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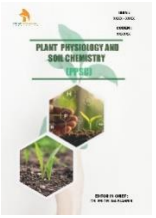

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RESEARCH ARTICLE

INVITRO EFFICACY OF TRICHODERMA ISOLATES ON SCLEROTINIA SCLEROTIORUM CAUSING WHITE MOLD OF KIDNEY BEAN

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ABSTRACT

The experiment was conducted in plant pathology laboratory of Nepal polytechnic institute for studying in invitro efficacy of *Trichoderma* isolates on *Sclerotinia sclerotiorum* causing white mold of kidney bean, Bharatpur, Chitwan, Nepal by dual culture technique. The experiment was carried out in completely randomized design (CRD) with four replications. The *Trichoderma* isolates namely Kapilvastu Isolate, Kavre Isolate, Salyan Isolate, Lalitpur Isolate and Taplejung Isolate were used in the experiment. The mycelium growth was measured at 2DAI, 4DAI, 6DAI, 8DAI and 10DAI. Also the number of Sclerotia, days to Sclerotia formed and width of the browning area at the region of interception were measured in 10DAI. All the *Trichoderma* isolates shows significant effect on the mycelium growth and number of Sclerotia formed. Among all the *Trichoderma* isolates, Lalitpur Isolate shows good result with 69.33% inhibition in the mycelium growth and number of Sclerotia (9.6~10) formed.

KEYWORDS

Trichoderma isolates, Sclerotia, Sclerotinia sclerotiorum

1. INTRODUCTION

Kidney beans (Phaseolus vulgaris) are also known as the common bean (Rajma) and French beans, belong to the family Fabaceae, and are an annual herbaceous plant grown worldwide for its unripe fruit or edible dried seeds. In 2016/17, green bean production was 296 tons under the 108 hectare cultivated areas producing 27407 kg/ha. It grows the next year 2017/18 to 320 tons under 115 hectares producing 27826 kg/ha. By 2018/19 production increased to 344 tons under 123 ha in a fertile area producing 27967 kg/ha. Although the production of dry beans in 2016/17 was 20056 tons of less than 23464 ha in the cultivated area producing 8548 kg/ha. Production decreased the following year of 2017/18 to 19576 tons less than 22757 ha in the cultivated area producing 8602 kg/ha. And the production was reduced to 19095 tons below 22058 ha in the cultivated area producing 8657 kg/ha (*FAO, 2022*).

Boiled kidney beans contain 67% water, 23% carbohydrates, 9% protein, and contain saturated fats (table). At a reference value of 100 grams, cooked kidney beans provide 530 kJ (127 kcal), and are not a rich source (20% or more of Daily Value, DV) of protein, and folate (33% DV), iron (22% DV).), and phosphorus (20% DV), with moderate amounts (10-19% DV) of thiamin, copper, magnesium, and zinc (11-14% DV). When the temperature is between 12-15 ° C, the vegetation is strong and when it is below 15 ° C, most fruits take the form of "chicken". Above 30 ° C, deformed pods emerge and flower abortions occur. Beans can grow in a variety of soils but are best cultivated with lightweight and sandy loam texture and well-drained soil rich in organic matter.

Sclerotinia sclerotiorum is a necrotrophic pathogen that attacks more than 400 species of plants, including a few economically important plants (Allan et al., 2019). The fungus that causes the plant can also cause a disease called white mold and cottony rot, soft water rot, stem rot, fall, crown rot, and flower rot. It is part of Ascomycota. Its wide variety and ability to infect plants at any stage of growth make white mold a very serious disease. The fungus can live on infected tissue, soil, and living plants. A key feature of this pathogen is its ability to produce a dark resting structure known as Sclerotia and a dull white growth of mycelium in an infected plant. White mold can spread quickly from one crop to another, and it may even spread to other areas throughout the harvest. Other important crops commonly affected are soybeans, green beans, sunflowers, canola, and peanuts.

Trichoderma is a fungus belonging to the genus Hypocreaceae found in all soils, where it is the most commonly grown fungus. Many species of this genus can be seen as potential predators of a variety of endangered plants (Harman et al., 2004)This refers to the ability of several Trichoderma species to form harmonious endophytic relationships with several plant species (Bae et al., 2011). The genome of many Trichoderma spp. edited and publicly available at the Joint Genome Institute (Mycocosm, 2021)

2. MATERIALS AND METHODS

2.1 Collection and Isolation of the Pathogen

All the experiments were done at the research laboratory of Nepal Polytechnic Institute (NPI), Bhojard-11, Bhartpur, and Chitwan Nepal. The glassware's were sterilized in a hot air oven at 140-180 degree centigrade for 2 hours and media wares are sterilized in an autoclave at 15psi for 15-30min.

