



Management of Powdery Scab (*Spongospora subterranea*) of Potato in Dailekh, Nepal

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ABSTRACT

Potato powdery scab has been reported in a severe form in different potato-growing areas of Nepal, particularly in western hills. An experiment was carried out to investigate the eco-friendly management options using bio-fungicide fortified organic amendments and safer chemicals for powdery scab disease of potato under field conditions in Dailekh. The experiment was conducted in a randomized complete block design with 12 treatments and 3 replications. Three Biocontrol Agents viz; *Trichoderma viride*, *Pseudomonas fluorescens*, and *Bacillus subtilis* were used in three different organic amendments viz; farmyard manure, vermicompost, and spent mushroom substrate as soil application along with potato tuber dip with respective BCAs. Also, boric acid was used for tuber treatment. A fungicide Metalaxyl+Mancozeb was used for tuber treatment along with soil treatment by Flusulfamide (Nebujin). The field efficacy of bio-fungicide fortified organic amendment and safer chemicals for management of powdery scab of potato were evaluated along with B:C ratio of different management options for powdery scab of potato over untreated control. Among different treatments, potato seed tuber dip with *Trichoderma viride* @ 10 gm/kg coupled with soil application of *Trichoderma viride* fortified vermicompost @ 100 kg/ha was found highest percent disease control (51.3%) followed by potato seed dip with boric acid @ 3 percent with 36.54 percent disease control. Highest B:C ratio (12.7) was found in case of potato seed dip with boric acid @ 3 percent followed by seed tuber dip with *Trichoderma viride* @ 10 gm/kg coupled with soil application of *Trichoderma viride* fortified spent mushroom substrate @ 100 kg/ha as compared to other management treatments. The study revealed the effective powdery scab disease control using consortia of seed tuber dip with Bio-Control Agents (BCA) and BCA fortified organic amendments.

Keywords: *Spongospora*; Powdery scab; Bio control agent; Bio fortified compost; Boric acid

INTRODUCTION

Powdery scab caused by *Spongospora subterranea* (Wallroth) Lagerheim f.sp. *subterranea* is a serious disease of potato worldwide. The disease has been reported in a severe form in different potato growing areas of Nepal, particularly western hills. At present, it is a major problem for fresh and seed tuber producer as it reduces substandard appearance and marketability of tuber which makes it as a quarantine potato disease in Nepal. The disease is a major reason for limiting the export potato from western hills. The infected tubers were planted in same field year after year. When the monsoon brings abundant rain during growing season, condition

was often favourable for the development of powdery scab. The disease causes powdery lesion on the surface which appears like scab containing mass of powdery spore balls. The initial visible symptom is the development of purple to brown pimple like swellings at the rose end of the tuber later on the pustules can increase in size and rupture the epidermis. The individual circular scab lesions can develop to approximately 10 mm in size and the shapes may become irregular when the lesions become large and merge together. Other symptoms may include galls and cankers which develop on the roots. The gall formations are seen in roots where there is no drainage facility and swampy field conditions. But, in hot dry condition of field no gall formation in roots. Galls may

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also develop on potato stolons and roots which become dark brown as they mature. The pathogen may be seed borne or soil borne prevalent in both cool temperate and hot dry climatic regions. In Nepal, potato powdery scab was first reported from a potatoes which grown about 3650 m above sea level in the Sherpa winter village, Jabluk near Beding in the Rolwaling valley in the North East. The development of disease in the tuber is either by planting disease free seed tubers in infested soil or infected seed tuber in non-infested soil. The disease is becoming a major constraint for profitable cultivation of potato under field conditions. Increasing concern regarding chemical residue, environment and health hazard, the impact of excessive pesticide usage on fragile Hilly ecosystem and mainstreaming of organic agriculture in Karnali Province at policy level demand alternative ecofriendly management method for powdery scab. Moreover, chemical treatments have been reported to provide limited control over disease [1].

