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EVALUATION OF NUTRITIONAL AMENDMENTS, PLANT EXTRACTS AND CHEMICALS FOR THE MANAGEMENT OF STEM ROT OF RICE

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A B S T R A C T

Rice is the staple food of more than one third of world population. The emerging threat to this crop is the stem rot of rice. It is one of the dangerous disease causing huge losses to rice crop worldwide. *In-vitro* experiments were performed for evaluation of three plant extracts (*Eucalyptus globules*, *Azadirachta indica* and *Alstonia scholaris*) was done at three concentrations (10%, 5% and 2.5%) by poison food technique against the causal fungus, *Sclerotium oryzae.* Eucalyptus extract was found the most effective followed by neem and devil. Similarly, three fungicides, Thiophenate methyl 70% WP, Difenoconazole 10% WDG and Azoxystrobin 50% WDG were also tested at three concentrations (R, R/2 & R/4) and among these Thiophenate methyl 70%WP was the most effective followed by Difenoconazole 10% WDG and Azoxystrobin 50% WDG. The field trial based on 11 treatments comprising of organic nutritional amendments, plant extracts and fungicides was conducted using RCBD. It revealed that mixture of all organic nutritional amendments in combination with Thiophenate methyl fungicide showed significant response in the reduction of disease incidence. Moreover, these amendments also improved the plant growth and yield parameters i.e. plant height, panicle length (cm), number of tillers per plant, number of kernels per panicle, 1000-grain weight and yield/plant.

Keywords: Rice, Stem rot, *Sclerotium oryzae*

INTRODUCTION

Stem rot incited by *Sclerotium oryzae* Catt. is one of the most devastating disease which prevails in all rice growing areas worldwide (Konthojam *et al*., 2007). It was first reported on rice from Italy in 1876 (Cattaneo, 1877). Worldwide 5-80 % yield loss has been reported due to this disease (Gopika *et al*., 2016). In Pakistan stem rot was reported in 1970 with 72% yield losses from different areas of Punjab (Shafi, 1970).

S. oryzae is a soil-borne fungus that produces sclerotia with 170-580μm diameter (Park and Bertus, 1932) which are black at maturity and covered with cotton like mycelium (Cralley and Tullis, 1935). These sclerotia serve as primary source of inoculums and survive in the soil and crop residues up to six years (Krause and Webster, 1973).

Tillering and internodes elongation is the most susceptible stages of rice against stem rot disease (Krause and Webster, 1973). The fungus mainly affects the stem near soil line (Kumar *et al.,* 2003). Initially, it infects leaf sheath, followed by rottening of stem tissues and produce new sclerotia which release in soil at harvesting and cause infection to next season rice crop. Infection usually occurred at hollow internodes of stem (Keim and Webster, 1974; Bockus *et al*., 1978). Black angular lesions are produced just

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after tillering that causes the death of leaf sheath (Groth and Hollier, 2010).

The host plant resistance is the most valuable source of disease management but rice plant resistance against stem rot is rarely determined from all the rice producing countries of world (Raina *et al*., 1980; Cother, 1998; Cother and Nicol, 1999). The present study was aimed to find out the eco-friendly management strategies particularly based on organic nutritional amendments and plant extracts which decreases the use of chemicals.

MATERIALS AND METHODS

In-vitro evaluation of fungicides and plant extracts

Plant extracts preparation: The leaves of three plants i.e. *Azadirachta indica* (Neem), *Alstonia scholaris* (Devil's tree) and *Eucalyptus globolus* (Sufaida) were collected and thoroughly washed. The leaves were extracted in distilled water; 10grams of leaves were added in 100ml of distilled water and grinded in a grinder for preparation of standard/stock solution "S (10%)". This mixture was filtered using whatman No.1 filter paper (Odey *et al*., 2012).

Isolation of Pathogen*:* Stem rot diseased samples were collected from Rice Research Institute, Kala Shah Kaku (Pakistan). The samples were cut into small pieces of 3-5 mm^2 size and disinfected in 1.0% sodium hypochlorite solution followed by two washings in sterilized distilled water. The sterilized pieces were dried by placing on blotter paper and placed on potato dextrose agar (PDA) plates in laminar air flow chamber. These plates were incubated at 25±2ºC for 5-7 days and fungal growth was observed daily. Initially whitish or grayish black mycelial growth was observed which turned into black at maturity with the formation of black sclerotia.

