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In vivo study on preventive effects of *Polyalthia longifolia* bioformulations against Black Scurf Disease of potato

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In the present study, bioformulation prepared by combining *Polyalthia longifolia* leaf extract with elicitors (neem oil cake) and binders (cow dung) were tested for management of black scurf disease of potato caused by *Rhizoctonia solani*. Different application methods like seed dipping and soil amendment were used for *in vivo* study. The preventive action was studied as a function of decrease in disease severity by PDI (Percent Disease Index) and PEDC (Percent Efficiency of Disease Control) methods, change in growth characteristics of host plant such as number of leaves/plant, plant height, number of branches/plant, number of flower/plant, number of fruit/plant and weight of fruits in control and treated plants. Six treatments T1 (formulation no.12), T2(formulation no.7), T3(formulation no.26), T4 (formulation no.17) were applied in different combinations and four different control were also maintained. Potato plants were examined for symptoms infected tuber (sclerotia) were quantitatively assessed after 90 DAS (days after sowing). Results of present study indicates that treatment with bioformulation especially T4 treatment not only reduces the infection but also leads to increased growth, health and vigour of the host plant as compared to untreated and synthetic fungicide control. Results were subjected to statistical analysis of student t test, one way ANOVA and post-Hoc comparisons analysis. Significance was measured at $p<0.001$ and showed highly significant. Statistical analysis at 1% and 5% CD revealed all the treatment are significant. Results of present study clearly indicate that treatment with bioformulations appreciably reduces the disease index as compare to control with significant improvement of growth of host plant. The T4 treatment showed significant inhibitory activity against *Rhizoctonia solani* and can help to minimize the economical loss of potato crop.

Keywords: Antifungal activity, bioformulation, plant extract, *Polyalthia longifolia*, *Rhizoctonia solani.*

INTRODUCTION

Potato (*Solanumtuberosum* L.) is tuberous crop and belongs to Solanaceae family. India ranks third in potato cultivation area and second in production in the world after China. During 2021-2022 the volume of potato produced across India was estimated to be around 53.58 million metric tons while 2020-21 potato cultivation in India was 22.48 lakh ha and production was 542.3 lakh tons (Horticulture Statistics Division, 2021).

Majority of the Indian potatoes came from northern state of Uttar Pradesh, West Bengal, Bihar, Punjab and Gujarat.Potato tubers comprise of 79% moisture, 21% dry matter and 60-80% is starch. Nutritionally, potato is rich in vitamins (B1, B2 and B6), minerals (potassium, phosphorus and magnesium), pantothenic acid and riboflavin (Siddique *et al*.2020). Black scurf disease on potatoes causes severe damage to plant parts in different stages of growth.Potato black scurf disease is one of the most important disease globally as it has a great economic impact and considered as a serious problem in the potato tuber production system. Major symptoms of this disease is damaging stem tissues from the grown area, deformation of tubers and development of black scurf/sclerotia on the surface of tubers hence decline the quality of tubers (Betancourth *et al*. 2021). Black scurf of potato caused by the pathogen *Rhizoctonia solani* Kuhn (Teleomorph *Thanatephorus cucumeris* Donk) causes severe losses globally, whereas 10-25 % crop loss reported in India (Sharma, 2015). Farmers faces economic losses upto 5-7% due to defacing of the tuber and sclerotia

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deposition. Infection on potato spread by tubers, soil or the remains of infected plants. The source of the primary infection is infected seeds, which is characterized by the presence of sclerotia covering the outer crust of the tubers, the distinguishing signs of infection with black scurf disease (Ferrucho *et al*. 2012).

Bavistin and mancozeb are the most commonly used fungicides to inhibit *Rhizoctonia solani* either by destroying their cell membrane or its permeability or by inhibiting metabolic processes and hence are extremely effective (Osman and Al-Rehiayam, 2003). Chemical control methods possess many harmful effects on the environment and human health besides causing pathogen resistance and their non-target effects disrupts natural biodiversity. Therefore, management of black scurf disease on potato is necessary. In recent years, the biological control of plant pathogens has been considered as an alternative strategy because chemical control results in the accumulation of harmful chemical residues, which may lead to serious ecological problems (Madbouly, 2018). The solution to this problem is to use alternate method that are both sustainable and harmless to the environment.

