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IN VIVO STUDY ON PREVENTIVE EFFECTS OF *POLYALTHIA LONGIFOLIA* BIOFORMULATIONS AGAINST BLACK SCURF DISEASE OF POTATO

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ABSTRACT

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*. Two different application methods- 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 were recorded 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% cadence levels revealed all the treatment are significant clearly indicating 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 economic loss of potato crop.

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

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most important food crop grown in more than hundred countries across the world. India ranks third in cultivation area and second in production of potatoes in the world after China. In 2020-2021 India produced around 542.3 lakh tons potatoes in 22.48 lakh ha (Horticulture Statistics Division, 2021). Majority of the Indian potatoes come from northern states of Uttar Pradesh, West Bengal, Bihar, Punjab and Gujarat.

Potato tubers comprise of 79% moisture, 21% dry matter and 60-80% starch. Nutritionally, potato is rich in vitamins (B1, B2 and B6), minerals (potassium, phosphorus and magnesium), pantothenic acid and riboflavin (Siddique *et al*., 2020).

Potato black scurf caused by the pathogen *Rhizoctonia solani* Kuhn (Teleomorph *Thanatephorus cucumeris* Donk) is one of the most important potato crop diseases globally as it has a great economic impact and considered as a serious problem in the potato tuber production. In India alone up to 10-25 % crop loss has been reported from this disease (Sharma, 2015).

Major symptoms of this disease are degenerated stem tissues, deformation of tubers and development of black scurf/sclerotia on the surface of tubers hence decline the quality of tubers (Betancourth *et al*., 2021). Farmers face economic losses upto 5-7% due to defacing of the tuber and sclerotia deposition (Shekhawat *et al*., 1993; Singh and Shekhawat, 1994). Infection on potato spread by tubers, soil or the remains of infected plants.

As soon as appropriate conditions are available low temperatures and high soil moisture begin to grow and attack the stems and grown area causing tissue damage as they are in the form of spots and ulcers on the nearby leg from the surface of the soil. Once the infection progresses these spots become brown and then black. 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, 2013; Hada and Sharma, 2015). One of these alternative strategies is the use of natural formulations prepared from plants or plant parts/extract. 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 use *in vivo* experiment. 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.

Inoculum Development

Rhizoctonia solani was cultured on potato dextrose agar (PDA) medium that had been sterilised at 15 psi for 20 minutes seven-day incubation period 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 diameter) 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 (Hada and Sharma, 2017). Herbal formulations were prepared by using *Polyalthia longifolia* leaf extracts, *Polyalthia longifolia* leaf powder, elicitor like neem oil cake (20 gm in 100 ml sterile water) and binder like cow dung (20 gm in 100 ml sterile water) were mixed with 100% alcoholic crude extract, partially purified

petroleum ether extract and dried leaf powder of *Polyalthia longifolia*. All ingredients of herbal formulation were used in following combinations:

- 100% Crude extract (20gm dried plant leaf material dissolve in 100ml alcohol) + 20gm elicitor(neem oil cake) dissolve in 100ml sterile water + 20gm binder (cow dung) dissolve in 100 ml sterile water
- 100% Partially purified extract (40gm dried plant leaf material dissolve in 280ml petroleum ether) + 20gm elicitor (neem oil cake) dissolve in 100 ml sterile water + 20 gm binder (cow dung) dissolve in 100 ml sterile water
- *Polyalthia longifolia* leaf powder+20gm elicitor (neem oil cake) dissolve in 100ml sterile water + 20gm 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 (Ganie *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 (60gm): neem oil cake (20gm): cow dung (20gm)).

T6-combination of leaf powder (40gm): neem oil cake (30gm): cow dung (30gm)).