Anti-fungal activity test of extracts: *In-vitro* evaluation of plant extracts was performed by using a standard poisoned food technique. Three concentrations (S, S/2 and S/4) of plant extracts were used. The concentration S/2 and S/4 were made from the standard solution "S". S/2 was half of standard while third S/4 was the quarter of standard. The mature sclerotia of the fungus were inoculated in the centre of the plate and incubated at 28±2˚C for 7-10 days. The radial growth of the desired fungus was recorded daily. Effects of three concentrations of three plant extracts were recorded on the basis of colony diameter and percent inhibition of pathogen by using following formula:

$$\%\ \text{inhibition} = \frac{\text{Diameter of colony in control} - \text{Diameter of colony in treatment}}{\text{Diameter of colony in control}} \times 100$$

The best proved extract was further evaluated in field condition separately as well as in-combination with other treatments.

Preparation of fungicidal formulations: Three fungicides Difenoconazole (10% WDG), Azoxystrobin (50% WDG) and Thiophenate methyl (70% WP) were tested against *Sclerotium oryzae.* Three doses/concentrations R, R/2 and R/4 of each fungicide were prepared for testing against *S. oryzae*. For preparation of dose R the recommended dose of fungicides was used. The recommended doses of Thiophenate methyl 70% WP, Azoxystrobin 50% WDG and Difenoconazole 10% WDG were 2g/L, 1g/L and 1.2g/L, respectively. The second dose R/2 was half of standard solution and the third dose R/4 was quarter of the recommended dose.

Anti-fungal activity test of fungicides: Poisoned food technique was used for the evaluation of three concentrations of three different fungicides. The mature Sclerotia of the fungus were inoculated in the centre of the plate and incubated at 28±2˚C for 7-10 days. The radial growth of the desired fungus was recorded daily. Colony diameter and percentage of inhibition of pathogen by using following formula:

$$\%\ \text{inhibition} = \frac{\text{Diameter of colony in control} - \text{Diameter of colony in treatment}}{\text{Diameter of colony in control}} \times 100$$

The best proved fungicide was further evaluated in field condition separately as well as in-combination with other treatments.

In-vivo evaluation of nutritional amendments, plant extract & fungicide

Field design: A field study was conducted to determine and compare the effects of alternative treatments against *S. oryzae* in order to avoid the increased use of fungicides for reduction of stem rot disease incidence and severity as well as for increasing yield. Soil was prepared by using rotavator. After tillage operations, the field was flooded with water and one month old seedlings were transplanted into field under (Randomized Complete Block Design) RCBD. For this the sub-plots were made in each block so that each treatment was applied in a single sub-plot. The size of each sub-plot was 45 inches long and 36 inches wide. Each treatment was replicated thrice within blocks. Each sub-plot contained 20 plants with the same row to row and plant to plant distance (22.5cm).

Treatments applied after in-vitro evaluation in the field

Treatments	Remarks
Sterculia alata leaves (T_1)	Organic amendment
Farm yard manure (T_2)	Organic amendment
Mushroom compost (T_3)	Organic amendment
Kitchen waste (T_4)	Organic amendment
Eucalyptus extract (T_5)	Best extract after in-vitro evaluation
Thiophenate methyl (T_6)	Best fungicide after in-vitro evaluation
$T_5 + T_6$	Combination of extract and fungicide
$T_1 + T_2 + T_3 + T_4$	Combination of organic amendments
$T_1 + T_2 + T_3 + T_4 + T_5$	Combination of organic amendments + extract
$T_1 + T_2 + T_3 + T_4 + T_6$	Combination of organic amendments + fungicide

Treatments application: Following treatments were applied in the field after in-vitro evaluation.

Three replications were maintained for all these treatments and single variety Shaheen-Basmati was sown in the management trials.

All the organic soil amendment separately as well as in combination was applied before transplanting while extract was applied at transplanting. Thiophentae methyl spray separately as well as in combination with other treatments was applied when the disease began to appear in the control.

Data recording:

Plant samples were selected randomly from each sub-plot for data recording. Data were recorded at 10 days interval. Effect of each treatment on the basis of disease incidence and disease decrease over control were recorded on selected plants. The formula for incidence and disease decrease over control is:

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected tillers}}{\text{Total no. of selected tillers}} \times 100$$

$$\text{Percentage disease decrease over control} = \frac{\text{Disease in control} - \text{Disease in treatment}}{\text{Disease in control}} \times 100$$

Plant yield and growth parameters: For yield and other plant growth parameters 10 plants were randomly selected. The selected plants were tagged and labeled for each parameter and treatment. These parameters include; number of tillers per plant, panicle length (cm), number of branches per panicle, number of kernels per panicle, plant height, 1000 grain weight (g) and yield per plant (g).