Thus current scheme for the plant and environment protection suggests various alternative strategies to pesticides in addition to well-known disease management methods such as crop rotation, use of resistant cultivars, planting disease free seeds, biological control etc. for control of fungal diseases (Sharma *et al*. 2021; Mehta and Sharma, 2018;

Hada and Sharma, 2017). One of these alternative strategies is the use of natural formulations prepared from plants or plant parts/extract.

Since plants possess different secondary metabolites which are antimicrobial in nature, plants or their parts/extract can be used to develop eco-friendly and effective fungicidal formulations. Therefore, the main objective of this study includes preparation of bioformulation by combining *Polyalthia longifolia* leaf extract with neem oilcake as elicitor and cow dung as binder and also test its efficacy in controlling the infection of *Rhizoctonia solani* in potato. In present study fungicidal potential of bioformulation was evaluated by performing an *in vivo* experiment. Bioformulations were applied as seed dressing and soil amendment for management of black scurf disease of potato. A comparison with synthetic fungicide has also been done. Innovative part of the study is that the use of combination of plant extract with elicitors and binders to develop protective measure against the black scurf disease of potato.

MATERIALS AND METHODS

Test crop and fungus

Infected tuber with sclerotia were collected from local vegetable market of Udaipur. Sclerotia was surface sterilized with 0.1% $HgCl_2$ solution for 1 min and then three time rinsed with sterile water. Then dried it well and placed on potato dextrose agar medium in petri plates which were incubated at 22°C for 4 days. Fresh potato tubers (no sclerotia visible) were collected having same size and 3-5 eyes were used for *in vivo* experiment(Tsror, 2010).

Inoculum development

Rhizoctonia solani was cultured on potato dextrose agar (PDA) medium that had been sterilised at 15 psi for 20 minutes and incubated for sevendays at 22 °C. Pure culture obtained were further used for mass culture of *Rhizoctonia solani*. Five grams of this mycelial mat was added to one liter of sterile distilled water and stirred constantly. The homogenized suspension thus prepared was used for inoculation at 25ml /gram of soil (Buttner*et al*.2004).

Preparation of pots and soil

Pots (30 cm dia) were sterilized with 20% copper sulphate solution whereas soil was autoclaved and then cooled. Sterile soil was used after 5 to 6 days. Sterile soil was mixed with inoculum and filled in the pre-sterilized pots. Since autoclaving of soil makes it nutrient deficient hence organic manure was added to the soil of each pot before sowing (El-Mougy and Abdel-Kader, 2009).

Preparation of herbal formulations and standard fungicide

In vivo study of preventive /protective action of herbal formulation was based on results of *in vitro* studies. Herbal formulations were prepared by using *Polyalthia longifolia* leaf extracts, *Polyalthia longifolia* leaf powder, elicitor like neem oil cake (20 g in 100 ml sterile water) and binder like cow dung (20 g in 100 ml sterile water) were mixed with 100% alcoholic crude extract, partially purified petroleum ether extract and dried leaf powder of *Polyalthia longifolia*(Mehta and Sharma, 2018; Meena and Sharma, 2021). All ingredients of herbal formulation were used in following combinations:

100% Crude extract (20gm dried plant leaf material dissolve in 100ml alcohol) + 20g elicitor(neem oil cake) dissolve in 100ml sterile water + 20g binder (cow dung) dissolve in 100 ml sterile water

100% Partially purified extract (40g dried plant leaf material dissolve in 280ml petroleum ether)+ 20g elicitor(neem oil cake) dissolve in 100 ml sterile water + 20 g binder(cow dung) dissolve in 100 ml sterile water

Polyalthia longifolia leaf powder+20g elicitor(neem oil cake) dissolve in 100ml sterile water + 20g binder(cow dung) dissolve in 100ml sterile water

Mancozeb was used as standard antifungal and 10 mg/ml concentration was prepared in sterile water.