The use of bio control agents and organic amendments has been suggested as an alternative to chemical for controlling plant disease. The recent study suggested that organic amendments have disease suppressing properties without chemical residue especially those caused by soil borne pathogen. The amendments commonly result in a highly metabolically active micro biota which can be antagonistic towards many pathogens. The mechanism of disease control for high nitrogen-containing amendments is the generation of ammonia and or nitrous acid following degradation of the amendments by microorganisms [2]. The formation of these products to concentrations lethal to pathogens is regulated by the soil pH, organic matter content, and nitrification rate, sand content and buffering capacity. Similarly, biological control agent,

Trichoderma spp has potential to parasitize spore balls of *Spongospora* under controlled experimental growing conditions. Recent study has revealed the ability of endophytes to suppress this disease which includes *Bacillus subtilis*, *Gliocladium catenulatum*, *Heteroconium chaetospora*, *Microbispora rosea* sp., *Streptomyces griseoruber*, *S. griseoviridis*, *Streptomyces lydicus*, *S. olivochromogenes*, *Trichoderma atroviride*, *T. harzianum* and *T. Viride*.

Research and studies against management of potato powdery scab in Dailekh are limited. The main objectives of the research was to investigate the eco-friendly management options using bio-fungicide fortified organic amendments and safer chemicals for powdery scab disease of potato under field condition in Dailekh [3].

MATERIALS AND METHODS

Experimental location

The experiment was carried out at Gurash Rural Municipality ward number 4, Dailekh district Nepal during 2020/2021. The study site is located at 28.74 °N latitude 81.65 °E longitudes with elevation of 1360 masl.

Experimental design

The experimental design details for this experimental trial are given in Table 1 [4].

Treatments details

The treatments used in the experiment along with application dosage and application method are given in Table 2.

Table 1: Details of experimental design used in study site at Rittha, Gurash Rural Municipality-04, Dailekh, Nepal, 2021.

SN.	Particulars	Trial details
1.	Design	Randomized Complete Block Design (RCBD)
2.	Replication (Block)	3
3.	Treatments	12
4.	Plot size	2.8 m × 1 m
5.	Spacing between replication	50 cm
6.	Spacing between plot within replication	30 cm
7.	Total number of plots	36
8.	Number of plants per plot	16
9.	Total number of plants	576
10.	Spacing	70 cm × 25 cm (row to row and plant to plant respectively)
11.	Sample	4

Table 2: List of treatments, application dosage and application method.

SN.	Treatment details
1.	Seed tuber dip with <i>Trichoderma viride</i> formulation (10 gm/kg)+Soil application of <i>Trichoderma viride</i> enriched well decomposed farm yard manure (5 kg <i>Trichoderma</i> mixed in 100 kg cow dung slurry per hectare)-incubate for 7 days in shade
2.	Seed tuber dip with <i>Trichoderma viride</i> formulation (10 gm/kg)+Soil application of <i>Trichoderma viride</i> in vermicompost (5 kg <i>Trichoderma</i> mixed in 100 kg vermicompost per hectare)
3.	Seed tuber dip with <i>Trichoderma viride</i> formulation (10 gm/kg)+Soil application of <i>Trichoderma viride</i> in spent mushroom substrate (5 kg <i>Trichoderma</i> mixed in 100 kg spent mushroom substrate per hectare)
4.	Seed tuber dip with <i>Pseudomonas fluorescens</i> formulation (10 gm/kg)+Soil application of <i>Pseudomonas fluorescens</i> enriched well decomposed farm yard manure (5 kg <i>Pseudomonas fluorescens</i> mixed in 100 kg cow dung slurry per hectare)
5.	Seed tuber dip with <i>Pseudomonas fluorescens</i> formulation (10 gm/kg)+soil application of <i>Pseudomonas fluorescens</i> in vermicompost (5 kg <i>Pseudomonas fluorescens</i> mixed in 100 kg vermicompost per hectare)

