, foliar application of 0.1 per cent chitosan (T_8) treated plants had the highest plant height and per cent increase over control (118.93 cm and 14.73 %), which was higher than the chemical hexaconazole 5 EC (T_9) treated plot (117.47 cm and 11.75 %) followed by the nano silicon (T_7) (114.33 cm and 10.29%) and the least was recorded in *B. pumilis* (T_3) (104.47 and 0.77 %), followed by *Pseudomonas fluorescens* (T_2)

Table 3. Effect of treatments on sheath blight severity in rice under field condition

Treatment No.	Treatment details	*Kharif* 2019-20		*Kharif* 2020-21	
		Mean disease severity	Per cent reduction over control	Mean disease severity	Per cent reduction over control
T_1	*Bacillus subtilis* at 5g/l	18.30	36.29	43.21	16.27
T_2	*P. fluorescens* at 5g/l	18.95	34.04	44.20	14.35
T_3	*B. pumilis* at 5g/L	18.15	36.82	42.22	18.18
T_4	*T. harzianum* at 5g/l	15.71	45.31	24.44	52.63
T_5	*T. viride* at 5g/l	19.00	33.86	34.81	32.54
T_6	Seaweed extract at 1ml/ l	18.81	34.52	37.00	28.35
T7	Nano silicon at 2ml/ l	14.79	48.52	23.95	53.59
T8	Chitosan at 1ml/ l	12.50	56.49	18.77	63.64
T_9	Hexaconazole 5EC at 1ml/ l	13.89	51.65	23.21	55.02
T_{10}	Untreated control	28.73	0.00	51.60	0.00

(105.67cm and 1.89%) and seaweed (T_6) (106.80 and 2.93%) (Figure 3).

Islam *et al.* (2016) recorded a similar pattern that foliar application of chitosan at 50, 75, 100 and 125 ppm on soyabean and rice in green house and field condition. They found that the plants treated with chitosan had the highest plant height and dry matter. The increase in vegetative growth in chitosan treated plants could be due to increased activity of key enzymes activities of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease), which are responsible for plant growth and development (Ke *et al.*, 2001). Chitosan and nano silicon were also reported to boost plant growth *via* stimulating auxin and gibberellin signalling pathways.

Effect of treatments against rice sheath blight on yield parameters

The yield parameters and B:C ratio of different treatments are represented in Table 4. During *Kharif* 2019-20, on the basis of test weight (g) and per cent chaffiness, the foliar application of 0.1 per cent chitosan (T_8) was found to be more effective (16.01g and 7.6 %) than 0.1 per cent hexaconazole 5 EC (T_9) (18.26 gm and 8.0 %) followed by 0.2 per cent of nano silicon (T_7) (18.14 g and 8.67%) and 0.5 per cent *T. harzianum* (T_4) (17.74 gm and 9.0 %) and the least effective treatment was found to be *P. fluorescens*(T_2) (15.97 g and 13.6%), followed byseaweed extract (T_6) at 0.2 per cent (16.01 gm and 12.27 %) compared to the untreated control (T_{10})(13.50 g and 15.33 %). Significant difference was observed among the treatments with respect to grain yield. Foliar spray of 0.1 per cent chitosan (T_8) was recorded highest per hectare straw and grain yield (36091.75 kg and 5350.0 kg) followed by the chemical hexaconazole 5 EC treatment (T_9) (33789.65 Kg and 5157.3 Kg), nano silicon (T_7) (36185.50 Kg and 4666.6 Kg) and *T. harzianum* (T_4) (32310.5 Kg and 4644.17 Kg) and the least yield was recorded in the seaweed extract treatment (T_6) (28529.25 Kg and 4082 Kg) followed by *P.fluorescens* (T_2) treatment (30000.1g and 4150 kg).