Treatments and Experimental Design

The study was conducted during December, 2021 to March 2022 in the Botanical Garden, University College of Science, M.L.S. University, Udaipur (Rajasthan). Seed dipping method and soil treatment were used to study preventive effect of herbal formulations (Ganiet*et al*. 2013). On the basis of *in vitro* screening of 30 herbal formulations (need citation of appropriate references), following six formulation showed best activity against test fungi and hence used for *in vivo* studies in following combinations:

T1: (formulation no.12) Combination of 6 ml (100% alcoholic crude extract), 2 ml (neem oil cake) and 2 ml (cow dung).
T2 (formulation no.7) combination of 2 ml (100% alcoholic crude extract), 6ml (neem oil cake) and 2ml (cow dung).
T3 (formulation no.26) combination of 1ml (partially purified petroleum ether extract), 1ml (neem oil cake) and 8ml (cow dung).
T4: (formulation no. 17) combination of 4ml (partially purified petroleum ether extract), 3ml (neem oil cake) and 3ml of (cow dung).
T5: combination of leaf powder (60g): neem oil cake (20g): cow dung (20g)).
T6: combination of leaf powder (40g): neem oil cake (30g): cow dung (30g).

Four different controls were also maintained respectively. These were as follows:

C1: Mancozeb used for treat to healthy tubers with 10mg/ml concentration sown in soil inoculated with *Rhizoctonia solani*.
C2: Unsterilized and uninoculated soil used for sown untreated healthy tubers.
C3: Untreated healthy tubers sown in sterilized soil inoculated with *Rhizoctonia solani*.
C4: Uninoculated soil untreated healthy tubers in sterilized

Healthy tubers were treated with all different formulations. 10 kg of soil infected with *Rhizoctonia solani* inoculum (25ml/pot) were put in pre-sterilized soil pots and healthy seed tubers dipped in respective herbal formulations were planted in disinfected soil. Three tubers were planted per pot at a depth of 5 cm. The pot were left for 90 days. 220 g urea was applied twice as nitrogen fertilizer in each pot after 28 and 42 days from date of sowing. The tubers were harvested after 90 days from the date of sowing and observations were recorded as the number of leaves/plant, plant height, tuber size, tuber weight /pot and number of tubers/pot. Percent disease infestation was recorded with the comparative study of positive controls. Data were subjected to analysis of CD and CV value. Three replicates were maintained with each treatment (El-Mougy and Abdel-Kader 2009; Mirkarimi*et al*. 2013).

Disease Rating and Percentage Disease Index (PDI)

The disease severity was defined as the percentage of infected area of tubers with black scurf. The severity was calculated on the basis following 0-5 rating scale(Muhammad *et al*.2020)

The Percentage Disease Index (PDI) was calculated using following formula **(**Mohammed*et al*. 2008**)**

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of all individual disease ratings}}{\text{Total numbers of plants assessed x maximum rating}} \text{X100}$$

The Percent Efficacy of Disease Control (PEDC) was determined by using formula given by Mohammed *et al*. 2008 (Table 1).

$$\text{Percent Efficacy of Disease Control PEDC} = \frac{\text{PDI Control- PDI in treatment}}{\text{PDI in control}} \text{X100}$$

Growth Parameters

Various growth parameters like number of leaves/ plant, plant height, tuber weight/pot, tuber size, number of tubers /pot have been determined using standard methods in healthy, infected, and treated plants, as reported in the Central Potato Research Institute's Technical Bulletin, ICAR, Shimla: Number of leaves/plant.

RESULTS

Disease severity

Results of effect of bioformulations on disease severity of black scurf on potato are given in Table2. Maximum disease index/ incidence was observed with control treatment C3 (88.76%) followed by C2 (58.14%) and C4 (55.14%) respectively. Amongst all treatments and control mancozeb*i.e.* control C1 found most effective in reducing disease index. In case of all treatments maximum reduction in disease incidence was observed with treatment T4 (10.3%) followed by C1 (16.75%), T3 (20.32%), T1 (28.50%), T2 (36.97%), T6 (41.24%), and T5 (45%) treatment . Results of percent disease index were comparable in control C1 (mancozeb) and treatment T4 (formulation no. 17) prepared from partially purified petroleum ether extract (4ml): 100% neem oil cake (3ml): 100% cow dung (3ml) and even treatment T4 exhibited better control then standard fungicide. Mancozeb showed well control of disease incidence then treatment T1, T2, T3, T5 and T6. Statistical analysis at 1% and 5% CD values reveals that all the treatments are significant to decrease disease severity in *in vivo* pot experiments.