Diseases plants infected with *Sclerotinia sclerotiorum* which is the pathogen for causing white mold in kidney bean, diseases samples were collected from farmer's field near NPI, Bharatpur. The diseased sample was placed on paper envelope and brought to the laboratory then the samples were preserved at 4 degree centigrade in the refrigerator for isolation of *Sclerotium rolfsii.* Similarly, Trichoderma isolates were obtained from AFU, Rampur. Potato dextrose agar media was prepared

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with the composition of 200gm peeled potato, agar 20gm, dextrose 20gm for one liter of final volume of water. The media was autoclaved at 121°C and 15 psi for 15 to 20 minutes and allowed to cool to bring around 50 to 60°C in room temperature before pouring sterilize the glass wares under the hot air oven. Prepared PDA powder also used with the composition of 42 gram for one liter of final volume of water. The media was sterilized on autoclave at 15 psi (125°C) for 15-20 minutes. The sterilized media was allowed to cool 50 to 60°C and pouring the sterilized media into the Petridis.

The Sclerotia from diseased sample were placed in 1% NaOCl (Sodium hypo chloride) solution for 30 seconds and transferred 3 times into distilled water. The fungus growth was transfer to culture plate containing PDA and allowed to grow for few days in incubator maintaining 24±2ºC for purification of pathogen. The mycelia growth covering the whole culture plate was observed in 4 days. The culture plate showing typical *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* growth was selected.

Table 1: List of Trichoderma Isolates

Treatments	Trichoderma Spp
T1	Kapilvastu Isolate
T2	Kavre Isolate
T3	Salyan Isolate
T4	Lalitpur Isolate
T5	Taplejung Isolate
T6	Control (Without Biological Control)

2.2 Dual Culture Technique

Culture discs (5 mm) of Trichoderma and the pathogen taken from the edges of the cultures grow vigorously and are evenly spaced approximately seven inches from 9 mm Petri plates containing a 20 ml PDA. With each treatment a minimum of four responses were maintained and controls were maintained by placing the pathogen disc only in the center. Petrol plates are then heated to 24 ± 2ºC. The growth of the pathogen and the ability of Trichoderma to prevent the pathogen, were documented from time to time. Reduced growth rate (Pi) of the experimental pathogen is calculated when the growth of the Sclerotium sclerotiorum is filled with control plates using the following formula (Vincent, 1947).

$$Pi = \frac{C-T}{C} \times 100$$

Where,

Pi = Percent growth reduction of test pathogen

C = Radial growth of test pathogen in control (mm)

T = Radial growth of test pathogen in treatment (mm)

2.3 Statistical Analysis

All the recorded data were processed to fit into R-studio and analyses were conducted using R 4.0.4 (R Core Team, 2013) and the Agricola version 1.1-8 package (De Mendiburu, F., 2014). The data entry was done to develop ANOVA table and different treatments were compared by Duncan's multiple range test and least significant difference at 5% level of significance. All the figures and graphs were prepared by using Microsoft excel 2013.

3. Result and Discussion

The experiments were conducted in the laboratory of NPI, Bharatpur of samples collected from white mold of kidney bean and collar rot of chili and the data were recorded on the inhibition percentage, number of days of Sclerotia formed, number of Sclerotia formed and width of point of interaction.

CV= coefficient of variation, LSD= Least significant difference, SEm= Standard error of mean. Figures denoted by same letter do not differ significantly

The redial mycelium growth of *Sclerotinia sclerotiorum* for all treatment varied significantly at 2,4,6,8 and 10 DAI (P≤0.001). The highest inhibition percentage of mycelia growth was found in Kavre Isolate i.e. 56.01% on 2 DAI followed by Kapilvastu isolate 51.84%. The highest inhibition percentage of mycelia growth was found in Kapilvastu Isolate (67.11%), (70.67%), (74.22%), (74.22%) after 4, 6, 8, 10 days of incubation respectively whereas Salyan isolates, taplejung isolate, kavre isolate and Lalitpur isolate were found at par.

This result is supported by (Elad et al., 1984). *Trichoderma* species are capable of producing extracellular lytic enzymes that are responsible for their antagonistic activity. They also reported similar positive effect of *Trichoderma* species. They also reported similar positive effect of *Trichoderma* species. It can be assumed that *T. harzianum* attacks the pathogen's mycelium first by dissolving its cell wall in certain location by penetration (Chet et al., 1981)

In field condition, *Trichoderma* viride reduce diseases incidence by 75.54% (Jegathambigai et al., 2010).

Table 1: Inhibition Percentage of Sclerotinia Sclerotium Against Trichoderma Isolates