6.	Seed tuber dip with <i>Pseudomonas fluorescens</i> formulation (10 gm/kg)+Soil application of <i>Pseudomonas fluorescens</i> in spent mushroom substrate (5 kg <i>Pseudomonas fluorescens</i> mixed in 100 kg spent mushroom substrate per hectare)
7.	Seed tuber dip with <i>Bacillus subtilis</i> formulation (10 gm/kg) for 30 minutes followed by shade drying+Soil application of <i>Bacillus subtilis</i> enriched well decomposed farm yard manure (5 kg <i>Bacillus subtilis</i> mixed in 100 kg cow dung slurry per hectare)
8.	Seed tuber dip with <i>Bacillus subtilis</i> formulation (10 gm/kg)+Soil application of <i>Bacillus subtilis</i> in vermicompost (5 kg <i>Bacillus subtilis</i> mixed in 100 kg vermicompost per hectare)
9.	Seed tuber dip with <i>Bacillus subtilis</i> formulation (10 gm/kg)+soil application of <i>Bacillus subtilis</i> in spent mushroom substrate (5 kg <i>Bacillus subtilis</i> mixed in 100 kg spent mushroom substrate per hectare)
10.	Seed tuber dip with Boric acid @3% (30 gm/l)
11.	Seed tuber dip with Metalyxal+Mancozeb (2.5 gm/kg)+soil application of Nebujin (Flusulfamide) (3 gm/10 square meter)
12.	Untreated control

Note: In each treatment, seed tubers were dipped for 30 minutes and followed by shade drying before planting.

Experiment details

Potato seed tubers were collected from Nepal potato seed production farmer group, Gurash-05, Dailekh. Also, the required treatments *Trichoderma viride*, *Pseudomonas fluorescens*, *Bacillus subtilis*, boric acid, Metalyxal+Mancozeb, Flusulfamide, well decomposed farmyard manure, vermicompost and spent mushroom substrate were collected. *Trichoderma viride*, was mixed with well decomposed farm yard manure and incubates for 7 days [5].

In each treatment, seed tubers were dipped for 30 minutes and followed by shade drying before planting.

To make soil free from large soil clods and weeds, about 3-4 deep ploughing followed by harrowing was done. The respective bio control agents were fortified with respective organic amendments. The soil application was done by incorporating bio fortified organic amendments after land preparation. The seed tubers were dipped for 30 minutes and followed by shade drying before planting. After sowing the tubers, ridges and furrow were made in the field and complete plantation procedures were done. The recommended dose for potato as enlisted in was used: FYM: 30 ton ha⁻¹, Urea: 140 kg ha⁻¹, DAP: 220 kg ha⁻¹, Potash: 100 kg ha⁻¹. All the fertilizer doses were incorporated into the field before sowing except nitrogen which was used half dose as basal dose and half dose after 25 days of plantation [6]. Potato plants were irrigated in furrow. Irrigation was done after 30 days of planting. Flooded irrigation was avoided and water level was maintained below 2/4th of ridges while irrigating the field. The variety used was Desiree and its day to maturity is around 90 days. Therefore, potato from the experimental field was harvested after 90 days of plantation. The climatic data for the entire duration of the research is given in Figure 1.

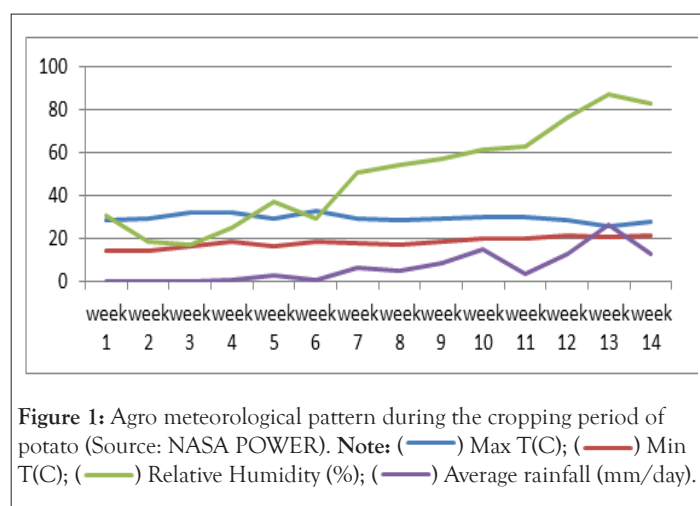


Figure 1: Agro meteorological pattern during the cropping period of potato (Source: NASA POWER). Note: (—) Max T(C); (—) Min T(C); (—) Relative Humidity (%); (—) Average rainfall (mm/day).