Tillers per plant: Ten plants were randomly selected from each sub-plot, their total and average number of tillers was calculated. Three replications were maintained in the similar way and mean taken. The number of tillers were calculated in the non-treated plots and compared with that of treated plots.

Panicle length (cm): Panicle length of 10 selected tillers was recorded with the help of measuring tape and average was calculated for final study.

Number of branches per panicle: Each panicle has different number of branches/spikelet which varies with environment, variety and time of planting. Number of branches per panicle was recorded for each panicle and average was calculated. Final evaluation is done by taking average of three replications.

Number of kernels per panicle: Number of kernels was calculated for each panicle per plant then average was calculated for each plant. Each replication was the average of 10 plants and three replications were maintained and their average was used for final evaluation.

Plant height: Plant height is measured with measuring tape. For this purpose, 10 plants were randomly selected from each sub-plot and the average height was calculated by taking mean of height of all the selected plants. Mean of three replications was considered for final evaluation.

1000-grain weight (g): For the calculation of 1000-grain weight the bulk yield of 20 plants of each treatment was sun-dried for three days.

Yield per plant: Yield per plant was determined after three days continuous sun-drying of the kernels. Average of 10 plants was taken for each replication and average of three replications was used for final evaluation.

Statistical analysis: Recorded data was statistically analyzed by statistics 8.0 software on the basis of ANOVA and least significant difference (LSD-test) at 5% level of significance.

RESULTS

Anti-fungal activity test of plant extracts: Among the three tested plant extracts eucalyptus extract was the most effective and it had high inhibitory potential than neem and devil extract. Eucalyptus extract showed percentage of inhibition 27.63% followed by neem extract 14.84% and devil extract 11.11%. The results on means are displayed in Table 1 while ANOVA is displayed in Table 2 which revealed significant results.

Table 1. *In vitro* evaluation of plant extracts against *Sclerotium oryzae*

Sr. No.	Treatments	Colony diameter (cm)	Inhibition percentage (%)
1.	Control	3.30 a	0%
2.	Devil extract	2.94 b	11.11%
3.	Neem extract	2.81 b	14.84%
4	Eucalyptus extract	2.39 c	27.63%

*Mean values are average of 3 replicates.

- Mean values followed by the same letters are not significantly different at (P < 0.05).