Growth parameters

Results of effect of bioformulations prepared from *Polyalthia longifolia* leaf alcoholic crude extract and partially purified petroleum ether fraction, dried leaf powder, neem oil cake (elicitors) and cow dung (binders) on following growth parameters of potato crop are summarized in (Tables 2 to 7). Data clearly indicate that, treatment with bioformulations reduces disease index which results into significant improvement of growth of host plant.

Number of leaves/plant

Results of Table3indicates the effects of different treatments and synthetic fungicides on the number of leaves/plant. The data show a slight decrease in the number of leaves per plant as a result of infection. However, treatment with herbal formulations and mancozeb resulted in a considerable increase in the number of leaves per plant. T4 (56.66) increased the number of leaves per plant the most, followed by C1 (54.66), T5 (50.33), T6 (49.33), T3 (48), and C4 (46.66). T1 and T2 results are comparable to C3 results. At 5% and 1% CD, statistical analysis demonstrated that all treatments are significant.

Plant height

Results of various treatments' effects on plant height has been presented in Table 4. After receiving herbal formulations, a similar pattern of growth improvement as shown in the number of leaves was observed for plant height. In comparison to the control, the treatment with formulations and mancozeb increased plant height**.**T4 treatment developed the tallest plants (27.33 cm), followed by T6 (26.33 cm), and T5

Table 1: Percentage Disease Index recorded on 1 to 5 Standard Disease Rating Scale for tubers

Disease Severity Grades	Percentage of Disease
0	No disease symptoms
1	< 1% tuber surface affected
2	1 to 10% tuber surface affected
3	11 to 20% tuber surface affected
4	21 - 50% tuber surface affected.
5	> 50% tuber surface affected

Table 2: Effect of different treatments on PDI and PEDC of Potato

Treatment	PDI (%)			Average ± SD	PEDC
	R1	R2	R3		(%)
T1	30	29	26	28.50±2.09	74.65%
T2	36	35	39	36.97± 2.30	58.34%
T3	20	22	18	20.32 ±1.855	77.10%
T4	11	10	9	10.30± 1.03	88.39%
T5	44	46	44	45.00 ±1.32	49.30%
T6	42	41	40	41.24± 1.19	53.53%
C1	16	17	16	16.75± 0.85	81.12%
C2	56	58	60	58.14±2.06	-
C3	85	88	85	88.76±85.29	-
C4	56	55	53	55.14±1.2	-
SE m^+	–	–	–	0.919	-
CD(P=0.05)	–	–	–	2.727	-
CD(P=0.01)	–	–	–	3.966	-
CV (%)	–	–	–	5.169	-

(25.33 cm) given in Fig. 3. Among the applied treatments, it was discovered that the plant height for T3, T1, and T2 was similar to C1. All of the treatments were discovered to be significant for field trials at 5% and 1% CD values.

Total tuber weight /pot

Results of the treatment's effect on average tuber weight are shown in Table5. Due to infection, a significant drop in tuber weight was seen (C2, C3, and C4). In comparison to maintained controls, the weight of the tubers is dramatically increased by all treatments. In line with prior observations, T4 treatment was shown to be the most effective and produced the greatest increase in tuber weight when compared to the C1 standard and the other controls (C2, C3, and C4). The C1 (201.44 gm) treatment outperformed T5 (191.21 gm) and T6 (187.5 gm) among controls. T4 (247.45 gm) was shown to be the most effective treatment, followed by T3 (225.47 gm), T2 (212.26 gm), and T1 (203.9 gm). 5% and 1% CD statistical study showed that all the treatments are significant.