Data collection

The data observation was done from 4 sample plant, centre of each plot, immediately after harvesting [6].

Pathological observations

Disease severity: The disease severity was determined using disease index given. All the tubers from the sample plant were washed in water and visually assessed for scoring the disease severity (Figure 2). The disease scores from disease severity assessment data sets were converted to proportions of surface area covered by diseased using the conversion. Percentage surface area less than 20% has different conversion compared to percentage surface area greater than 20% [7-10]. These conversions should be carried out using mean values after statistical analysis of disease scores. An example is that of potato tubers from each of two plants. The powdery scab severity scores for the seven tubers from one of the plants were 6,4, 5, 5, 6,7, and 6 (mean=5.57), whereas those for the nine tubers from the other plant were 2,2,1,2,1,2,1,2, and 1 (mean=1.56). Mean score converted to proportions of tuber surfaces diseased of 41% and 8% respectively.

These conversions will be made arithmetically using the equations: Y=5X (for disease scores<4); and Y=13.3X-33 (for disease scores>4); where Y=% of surface area diseased, and X=mean disease score.

Percentage Tuber Infection (PTI): Percentage tuber infection is a proportion of infected tuber in a plot [11,12]. The tuber with scores 0 and 1 were categorized as healthy tuber and remaining score were categorized as infected tuber. The PTI was calculated by given formula;

$$PTI = \frac{\text{Number of infected tubers in a plot}}{\text{Total number of tubers in a plot}} \times 100$$

Percent Disease Control (PDC): PDC was calculated by given formula;

$$PDC = \frac{\text{Disease severity in control plot} - \text{Disease severity in treated plot}}{\text{Disease severity in control plot}} \times 100$$

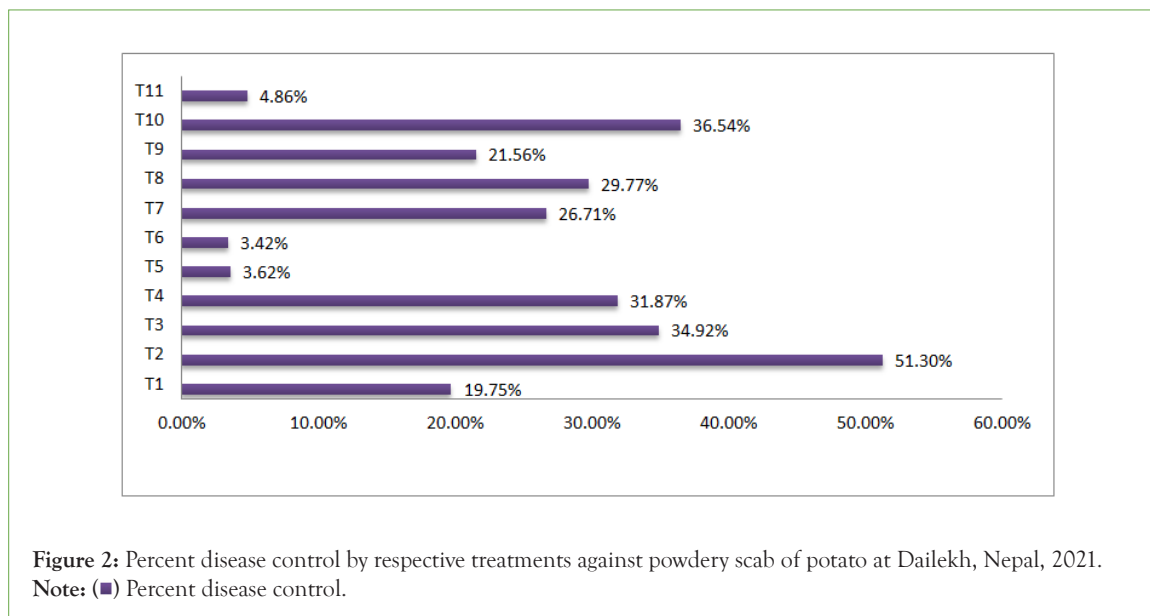
Biometric observations

Fresh root weight: The fresh root weight was recorded by removing soil particles from root zone.