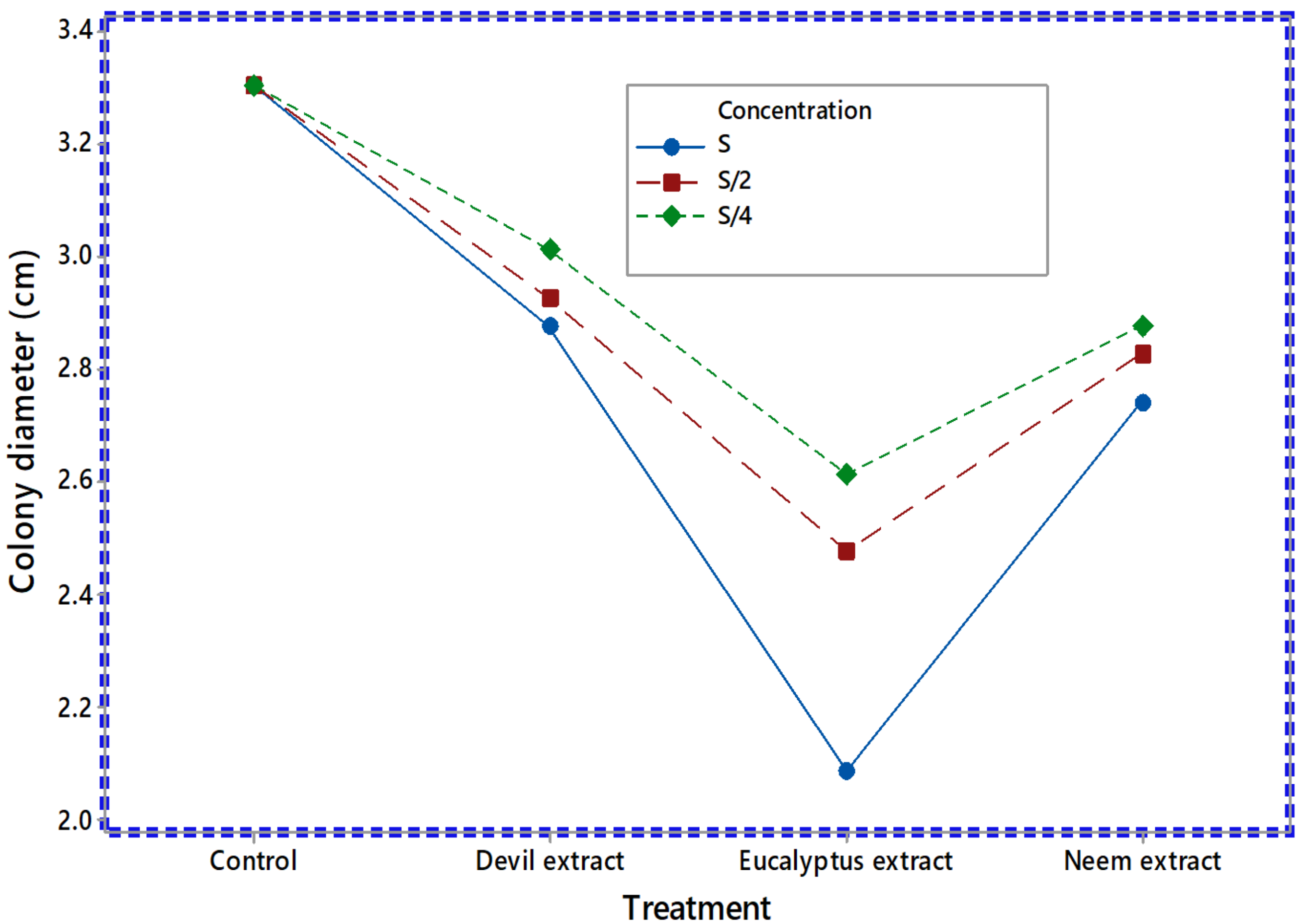

Figure 1. Graph for colony diameter of fungus for evaluation of extracts

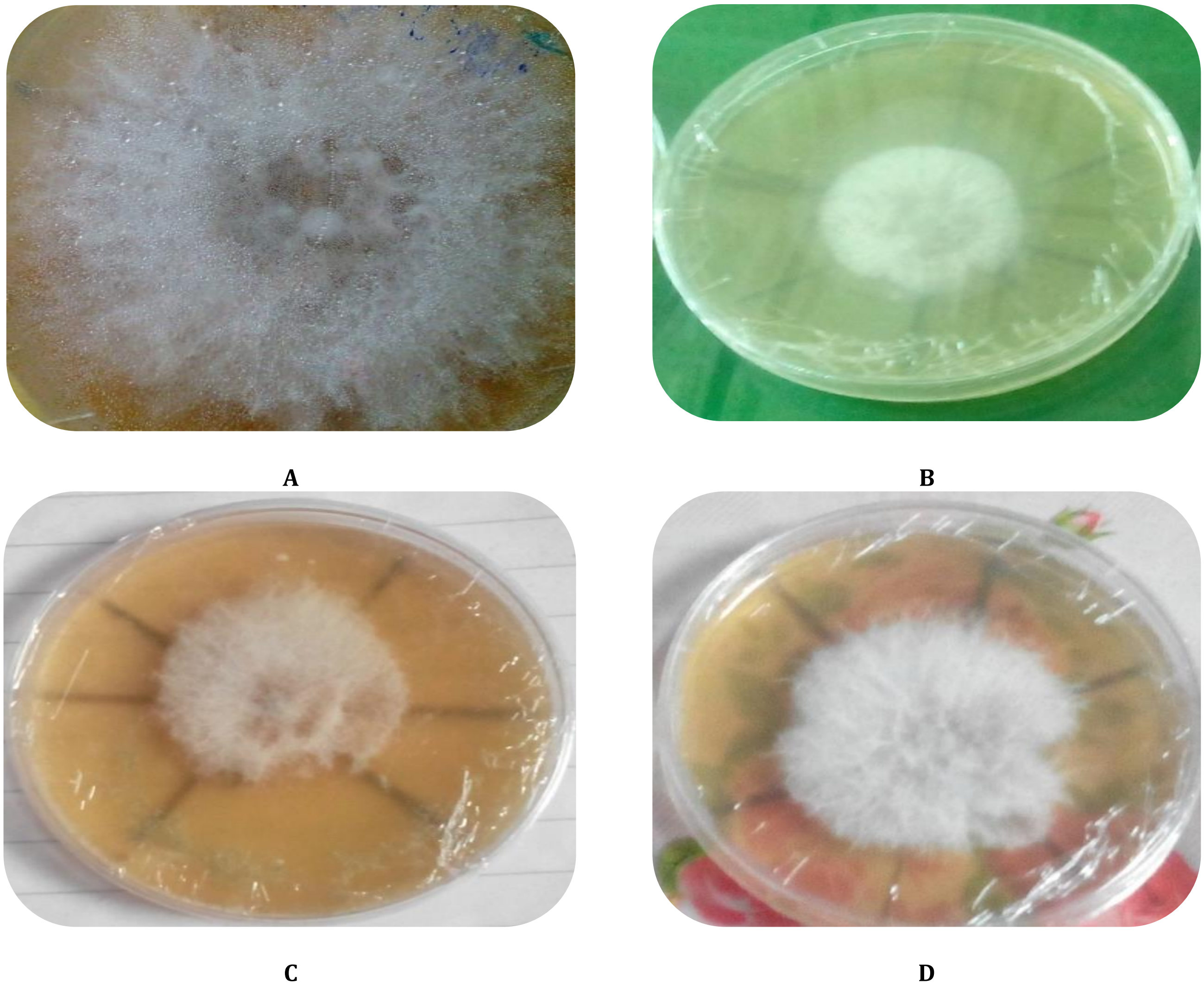

Figure 1. Poison Food Technique for evaluation of extracts **A.** Control plate, **B.** Eucalyptus extract treated plate **C.** Neem extract treated plate **D.** Devil extract treated plate

***In-vitro* testing of fungicides:** Among three tested fungicides Thiophenate methyl 70% WP was found the most effective and it had most inhibitory potential than Difenoconazole 10% WDG and Azoxystrobin 50% WDG. Thiophenate methyl 70% WP showed inhibition 42.11% followed by Difenoconazole 10% WDG 39.12% and Azoxystrobin 50% WDG 22.29%. The results based on means are displayed in table 3 while that of ANOVA is in table 4 which revealed significant results.

Table 3. *In vitro* evaluation of fungicides against *Sclerotium oryzae*

Sr. No.	Treatments	Colony diameter (cm)	Inhibition percentage (%)
1.	Control	3.31 a	0%
2.	*Azoxystrobin 50%* WDG	2.57 b	22.29%
3.	*Difenoconazole10%* WDG	2.02 c	39.12%
4.	Thiophenate methyl 70% WP	1.91c	42.11%

*Mean values are average of 3 replicates.
- Mean values followed by the same letters are not significantly different at (P < 0.05).