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 treated with all different formulation. 10 kg soil infected with *Rhizoctonia solani* inoculum (25ml/pot) 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 pots were left for 90 days. 220 gm. 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/plants, plant height, tuber size, tuber weight /pot and numbers 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 Percent Disease Index

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 as showed in table 1 (Muhammad *et al*., 2020)

Table 1. The Percentage Disease Index (on 0 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

The percentage disease Index (PDI) was calculated using following formula **(**Muhammad *et al*., 2020**)**

$$\text{Percent Disease Index} = \frac{\text{Sum of all individual disease ratings}}{\text{Total numbers of plants assessed} \times \text{Maximum rating}} \text{X100}$$

The Percent Efficacy of Disease Control (PEDC) was determined by using formula given by Muhammad *et al*., 2020

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

Growth Parameters

Various growth parameters like number of leaves/plants, plant height, tuber weight/pot, tuber size, number of tubers /pots 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/plants

RESULTS and DISCUSSION

Disease Severity

Results of effect of bioformulations on disease severity of black scurf on potato are given in Table 2. 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. Statistical analysis at 1% and 5% CD values reveals that all the treatments are significant to decrease disease severity in *in vivo* pot experiments.

Table 2. Effect of Treatments on Percentage Disease Index and PEDC

S.N.	Treatment	PDI (%)			Average ± SD	PEDC (%)
		R1	R2	R3		
1.	T1	30	29	26	28.50±2.09	74.65%
2.	T2	36	35	39	36.97± 2.30	58.34%
3.	T3	20	22	18	20.32 ±1.855	77.10%
4.	T4	11	10	9	10.30± 1.03	88.39%
5.	T5	44	46	44	45 ±1.32	49.30%
6.	T6	42	41	40	41.24± 1.19	53.53%
7.	C1	16	17	16	16.75± 0.85	81.12%
8.	C2	56	58	60	58.14±2.06	-
9.	C3	85	88	85	88.76±85.29	-
10.	C4	56	55	53	55.14±1.2	-
11.	SE m^+	–	–	–	0.919	-
12.	CD(P=0.05)	–	–	–	2.727	-
13.	CD(P=0.01)	–	–	–	3.966	-
14.	CV (%)	–	–	–	5.169	-

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 Table no. 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/Plants

In Table 3 shows 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.

Table 3. Effect of Treatments on Number of Leaves/Plant

S.N.	Treatment	Number of Leaves			Average ± SD
		R1	R2	R3	
1.	T1	46	48	46	46.66±1.15
2.	T2	42	44	44	43.33±1.15
3.	T3	48	47	49	48±1
4.	T4	56	57	57	56.66±0.57
5.	T5	51	49	51	50.33±1.15
6.	T6	50	50	48	49.33±1.15
7.	C1	55	54	55	54.66±0.57
8.	C2	34	36	33	34.33±1.52
9.	C3	45	46	46	45.66±0.57
10.	C4	46	47	47	46.66±0.57
11.	SE m^+	–	–	–	0.546
12.	CD(P=0.05)	–	–	–	1.621
14.	CD(P=0.01)	–	–	–	2.357
15.	CV (%)	–	–	–	2.088

Plant Height

Table 4 showed In comparison to the control, the treatment with formulations and mancozeb increased plant height. The T4 treatment developed the tallest plants (27.33 cm), followed by T6 (26.33 cm), and T5 (25.33 cm). 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.

Table 4. Effect of Treatments on Plant Height (cm)

S.N.	Treatment	Plant Height (cm)			Average ± SD
		R1	R2	R3	
1.	T1	24	26	25	25±1
2.	T2	25	24	25	24.66±1
3.	T3	26	25	24	25±1
4.	T4	27	27	28	27.33±0.57
5.	T5	24	26	26	25.33±1.15
6.	T6	26	27	26	26.33±0.57
7.	C1	24	22	24	23.33±1.15
8.	C2	24	22	22	22.66±1.15
9.	C3	17	18	19	18±1
10.	C4	14	16	14	14.66±1.15
11.	SE m^+	–	–	–	0.540
12.	CD(P=0.05)	–	–	–	1.603
13.	CD(P=0.01)	–	–	–	2.331
14.	CV (%)	–	–	–	4.267

Total Tuber Weight /Pot

Table 5. Shows the T4 treatment was 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.

Table 5. Effect of Treatments on Total Tuber Weight (gm.)