Fresh shoot weight: Yield per plot: Yield per plot was recorded as the sum of healthy and infected tuber yield harvested from a plot.

Benefit cost return over control untreated:

$$B/C = \frac{\text{Additional benefit of the produce from treatment}}{\text{Additional cost (Variable cost) of the treatment}}$$



Statistical analysis

The collected data were systematically arranged and entered in MS Excel. Then the arranged data were analyzed using the software, R studio. The means were compared by using Duncan’s Multiple Range Test (DMRT) at 5% level of significance (Gomez and Gomez). Effectiveness of treatments was studied based on above mentioned parameter.

RESULTS

Disease severity and percentage tuber infection

The disease severity of powdery scab disease on potato was found significantly influenced by the application of the treatments [13]. Comparing all the treatment, the significantly lower disease severity was found on T2 (5.10) which were statistically at par with T10 and T3 while the significantly higher disease severity was found on T12 (10.48) which was statistically at par with T5, T6 and T11. The influence of different treatments on percentage tuber infection of

powdery scab of potato is shown in (Table 2). There was a significant difference in percentage tuber infection by the treatments (Table 3). The percentage tuber infection was significantly lower in T4 (19.22) which were statistically at par with T8 while the percentage tuber infection was significantly higher in T12 (76.25) which were statistically at par with T7 and T6 (Table 4).

Percent disease control

The bar diagram shows percent disease control by respective treatments against powdery scab of potato. The treatment seed tubers dipped with *Trichoderma viride* formulation+soil application of *Trichoderma viride* bio fortified with vermicompost (T2) showed higher (51.30) percent disease control followed by treatment seed tubers dipped with boric acid (T10) while the treatment seed tubers dipped with *Pseudomonas fluorescens* formulation+soil application of *Pseudomonas fluorescens* bio fortified spent mushroom substrate (T6) shows lower (3.42) percent disease control followed by treatment seed tuber dipped with *Pseudomonas fluorescens* formulation+soil application of *Pseudomonas fluorescens* bio fortified vermicompost (T5) (Table 5) [14].

Table 3: Influence of different treatments on disease severity and percentage tuber infection of potato powdery scab at 90 DAS in Dailekh, Nepal, 2021.

Treatments	Disease Severity	Percentage Tuber Infection
T1=Seed tuber dip with <i>Trichoderma viride</i> formulation+Soil application of <i>Trichoderma viride</i> enriched FYM	8.41	32.18
T2=Seed tuber dip with <i>Trichoderma viride</i> formulation+Soil application of <i>Trichoderma viride</i> in vermicompost	5.10	21.66
T3=Seed tuber dip with <i>Trichoderma viride</i> formulation+Soil application of <i>Trichoderma viride</i> in spent mushroom substrate	6.82	39.82
T4= Seed tuber dip with <i>Pseudomonas fluorescens</i> formulation+Soil application of <i>Pseudomonas fluorescens</i> enriched FYM	7.14	19.22
T5=Seed tuber dip with <i>Pseudomonas fluorescens</i> formulation+Soil application of <i>Pseudomonas fluorescens</i> in vermicompost	10.10	51.41
T6=Seed tuber dip with <i>Pseudomonas fluorescens</i> formulation+Soil application of <i>Pseudomonas fluorescens</i> in spent mushroom substrate	10.12	59.04
T7=Seed tuber dip with <i>Bacillus subtilis</i> formulation+Soil application of <i>Bacillus subtilis</i> enriched FYM	7.68	60.96
T8=Seed tuber dip with <i>Bacillus subtilis</i> formulation+Soil application of <i>Bacillus subtilis</i> in vermicompost	7.39	25.04
T9=Seed tuber dip with <i>Bacillus subtilis</i> formulation+Soil application of <i>Bacillus subtilis</i> in spent mushroom substrate	8.22	48.83

T10= Seed tuber dip with boric acid	6.65	39.50
T11=Seed tuber dip with Metalyaxyl+Mancoze +Soil application of Nebujin (Flusulfamide)	9.97	57.74
T12=Untreated	10.48	76.25
F-Probability	<0.01	<0.001
SEM (±)	0.270	2.48
LSD (p ≤ 0.05)	2.74**	23.09***
CV (%)	19.82	30.77
Grand Mean	8.177	44.31

Note: DAS: Days After Sowing; SEM: Standard Error of Mean; CV: Coefficient of Variation; LSD: Least Significant Difference. Means in the column with same letter (s) in superscript indicate no significant difference between treatments at 5% level of significance; '****' Significant at 0.1 % level of Significance; '***' Significant at 1 % level of Significance; '**' Significant at 5 % level of Significance.