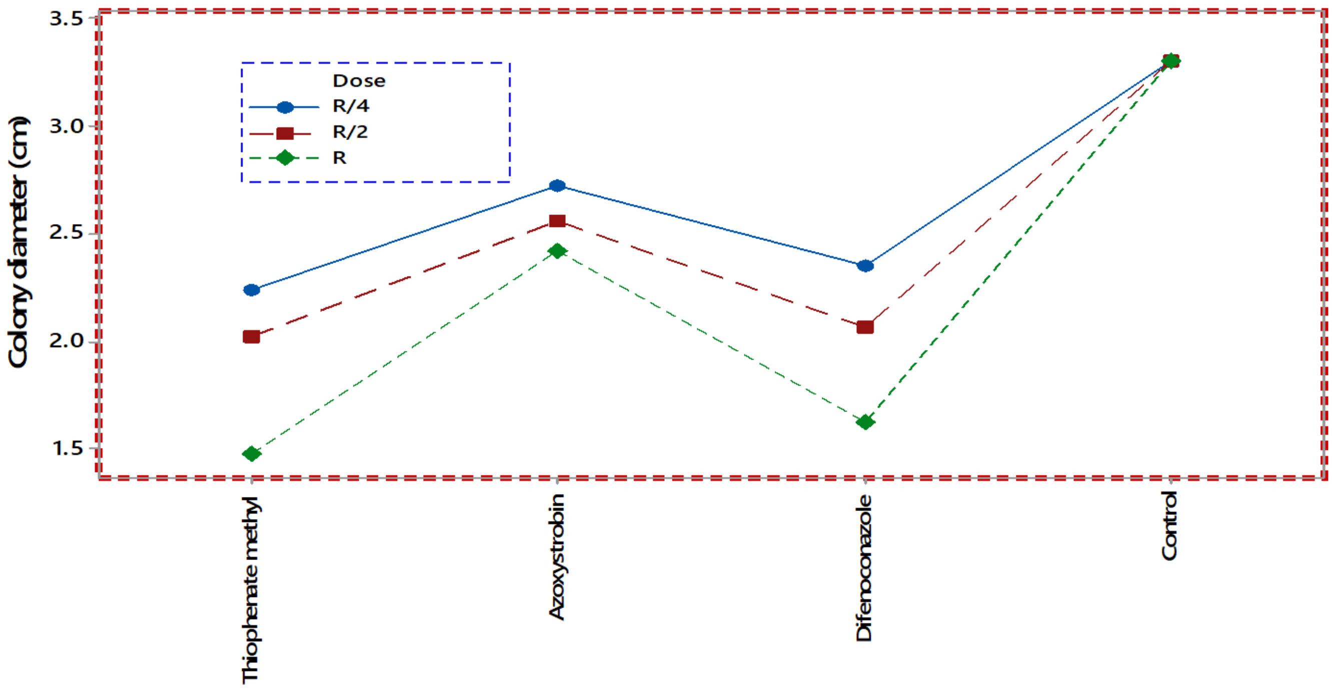

Figure. 2. Graph on colony diameter (cm) of pathogen for evaluation of fungicides

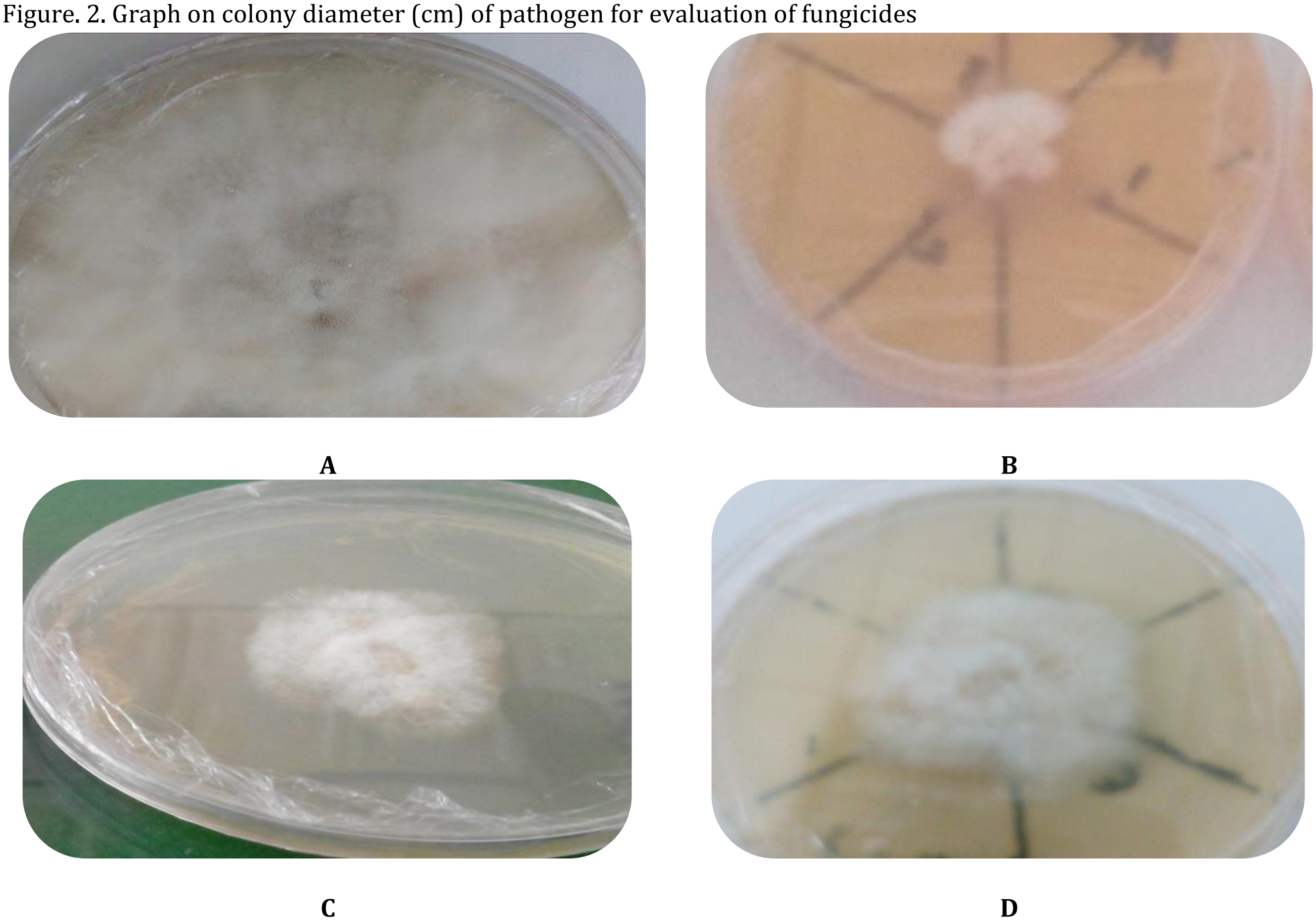

Figure 2. Poison Food Technique for evaluation of fungicides A. Control plate, B. Thiophenate methyl treated plate C. Difenoconazole treated plate D. Azoxystrobin treated plate