During *Kharif* 2020-21, test weight (g) and per

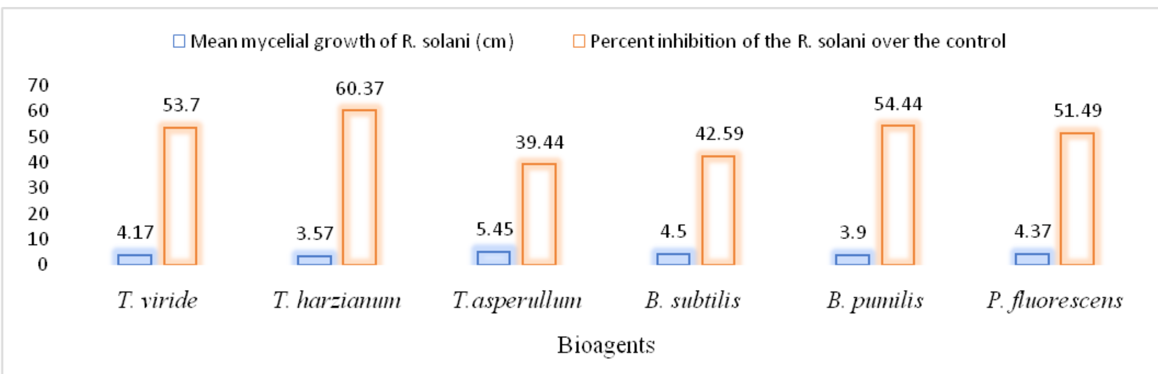
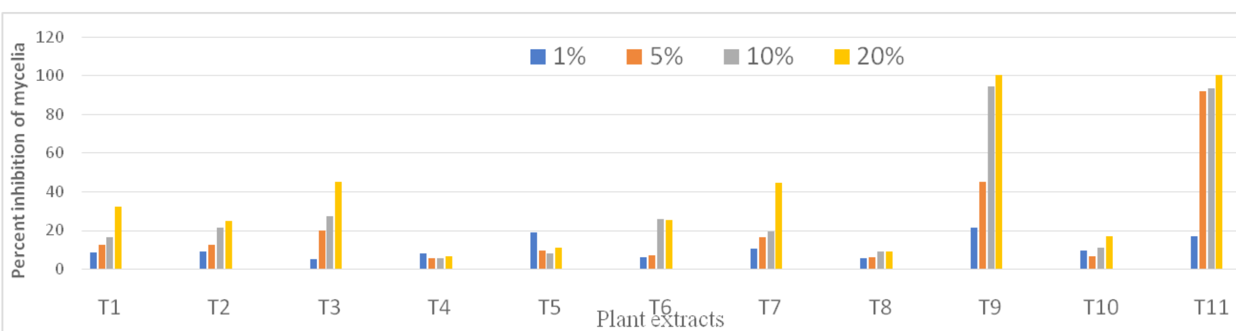


Fig. 1. Efficacy of bio agents against mycelial growth of *R. solani* of rice



T_1- *Catheranthus roseus* T_2- *Mentha arvensis* T_3- *Trigonella foenumgraecum* T_4- *Zingiber officinale* T_5- *Centella asiatica* T_6- *Cyprus rotundus* T_7- *Moringa oliefera* T_8- *Phyllanthus niruri* T_9- *Allium sativum* T_{10}- *Aloe vera* and T_{11}-*Seaweed extract*

Fig. 2. Efficacy of plant extracts against *R. solani* under *in vitro* condition

Table 4. Effect of treatments on rice yield parameters under field conditions

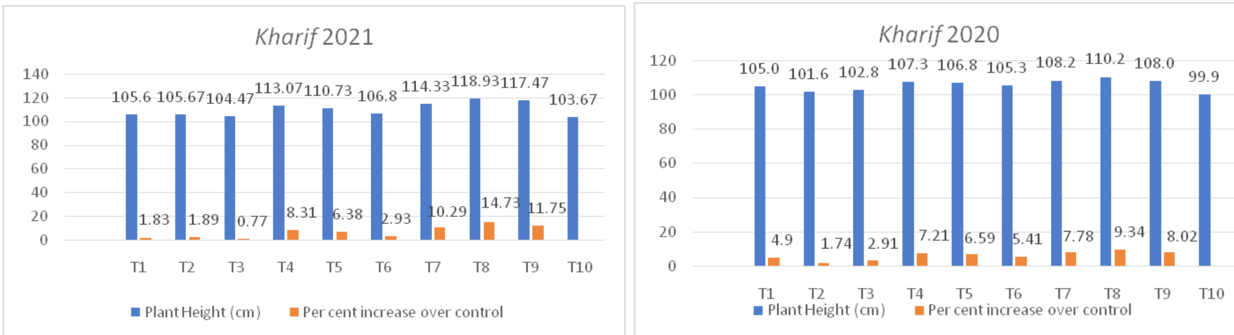
Treatment details	*Kharif* 2019-20					*Kharif* 2020-21				
	Test weight (gm)	Per cent chaffiness	Per hectare yield (Kg)		B: C ratio (gm)	Test weight	Per cent chaffiness	Per hectare yield (kg)		B: C ratio
			Straw	Grain				Straw	Grain	
T1:(*Bacillus subtilis* at 5g/l)	16.83	10.17 (18.61)	35331.21	4525.00	2.04	18.92	8.00 (16.44)	35358.34	4367.00	2.20
T2 : (*P. fluorescens* at 5g/l)	16.97	13.60 (21.65)	30000.01	4150.83	2.06	18.60	11.00 (19.38)	32308.31	4260.00	2.09
T3 : (*B. pumilis* at 5g/l)	17.94	12.83 (21.00)	33331.32	4312.50	2.12	18.54	13.00 (21.15)	31358.32	4210.00	2.10
T4 : (*T. harzianum* at 5g/l)	17.74	9.00 (17.47)	32310.50	4644.17	2.07	20.07	7.33 (15.72)	28556.25	4490.00	2.03
T5 :(*T. viride* at 5g/l)	15.27	13.47 (21.54)	36050.08	4187.50	2.17	18.76	11.33 (19.68)	36077.09	4320.00	2.21
T6 ;(Seaweed extract at 1ml/ l)	17.01	12.27 (20.52)	28529.25	4395.83	2.14	17.10	10.00 (18.44)	32337.50	4211.00	2.09
T7 :(Nano silicon at 2ml/ l)	18.14	8.67 (17.13)	36185.50	4666.67	2.31	22.33	6.33 (14.58)	36212.50	4607.00	2.29
T8 : (Chitosan at 1ml/ l)	19.70	7.60 (16.01)	36091.75	5350.00	2.49	25.60	5.00 (12.93)	36118.75	5090.00	2.42
T9:(Hexaconazole 5EC at 1ml/ l)	18.26	8.00 (16.44)	33789.65	5157.33	2.35	24.09	6.00 (14.19)	33816.67	4697.00	2.26
T10 : (Untreated control)	13.50	15.33 (23.06)	29947.25	3125.00	1.73	13.65	13.00 (21.15)	23034.01	3421.00	1.66
S.Em ±	0.30	1.01	0.69	10.67		1.09	0.86	1.10	8.44	
C.D @ 5 %	0.88	2.95	2.02	31.19		3.18	2.51	3.22	25.32	