Table 4: Influence of different treatments on biometric parameters against powdery scab of potato at 90 DAS in Dailekh, Nepal, 2021.

Treatments	Fresh root weight (g)	Fresh shoot weight (g)	Yield per plot (kg)
T1=Seed tuber dip with <i>Trichoderma viride</i> formulation+Soil application of <i>Trichoderma viride</i> enriched FYM	33.75	676.00	8.91
T2=Seed tuber dip with <i>Trichoderma viride</i> formulation+Soil application of <i>Trichoderma viride</i> in vermicompost	29.01	473.41	9.29
T3=Seed tuber dip with <i>Trichoderma viride</i> formulation+Soil application of <i>Trichoderma viride</i> in spent mushroom substrate	33.79	615.79	10.34
T4=Seed tuber dip with <i>Pseudomonas fluorescens</i> formulation+Soil application of <i>Pseudomonas fluorescens</i> enriched FYM	26.47	553.41	10.25
T5=Seed tuber dip with <i>Pseudomonas fluorescens</i> formulation+Soil application of <i>Pseudomonas fluorescens</i> in vermicompost	29.08	435.50	9.04
T6=Seed tuber dip with <i>Pseudomonas fluorescens</i> formulation+Soil application of <i>Pseudomonas fluorescens</i> in spent mushroom substrate	38.21	713.25	10.34
T7=Seed tuber dip with <i>Bacillus subtilis</i> formulation+Soil application of <i>Bacillus subtilis</i> enriched FYM	39.85	651.50	10.11
T8=Seed tuber dip with <i>Bacillus subtilis</i> formulation+Soil application of <i>Bacillus subtilis</i> in vermicompost	27.75	585.79	10.31
T9=Seed tuber dip with <i>Bacillus subtilis</i> formulation+Soil application of <i>Bacillus subtilis</i> in spent mushroom substrate	31.75	528.58	8.57
T10= Seed tuber dip with boric acid	30.21	578.58	9.36
T11=Seed tuber dip with Metalyaxyl+Mancozeb+Soil application of Nebujin (Flusulfamide)	29.42	448.61	8.86
T12=Untreated	29.58	514.75	8.15
F-Probability	~	0.05	~
SEM(±)	0.90	16.443	13.74
LSD(p ≤ 0.05)	Ns	167.06*	Ns
CV (%)	17.12	17.47	13.68
Grand Mean	31.57	564.59	9.41

Note: DAS: Days After Sowing, SEM: Standard Error of Mean; CV: Coefficient of Variation; LSD: Least Significant Difference. Means in the column with same letter (s) in superscript indicate no significant difference between treatments at 5% level of significance; '****' Significant at 0.1 % level of Significance; '***' Significant at 1 % level of Significance; '**' Significant at 5 % level of Significance.

Table 5: Benefit cost ratio of treatments over untreated control against potato powder scab in Dailekh, Nepal, 2021.

Treatments	Additional benefit of the produce from treatment (NRs./ha)	Additional cost of the treatment (NRs./ha)	B:C ratio
T1=Seed tuber dip with <i>Trichoderma viride</i> formulation+Soil application of <i>Trichoderma viride</i> enriched FYM	63598.31	17725	3.5
T2=Seed tuber dip with <i>Trichoderma viride</i> formulation+Soil application of <i>Trichoderma viride</i> in vermicompost	32242	20625	1.5
T3=Seed tuber dip with <i>Trichoderma viride</i> formulation+Soil application of <i>Trichoderma viride</i> in spent mushroom substrate	206008.31	17825	11.5