Treatments (*Trichoderma* Isolates)	Inhibition Percentage (%)				
	2DAI	4DAI	6DAI	8DAI	10DAI
Taplejung Isolate	39.13 ^{b} (38.32)	56 ^{b} (48.45)	62.22 ^{b} (52.09)	65.78 ^{bc} (54.21)	65.78 ^{bc} (54.21)
Salyan Isolate	20.74 ^{c} (26.54)	55.55 ^{b} (48.18)	59.33 ^{b} (50.38)	63.11 ^{c} (52.62)	63.33 ^{c} (52.75)
Lalitpur Isolate	49.88^{ab} (44.91)	61.33^{ab} (51.59)	66 ^{ab} (54.35)	70.67 ^{ab} (57.24)	72.44 ^{ab} (58.38)
Kavre Isolate	56.01 ^{a} (48.52)	63.55 ^{a} (52.95)	64.44^{ab} (53.48)	68.22^{abc} (55.78)	69.33^{abc} (56.52)
Kapilvastu Isolate	51.84^{ab} (46.04)	67.11 ^{a} (55.03)	70.67 ^{a} (57.22)	74.22 ^{a} (59.51)	74.22 ^{a} (59.51)
Control (*S. Sclerotiorum*)	0 ^{d} (2.56)	0 ^{c} (2.56)	0 ^{c} (2.56)	0 ^{d} (2.56)	0 ^{d} (2.56)
Mean	36.30 (34.48)	50.62 (43.12)	53.81 (45.01)	57.03 (46.99)	57.55 (47.32)
CV	19.77	7.73	6.98	6.45	6.80
LSD	8.90***	4.35***	4.10***	3.95***	4.20***
SEm (±)	4.31	2.11	1.99	1.91	2.04

Table 2: Days to Sclerotia Formed in Dual Culture

Treatments (*Trichoderma* isolates)	Days to Sclerotia Formation
Taplejung Isolate	5.2
Salyan Isolate	5.2
Lalitpur Isolate	4.8
Kavre Isolate	4
Kapilvastu Isolate	5.6
Control (*S. Sclerotiorum*)	5.6
Mean	5.07
CV	17.47
LSD	1.15 (NS)
SEm (±)	0.56

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CV= coefficient of variation, LSD= Least significant difference, SEm= Standard error of mean. Figures denoted by same letter do not differ significantly.

Since, the data shows that there was no significant difference in days to form Sclerotia, all the treatments were same and its mean days to form Sclerotia was 5.07~5. *Trichoderma* isolates killed 62-100% of the Sclerotia within 25 days of inoculation (Santos and Dhingra, 2011)

Table 3: Number of Sclerotia Formed in Dual Culture

Treatment (*Trichoderma* Isolates)	No. of Sclerotia
Taplejung Isolate	7.2 ^{cd} (2.74)
Salyan Isolate	10.6 ^{b} (3.31)
Lalitpur Isolate	6.2 ^{d} (2.56)
Kavre Isolate	9.6 ^{bc} (3.15)
Kapilvastu Isolate	3.6 ^{e} (2.01)
Control (*Sclerotinia Sclerotiorum*)	51.4 ^{a} (7.19)
Mean	14.77 (3.49)
CV	11.97
LSD	0.55***
SEm (±)	0.26

CV= coefficient of variation, LSD= Least significant difference, SEm= Standard error of mean. Figures denoted by same letter do not differ significantly

The number of Sclerotia formed in dual culture of *Sclerotinia sclerotiorum* for all treatment varied significantly at 10 DAI (P≤0.001). The highest number of Sclerotia was observed in Salyan isolate (10.6~11) followed by Kavre isolate (9.6~10), Taplegunj isolate (7.2~7), Lalitpur isolate (6.2~6) and lowest number of Sclerotia was observed in Kapilvastu isolate (3.6~4) whereas maximum number of Sclerotia was observed in control (51.2~51). The ability of *Trichoderma* viride to control *S. sclerotiorum* infection has been attributed to the ability of *Trichoderma* to parasitize the Sclerotia (Mukherjee and Raghu, 1997).

Table 4: Width of the Browning Area at the Region of Interception in Dual Culture

Trichoderma Isolates	Width of The Browning Area at the Region of Interception in Dual Culture (*Sclerotinia Sclerotiorum*)
Kapilvastu Isolates	0.30 ^{c}
Kavre Isolates	0.48 ^{ab}
Lalitpur Isolates	0.40 ^{b}
Salyan Isolates	0.54 ^{a}
Taplejung Isolates	0.52 ^{a}
Mean	0.45
CV	16.4
LSD	0.09***
Sem (±)	0.05

CV= coefficient of variation, LSD= Least significant difference, SEm= Standard error of mean. Figures denoted by same letter do not differ significantly

The number of Sclerotia formed in dual culture of Sclerotium rolfsii for all treatment varied significantly at 10 DAI (P≤0.001). The maximum width of the browning area at the region of interception in dual culture was observed in Salyan isolate (0.54) statistically at par with Taplegunj isolate (0.52) and the minimum width of the browning area at the region of interception in dual culture was observed in Kapilvastu isolate (0.30)

4. Conclusion

S. *sclerotiorum* appears to be among the most nonspecific, omnivorous and successful plant pathogen with broad host range in the world. It is also an important pathogen to be control by integrated pest management. As we know *Trichoderma* is echo-friendly fungus, nonhazardous to soil and human beings. Among all the *Trichoderma* isolates, Lalitpur isolate on an average shows good result to control this pathogen in invitro condition. Therefore, it is recommended that the biological agent Lalitpur isolate can be suggested to the farmers for the management of white mold of kidney bean.

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