***In-vivo* evaluation of nutritional amendments, plant extracts and fungicides against *Sclerotium oryzae*:** Among the 11 tested treatments mixture of all the amendments with that of thiophenate methyl gave maximum result among all other treatments while *Sterculia alata* leaves showed minimum result among all as compared to control. The treatments including mixture of all amendments showed a mean value of disease incidence 5.78% which had a significant impact on plant health and hindering the stem rot infection and *Sterculia alata* leaves showed a mean value of 17.57% as compared to control.

Table 6. Disease incidence and disease decrease over control under field conditions

Treatment	Disease incidence (%)	Disease decrease over control (%)
Sterculia alata leaves (T1)	17.57 b	47.653e
Farm yard Manure (T2)	16.94 b	49.578e
Mushroom Compost (T3)	12.38 c	63.291cd
Kitchen waste (T4)	14.08 cd	58.199d
Eucalyptus extract (T5)	11.53 de	65.694cd
Thiophenate methyl (T6)	10.27 de	69.467bc
T5+T6	7.15 e	78.711a
T1+T2+T3+T4	10.69 f	68.243bc
T1+T2+T3+T4+T5	8.00 fg	76.171ab
T1+T2+T3+T4+T6	5.78g	82.778a
Control	33.70 a	0 f

*Mean values are average of 3 replicates.

- Mean values followed by the same letters are not significantly different at (P < 0.05).

Effect on yield and growth parameters of rice plants: All the organic nutritional amendments as well as extract and fungicide showed significant impact on plant growth and yield parameters. Among eleven tested treatments mixture of all amendments along with fungicide (*Sterculia alata* leaves + farm yard manure + mushroom compost + kitchen waste + Thiophenate methyl) showed most effective results while *Sterculia alata* leaves showed least significant results when compared with control (Table 7).

Table 7. Effect of organic amendments, plant extracts and fungicides on yield and plant growth parameters

Treatment	No. of Tillers/plant	Panicle length (cm)	No. of Branches/panicle	No. of kernals/ panicle	Plant height Plant height(cm)	1000-grain Grain weight (g)	Yield/plant yield (g)
Sterculia alata leaves (T1)	17.13 e	18.65h	7.87 f	72.80 g	77.19d	18.97 fg	30.21 h
Farm yard manure (T2)	18.40 d	19.41gh	8.80 e	78.60 f	85.64c	19.51ef	34.16 g
Mushroom compost (T3)	19.40 c	21.29f	9.53 d	90.27 d	89.09c	20.25 cdef	36.51 f
Kitchen waste (T4)	19.13 cd	19.87g	9.00 e	82.47 e	86.18c	19.79 def	35.75 fg
Eucalyptus extract (T5)	19.40 c	23.44e	10.00 d	93.33 d	89.23c	20.69 cde	39.29 e
Thiophenate methyl (T6)	19.93 bc	26.51c	11.47 c	105.60 c	96.06b	21.22 abc	43.70 c
T5+T6	20.53 ab	28.33b	12.20 b	120.80 b	98.80b	22.10 ab	46.88 b
T1+T2+T3+T4	19.87 bc	25.03d	11.40 c	105.13 c	95.83b	20.92 bcd	41.92 d
T1+T2+T3+T4+T5	20.60 ab	27.83b	12.13 b	120.20 b	97.19b	21.50 abc	44.81 c
T1+T2+T3+T4+T6	21.40 a	30.33a	13.53 a	132.53 a	105.65a	22.28 a	50.93 a
Control	15.07 f	17.19i	6.80 g	65.53 h	61.91e	17.98 g	26.69 i

*Mean values are average of 3 replicates.

- Mean values followed by the same letters are not significantly different at (P < 0.05).