T4=Seed tuber dip with <i>Pseudomonas fluorescens</i> formulation+Soil application of <i>Pseudomonas fluorescens</i> enriched FYM	176136.73	21250	8.2
T5=Seed tuber dip with <i>Pseudomonas fluorescens</i> formulation+Soil application of <i>Pseudomonas fluorescens</i> in vermicompost	74247.17	24150	3.07
T6=Seed tuber dip with <i>Pseudomonas fluorescens</i> formulation+Soil application of <i>Pseudomonas fluorescens</i> in spent mushroom substrate	184079.46	21350	8.62
T7=Seed tuber dip with <i>Bacillus subtilis</i> formulation+Soil application of <i>Bacillus subtilis</i> enriched FYM	212601.74	19840	10.74
T8=Seed tuber dip with <i>Bacillus subtilis</i> formulation+Soil application of <i>Bacillus subtilis</i> in vermicompost	181622.03	22740	7.98
T9=Seed tuber dip with <i>Bacillus subtilis</i> formulation+Soil application of <i>Bacillus subtilis</i> in spent mushroom substrate	35573	19940	1.78
T10= Seed tuber dip with boric acid	101547	7995	12.70
T11=Seed tuber dip with Metalaxyl+Mancozeb+Soil application of Nebujin (flusulfamide)	59542	10200	5.83

Benefit cost ratio of treatments over untreated control

The highest B:C ratio was observed in treatment seed tubers dip with boric acid T10 (12.70) followed by the treatment seed tubers dip with *Trichoderma viride* formulation+soil application of *Trichoderma viride* bio fortified spent mushroom substrate (T3) while the lowest B:C ratio (1.5) was observed in treatment seed tubers dip with *Trichoderma viride* formulation+soil application of *Trichoderma viride* bio fortified with vermicomposting (T2) [15-17].

DISCUSSION

The biocontrol agent *Trichoderma viride* bio fortified with vermicompost @100 kg/ha reduces higher disease severity and highest percent disease control against soil borne disease. Similar result was also found by Subashini, 2021. The antagonistic microbes *Trichoderma* spp has the ability to parasitized spore balls of *Spongospora subterranea* under control experimental condition. The report aligns with the experimental findings. The seed treatment with boric acid @3% also reduces disease severity along with higher percent disease control. Similar result was reported in Jan, 2002 on the experiment on effect of seed or soil treatment with fungicides on the control of powder scab of potato. The bio control agent *Bacillus subtilis* in green house and field condition reduces disease severity against seed and soil borne infection. The report aligns with findings. The reduction of disease severity ultimately had decreased percentage tuber infection and had increased percent disease control in the plot treated with bio control agent and biocontrol agent fortified with organic amendments. The treatment seed tubers dipped with (Metalaxyl+Mancozeb)+soil application of Flusulfamide was found to be incapable in reducing disease severity, lower percent disease control and higher percentage tuber infection. Moreover, chemical treatments have been reported to limited control over powdery scab of potato. Falloon trial had confirmed various chemicals, including, Mancozeb reduced the proportion of diseased tubers in the potato. There is no significant reduction in the yield as no gall formations were seen in root zone in the experimental plot. Study indicates that there is no reduction in yield as powdery scab effect the substandard appearance of tuber. The study is aligns with the findings. For controlling powdery scab no sole method is adequate as suggested by Harrison JG. So, the consortia of seed tuber dip with biological agent and biological agent fortified organic amendments reported effective management in findings.

CONCLUSION

Among different management treatments, potato seed tuber dip with *Trichoderma viride* @10 gm/kg coupled with soil application of *Trichoderma viride* fortified vermicompost @100 kg/ha was found most promising in terms of disease control followed by potato seed dip with boric acid @3 percent. The highest B:C ratio was found in case of potato seed dipped with boric acid @3 percent followed by seed tuber dipped with *Trichoderma viride* @10 gm/kg coupled with soil application of *Trichoderma viride* fortified spent mushroom substrate @100 kg/ha as compared to other management treatments. The study revealed the effective control of powdery scab disease using consortia of seed tuber dip with Bio-Control Agents (BCA) and BCA fortified organic amendments.

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