S.N.	Treatment	Total Tuber Weight (gm.)			Average ± SD
		R1	R2	R3	
1.	T1	204.10	204.40	203.20	203.9±0.62
2.	T2	212.24	211.10	213.46	212.2667±1.18
3.	T3	226.29	225.16	224.96	225.47±0.71
4.	T4	248.16	247.00	247.20	247.4533±0.62
5.	T5	192.28	191.16	190.20	191.2133±1.04
6.	T6	187.48	186.16	188.86	187.5±1.35
7.	C1	202.00	201.46	200.86	201.44±0.57

S.N.	Treatment	Total Tuber Weight (gm.)			Average ± SD
		R1	R2	R3	
8.	C2	168.24	167.24	168.18	167.8867±0.56
9.	C3	128.00	126.00	127.49	127.16±1.03
10.	C4	158.00	156.00	157.15	157.05±1.00
11.	SE m^+	–	–	–	0.503
12.	CD(P=0.05)	–	–	–	1.492
13.	CD(P=0.01)	–	–	–	2.170
14.	CV (%)	–	–	–	0.477

Tuber Size

Table 6 shows the results of the impact of various treatments on tuber size. With C3, the smallest tuber size was noticed. C4 was followed by C2 in terms 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 (4.76 cm). All of the treatments are significant, according to statistical analysis at 5% and 1% CD.

Table 6. Effect of Treatments on Tuber Size (cm)

S.N.	Treatment	Tuber Size (cm)			Average ± SD
		R1	R2	R3	
1.	T1	5.3	5.2	5.3	5.26±0.05
2.	T2	5.7	5.7	5.6	5.66±0.05
3.	T3	6.0	6.1	6.2	6.1±0.1
4.	T4	6.7	6.6	6.7	6.66±0.05
5.	T5	5.0	5.0	4.9	4.96±0.05
6.	T6	4.8	4.8	4.7	4.76±0.05
7.	C1	5.2	5.2	5.1	5.16±0.05
8.	C2	4.7	4.6	4.7	4.76±0.05
9.	C3	3.0	2.9	3.0	2.96±0.05
10.	C4	4.1	4.0	4.0	4.03±0.05
11.	SE m^+	–	–	–	0.036
12.	CD(P=0.05)	–	–	–	0.106
13.	CD(P=0.01)	–	–	–	0.154
14.	CV (%)	–	–	–	1.273

Number of Tubers/Pot

Table 7 shows the results of the impact of various treatments and synthetic fungicide on the quantity 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 (15.66) among the treatments used. The treatments T5 (13.66) and T6 (11.66) had the fewest tubers per pot. All of the treatments were shown to be statistically significant for field trials at 5% and 1% CD values.

Table 7. Effect of Treatments on Number of Tubers

S .N.	Treatment	Number of Tubers			Average ± SD
		R1	R2	R3	
1.	T1	15	15	14	14.66667±0.57
2.	T2	16	16	15	15.66667±0.57
3.	T3	18	18	17	17.66667±0.57
4.	T4	19	20	19	19.33333±0.57
5.	T5	14	13	14	13.66667±0.57
6.	T6	12	12	11	11.66667±0.57
7.	C1	15	14	15	14.66667±0.57
8.	C2	14	15	14	14.33333±0.57
9.	C3	14	14	13	13.66667±0.57
10.	C4	11	11	10	10.66667±0.57
11.	SE m^+	–	–	–	0.333
12.	CD(P=0.05)	–	–	–	0.990
13.	CD(P=0.01)	–	–	–	1.439
14.	CV (%)	–	–	–	4.065

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 (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 extracts have been shown to exhibit fungicidal action (Ashraf et al., 2011; Rajamanickam and Sudha, 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. According to El-Bakali et al. (2006), a successful disease control program involves cultural techniques, fungicides, biological control, and solarization. Several researchers have reported using plant extracts to treat plant diseases (Jabeen et al., 2021; Asuquo et al., 2017). Different induced defense mechanisms are present in plants to defend against pathogen attack. Neem kernel cake powder (NKCP) was studied by Kimaru et al. (2004) in relation to tomato Fusarium wilt growth. Hamid et al. (2011) successfully suppressed the potato black scurf disease using manure and soil solarization treatment.

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 present in plant extract, elicitor and binder. *Polyalthia longifolia* contain several secondary metabolite like terpenoid, flavanoids, volatile oil, alkaloids, saponin, phytosterols, and tannins. (Parveen and Sharma, 2015; Mehta and sharma, 2016; Kaur *et al*., 2022 ; Karak, 2019; Salvatore *et al*., 2020; Meena *et al*., 2021). Review of literature suggests that several reports are available on 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). 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 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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