Tuber size

In case of tuber size,with C3, the smallest tuber size was noticed. C4 was followed by C2 in terms

Table 3: Effect of various treatments on number of leaves/ plant

Treatment	Number of Leaves/plant			Average ± SD
	R1	R2	R3	
T1	46	48	46	46.66±1.15
T2	42	44	44	43.33±1.15
T3	48	47	49	48.00±1.00
T4	56	57	57	56.66±0.57
T5	51	49	51	50.33±1.15
T6	50	50	48	49.33±1.15
C1	55	54	55	54.66±0.57
C2	34	36	33	34.33±1.52
C3	45	46	46	45.66±0.57
C4	46	47	47	46.66±0.57
SE m^+	–	–	–	0.546
CD(P=0.05)	–	–	–	1.621
CD(P=0.01)	–	–	–	2.357
CV (%)	–	–	–	2.088

of observing a slight rise in tuber size. The tuber size was shown to be increased by all treatments and mancozeb. T4 (6.66 cm) showed the greatest increase in tuber

size, followed by T3 (6.1 cm), T2 (5.66 cm), and T1 (5.26 cm), in that order. T5 (4.96 cm) and T6 (4.76 cm) treatments were found to be less effective than C1 (5.16 cm) (Table 6). All of the treatments are significant, according to statistical analysis at 5% and 1% CD.

Number of tubers/pot

Due to infection (C3 and C4), there was a nearly 50% decrease in the number of tubers. With T4 therapy, 18.33 more tubers were found, which a significant improvement is. T2 (15.66) and T3 (17.66) were the next two most efficient treatments. The number of tubers/pot observed for T1 (14.66) was similar to that for C1 (14.66) and C2 (14.33) among the treatments used. The treatments T5 (13.66) and T6 (11.66) had the fewest tubers per pot (Table 7). All of the treatments were shown to be statistically significant for field trials at 5% and 1% CD values.

A total of 30 bio formulations were assayed for antifungal activity against *Rhizoctonia solani* among all 30 bio formulations prepared, optimum activity was observed with formulation no. 7, 12, 17 and 26 and percent mycelia growth inhibition was 80.47%, 78.51%, 83.98%, 82.03% respectively as shown in Table 7. The second

Table 4: Effect of bioformulations and chemical treatments on plant height

Treatment	Plant Height (cm)			Average ± SD
	R1	R2	R3	
T1	24	26	25	25.00±1.00
T2	25	24	25	24.66±1.00
T3	26	25	24	25.00±1.00
T4	27	27	28	27.33±0.57
T5	24	26	26	25.33±1.15
T6	26	27	26	26.33±0.57
C1	24	22	24	23.33±1.15
C2	24	22	22	22.66±1.15
C3	17	18	19	18.00±1.00
C4	14	16	14	14.66±1.15
SE m^+	–	–	–	0.540
CD(P=0.05)	–	–	–	1.603
CD(P=0.01)	–	–	–	2.331
CV (%)	–	–	–	4.267

Table 5: Effect of bioformulations and chemical treatments total tuber weight

Treatment	Total Tuber Weight (gm.)			Average ± SD
	R1	R2	R3	
T1	204.10	204.40	203.20	203.9±0.62
T2	212.24	211.10	213.46	212.2667±1.18
T3	226.29	225.16	224.96	225.47±0.71
T4	248.16	247.00	247.20	247.4533±0.62
T5	192.28	191.16	190.20	191.2133±1.04
T6	187.48	186.16	188.86	187.5±1.35
C1	202.00	201.46	200.86	201.44±0.57
C2	168.24	167.24	168.18	167.8867±0.56
C3	128.00	126.00	127.49	127.16±1.03
C4	158.00	156.00	157.15	157.05±1.00
SE m^+	–	–	–	0.503
CD(P=0.05)	–	–	–	1.492
CD(P=0.01)	–	–	–	2.170
CV (%)	–	–	–	0.477

highest inhibition was shown by formulation no. 1 (70.31%), 18 (70.04%), 24 (69.92%) 25 (68.75%), 5 (66.79 %), 4 (66.79%), 13 (66.40%), 3 (66.00%), 23 (62.10%) given in table no. 8. Water was used as control in which no bio formulation was present and all data were compared with water.

In vivo study of preventive effect of different treatments like T1, T2, T3, T4,T5 and T6 for control of *Rhizoctonia solani*(72 days after sowing) given in Fig.1. Effect of different bioformulations on potato tuber number and size given in Fig. 2.