Note: Figures in the parenthesis are arcsine transformed values

cent chaffiness found to be high in the foliar application at 0.1 per cent chitosan (T_8) (25.60 g and 5.0 %) than 0.1 per cent hexaconazole 5 EC (T_9) (24.09 g and 6.0 %) followed by 0.2 per cent of nano silicon (T_7) (22.33 g and 6.33 %) and 0.5 per cent *T. harzianum* (T_4) (20.07 g and 7.33 %) and the least effective treatment was found to be *P. fluorescens*(T_2) *(18.60 g and 11.0%)*, followed byseaweed extract (T_6) at 0.2per cent (17.10 g and 10.0 %) compared to the untreated control (T_{10})(13.65 g and 13.0 %). Significant difference was observed among the treatments with respect to grain yield.

Foliar spray of 0.1 per cent chitosan (T_8) was recorded highest per hectare straw and grain yield (36118.75 kg and 5090.0 kg) followed by the chemical hexaconazole 5 EC treatment (T_9) (33816.67 Kg and 4697.0 Kg), nano silicon (T_7) (36212.50 Kg and 4607.0 Kg) and *T. harzianum* (T_4) (28556.25 Kg and 4490.0 Kg) and the least yield was recorded in the seaweed extract treatment (T_6) (32337.50 Kg and 4211.0 Kg) followed by *P.fluorescens* (T_2) treatment (32308.31 Kg and 4260.0 kg).

As per the report of Khan *et al.* (2009) and (Zeng and Luo, 2012), the benefit from the chitosan in enhancing yield characters are due to the increased rate of photosynthesis in leaves and stimulation of metabolism at seedling stage. The findings of the current investigation validated the Abdallah *et al.* (2020) reports, foliar spray of chitosan has a



T1-Foliar spray of *Bacillus subtilis* @ 5g/l, T2-Foliar spray of *Pseudomonas fluorescens* @ 5g/l, T3-Foliar spray of *Bacillus pumilis* @ 5g/l, T4-Foliar spray of *Trichoderma harzianum* @ 5g/l, T5-Foliar spray of *Trichoderma viride* @ 5g/l, T6-Foliar spray of 0.1 % Seaweed (Red algae- *Kappaphycus alverezii)*T7- Foliar application of 0.2 % of nano silicon, T8-Foliar application of 0.1 % Chitosan, T9-Foliar application of 0.1% Hexaconazole 5 EC and T10-Untreated control

Fig. 3. Effect of treatments on Plant height under field condition during *Kharif* 2020 and *Kharif* 2021

positive influence on yield and yield indices irrespective of grain type. Significant increase in yield components and it is also known to aid nitrogen transport in functioning leaves. The increase in grain yield in the chitosan treated plants is might be due to the stimulatory impact of chitosan on physiological processes, may account for the significant increase in plant growth and yield components and it is also known to aid nitrogen transport in functioning leaves (Gornik *et al.*, 2008).

Conclusion

The current study found that foliar application of several bio-pesticides effectively decreased disease and also having a bio-stimulating effect on plant growth and productivity. Although the bioagents *P. fluorescens*, *T. viride* and *B. pumilis*, as well as seaweed extract, are effective *in vitro* at controlling *R. solani*, they are ineffective in the field. In both the field studies, when compared to the fungicide Hexaconazole (T_9), which is commonly used to control this disease, the treatments Chitosan (T_8), Nanosilicon (T_7) and *T. harzianum* (T_4) were found to be more successful in controlling the disease. These biopesticides were also found to be cost-effective, with a cost-benefit ratio that was nearly equal to that of the chemical hexaconazole. All of these are biodegradable, non-hazardous to the environment, and cost-effective, thus they can be utilised as an alternative to synthetic chemicals for sheath blight management in rice.

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