Data revealed that maximum number of tillers per plant were 21.40 which were produced by plants treated with mixture of all the amendments i.e. *Sterculia alata* leaves + farm yard manure + mushroom compost + kitchen waste with that of thiophenate methyl and *Sterculia alata* leaves showed least significant results with mean number of tillers per plant 17.13. Similarly, the maximum mean panicle length 30.33cm, branches per panicle 13.53, kernels per panicle were 132.53, plant height 105.65cm, 1000-grain weight 22.28(g), and yield/plant 50.93(g) was recorded in combined effect of soil amendments with that of fungicide and minimum mean panicle length was 18.65cm, number of branches per panicle 7.87, kernels per panicle 72.80, plant height 77.19cm, 1000-grain weight 18.97(g) and yield/plant 30.21 (g) was observed in case of *sterculia alata* leaves.

DISCUSSIONS

Medicinal plants have great effect for the control of sclerotial diseases. Some plants contain components that are toxic to pathogens (Gurjar *et al*., 2012). The antimicrobial efficacies of plant extracts against plant diseases have been studied by many scientists (Suberu, 2004). Three plants extracts *Alstonia scholaris* (Devil), *Azadirachta indica* (Neem) and *Eucalyptus globules* (Sufaida) were evaluated *in-vitro* against *S. oryzae* and Eucalyptus extract was the most effective in inhibiting the fungal growth followed by neem and devil tree. The results of this experiment have significant resemblance with other researcher's work. Antifungal properties of neem have been described by (Bansal and Sobti, 1990). Khalil (2001) demonstrated that eucalyptus extracts effectively reduces the spore germination of different fungi. Significant reduction in brown leaf spot disease incidence and increase in yield of rice was recorded in neem extracted treated plots (Harish *et al*., 2008). Jalal and Ghaffar (1992) found that sclerotial growth of *Sclerotium rolfsii* was significantly reduced by the leaf extracts of *Ocimum sanctum* L. Similarly, this extract was also used against other related fungi and proved effective as medicinal plant. Dubey *et al*., (2009) reported that extracts of *A. indica* and *E. globules* were proved best against suppression of *Macrophomina phaseolina* sclerotial germination. Moreover, these are not only cost effective but also eco-friendly for controlling plant diseases (Venkateswarlu and Sreeramulu, 2013). The plant extracts cause granulation of cytoplasm, membrane rupture in cytoplasm and inactivation of intracellular and extracellular enzyme synthesis (Cowan, 1999). Chung *et al*., (1998) stated that plant extracts contain tannins that arrest the growth of fungi.

Fungicides are most commonly used to reduce the economic losses caused by soil-borne diseases. Their ease of application and effectiveness has made them the most common mean to combat many fungal diseases (Vinale *et al.,* 2008; Dias, 2012). In the present experiment, three fungicides Thiophenate methyl 70% WP, Difenoconazole 10% WDG and Azoxystrobin 50% WDG were used. The results revealed that Thiophenate methyl 70% WP was the most effective even at lower concentration followed by Difenoconazole 10% WDG and Azoxystrobin 50% WDG. These results are in accordance with previous studies. Reddy (1984) conducted a trial on 16 fungicides evaluation against stem rot of rice and results revealed that, carbendazim (Bavistin 50 WP), tridemorph, tolfos – methyl, thiophanate methyl, carboxin (Vitavax 75 WP), ziram and cuman L-27 were highly effective against this disease and reduced the sclerotial germination even at lower concentration. Singh *et al*., (2002) also reported that Thiophanate methyl was the most effective against stem rot disease among many fungicides. Diafenoconzole and azoxystrobin completely controlled the growth of *Lasiodiplodia theobromae* on PDA medium (Rehana *et al*., 2014). Hossain *et al*., (2011) reported that azoxystrobin reduced the brown leaf spot of rice and increased the yield of rice. The fungicides cause demethylation of ergosterol that inhibits the cell wall formation of fungi and ultimately protects the plants from high disease incidence (Gupta *et al*., 2013).

Stem end rot disease reduced in the plots treated with organic amendments. The growth and yield parameters significantly increased in treated plots as well. In mature compost sclerotia killed by hyperparasitism (Chen *et al*., 1988). Salim *et al*., (2016) described the increased yield and decreased fungal growth by using spent mushroom compost along with *Trichoderma harzianum*. Melese *et al*., (2008) used different concentrations of farm yard manure and recorded low disease incidence and high yield of lettuce. Fuchs *et al*., (2008) concluded after different experiments that composts help in the mobilization and availability of nutrients in the soil that ultimately boosts up the plant defense.

CONCLUSION

The effect on plant growth and yield parameters was recorded and found that maximum promotion of growth and yield parameters was observed in combination of organic soil amendments, including Farm yard manure, *Sterculia alata* leaves, mushroom compost and kitchen wastes with that of fungicide, Thiophenate methyl.

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