DISCUSSION

Potato is most important protective food crops of India. Black scurf disease caused by *Rhizoctonia solani* inflicts serious damage to these crops by reducing the quantity and quality of tuber yield of potato (Bakali and Martín, 2006;Ghadsingh and Mandge, 2012). *Rhizoctonia solani* is a very harmful fungus for potato crops, however with the use of cutting-edge technology, it is now simpler to manage this global fungus. Fungicides are one of the most often utilized techniques. In order to manage various plant infections, some plant

Table 6 : Tuber Size of potatoes affected by bioformulations and chemical treatments

Treatment	Tuber Size (cm)			Average ± SD
	R1	R2	R3	
T1	5.3	5.2	5.3	5.26±0.05
T2	5.7	5.7	5.6	5.66±0.05
T3	6.0	6.1	6.2	6.1±0.1
T4	6.7	6.6	6.7	6.66±0.05
T5	5.0	5.0	4.9	4.96±0.05
T6	4.8	4.8	4.7	4.76±0.05
C1	5.2	5.2	5.1	5.16±0.05
C2	4.7	4.6	4.7	4.76±0.05
C3	3.0	2.9	3.0	2.96±0.05
C4	4.1	4.0	4.0	4.03±0.05
SE m^+	–	–	–	0.036
CD(P=0.05)	–	–	–	0.106
CD(P=0.01)	–	–	–	0.154
CV (%)	–	–	–	1.273

extracts have been shown to exhibit fungicidal action (Ashraf , 2011; Rajamanickam *et al*. 2013), yet these fungicides pose major risks to human health as well as environmental contamination. Because they are more practical, eco-friendly, and secure, different disease management techniques such as breeding disease-free plants, changing agronomic procedures, using plant and natural products, and bioformulation are currently receiving more attention.

In present study comparative effect bioformulation as well as chemical fungicide (mancozeb) to control black scurf disease of potato were evaluated by *in vivo* pot experiments. In soil containing mycelial suspension of *Rhizoctonia solani*, potato tuber buds treated with bioformulations and mancozeb were planted. Untreated tuber seeds planted in the same soil served as a positive control. The T4 treatment, or formulation no. 17 (partially purified petroleum ether extract (4ml): 100% neem oil cake (3ml): 100% cow dung (3ml), significantly lowers the percent disease index and also improves all growth parameters when compared to control, according to a comparative study of the effects of all bioformulation treatments on potatoes. The results of the comparison study between the preventive effects of bioformulations and synthetic fungicide show that treatment no. 4 provided better protection than mancozeb, a common fungicide. The T4 treatment considerably lowers *R. solani* infection as compared to other forms of treatment. Preventive effect of bioformulation on disease severity might be due to presence of antifungal secondary metabolites or compounds

Table 7: Number of Tubers after treatment with bioformulations and chemical treatments

Treatment	Number of Tubers			Average ± SD
	R1	R2	R3	
T1	15	15	14	14.66±0.57
T2	16	16	15	15.66±0.57
T3	18	18	17	17.66±0.57
T4	19	20	19	19.33±0.57
T5	14	13	14	13.66±0.57
T6	12	12	11	11.66±0.57
C1	15	14	15	14.66±0.57
C2	14	15	14	14.33±0.57
C3	14	14	13	13.66±0.57
C4	11	11	10	10.66±0.57
SE m^+	–	–	–	0.333
CD(P=0.05)	–	–	–	0.990
CD(P=0.01)	–	–	–	1.439
CV (%)	–	–	–	4.065

present in plant extract, elicitor and binder. *Polyalthia longifolia* contain several secondary metabolite like terpenoid, flavonoids, volatile oil, alkaloids, saponin, phytosterols, and tannins. (Gershenzon and Natalia, 2007; Parveen and Sharma, 2015; Mehta and Sharma, 2018; Kaur *et al*. 2022; Karak, 2019; Salvatore *et al*. 2020; Meena *et al*.2021). The antifungal activity of plant extracts, various elicitor (ground nut oil cake, mustard oil cake, cotton oil cake, clove bud oil cake, coconut oil cake, neem oil cake and sesame oil cake) and binders (guar gum, gum acacia and cow dung) separately or individually (Mandal *et al*.2022; Rajeswari *et al*. 2021; Modi *et al*. 2022; Meena *et al*. 2021), have been worked out previously. Neem oilcake and cow dung are used as elicitors and binders to enhance the antimicrobial activity of bioformulation. Combination of 100% cow dung and urine with *Vincarosea*, *Piper betle* and *Azadirachta indica* extract is reported to inhibit the conidial germination of *Bipolaris sorokiniana* which cause leaf blight in wheat (Jin and Jin, 2021).

According to the study's findings, treatment with bioformulation, particularly T4, not only reduces *R. solani* infection but also significantly outperforms synthetic fungicide treatment in terms of the host plant's growth, health, and vigour.

CONCLUSION

The reduction in percent disease index after treatment with bioformulations was found to be comparable to that of a synthetic fungicide (Mencozeb) in the present study. Hence these bioformulations can be used as an eco-friendly alternative for management of black scurf disease of potato. Among various treatments, T4 treatment (Formulation no.17) was found to be most effective against *Rhizoctonia solani*. This treatment was found to not only reduce disease incidence but also to improve all of the host plant's growth parameters. Effective bioformulations will be subjected to further field trials for control of

Table 8: Antifungal activity of different formulations against *Rhizoctonia solani*

Formulation	Ratio(ml)	Growth Diameter after 7 days(mm)±SD	% Mycelial Growth Inhibition
100% alcoholic crude extract: neem oil cake: cow dung	1:1:8	25.33±0.57	70.31%
100% alcoholic crude extract: neem oil cake: cow dung	2:2:6	35.33±0.57	58.59%
100% alcoholic crude extract: neem oil cake: cow dung	3:3:4	29.00±1.00	66.00%
100% alcoholic crude extract: neem oil cake: cow dung	4:4:2	28.33±0.57	66.79%
100% alcoholic crude extract: neem oil cake: cow dung	5:3:2	28.33±1.15	66.79%
100% alcoholic crude extract: neem oil cake: cow dung	8:1:1	32.66±0.57	61.72%
100% alcoholic crude extract: neem oil cake: cow dung	2:6:2	16.66±0.57	80.47%
100% alcoholic crude extract: neem oil cake: cow dung	4:3:3	34.66±0.57	59.37%
100% alcoholic crude extract: neem oil cake: cow dung	2:4:4	31.33±0.57	63.27%
100% alcoholic crude extract: neem oil cake: cow dung	2:5:3	37.66±1.15	55.86%
100% alcoholic crude extract: neem oil cake: cow dung	1:8:1	31.00±1.00	63.66%
100% alcoholic crude extract: neem oil cake: cow dung	6:2:2	18.33±0.57	78.51%
100% alcoholic crude extract: neem oil cake: cow dung	3:4:3	28.66±0.57	66.40%
100% alcoholic crude extract: neem oil cake: cow dung	4:2:4	35.66±0.57	58.20%
100% alcoholic crude extract: neem oil cake: cow dung	3:2:5	34.33±0.57	59.76%
Petroleum ether extract: neem oil cake: cow dung	5:4:1	35.33±0.57	58.59%
Petroleum ether extract: neem oil cake: cow dung	4:3:3	13.66±0.57	83.98%
Petroleum ether extract: neem oil cake: cow dung	4:4:2	27.66±0.57	70.04%
Petroleum ether extract: neem oil cake: cow dung	4:2:4	33.66±0.57	60.54%
Petroleum ether extract: neem oil cake: cow dung	4:3:3	31.66±0.57	62.89%
Petroleum ether extract: neem oil cake: cow dung	1:4:5	33.66±1.15	60.54%
Petroleum ether extract: neem oil cake: cow dung	3:1:6	34.66±0.57	59.37%

(Contd. Part Table 8)

Petroleum ether extract: neem oil cake: cow dung	7:2:1	32.33±0.57	62.10%
Petroleum ether extract: neem oil cake: cow dung	2:2:6	25.66±0.57	69.92%
Petroleum ether extract: neem oil cake: cow dung	2:1:7	26.66±0.57	68.75%
Petroleum ether extract: neem oil cake: cow dung	8:1:1	15.33±0.57	82.03%
Petroleum ether extract: neem oil cake: cow dung	1:1:8	33.66±0.57	60.54%
Petroleum ether extract: neem oil cake: cow dung	2:7:1	34.66±0.57	59.37%
Petroleum ether extract: neem oil cake: cow dung	1:8:1	36.33±0.57	57.41%
Petroleum ether extract: neem oil cake: cow dung	6:2:2	38.33±0.57	55.07%
Control		80.32±0.57	

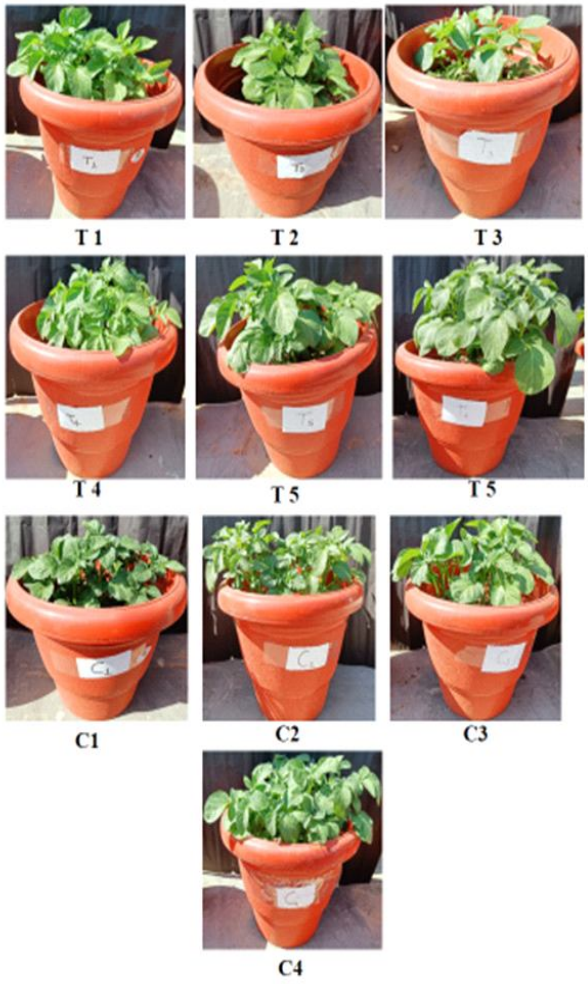

Fig.1: *In vivo* Study of Preventive Effect of Different Bioformulationsagainst *Rhizoctonia solani*(72 Days after sowing)

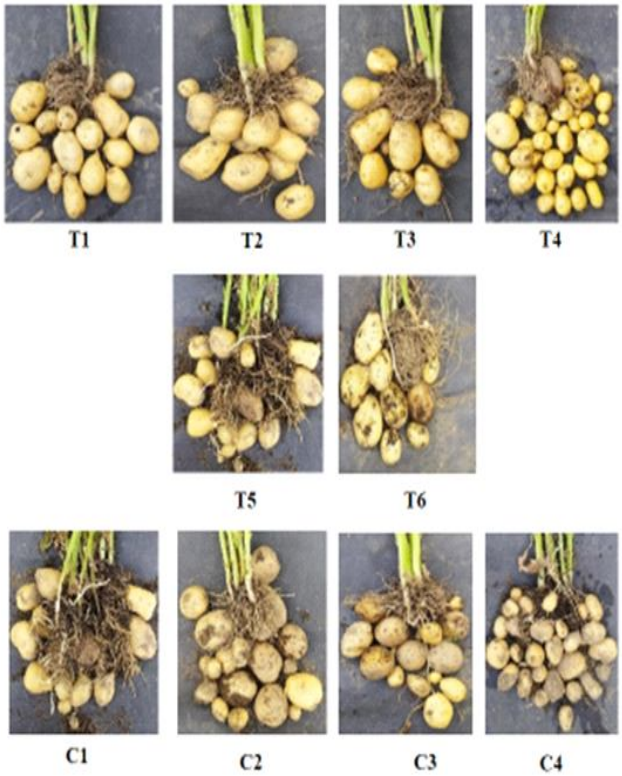

Fig. 2: Effect of Different Bioformulations on Potato Tuber Number and Size

black scurf disease of potato to incredulous the problem of poisonous and harmful fungicides. These bioformulations can be produced at large scale and can be supplied to the farmers either in the form of powder or solutions for application.

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DECLARATIONS

Conflict of Interest: Authors declare no conflict of interest

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