STM JOURNALS

International Journal of
Fungi

ISSN: 3049-1509
Volume 2, Issue 1, 2025
January–June
DOI (Journal): 10.37591/IJF

https://journals.stmjournals.com/ijf

Research IJF

Efficacy Evaluation of *Trichoderma Harzianum*, *T. Viride*, & *T. Asperellum*: Eco-Biocontrol Agents Against Dry Rot of Potato in Pakistan

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Abstract

Fusarium sambucinum is one of the most aggressive fusarium species initiating potato dry rot disease around the world and causing significant economic losses. To alleviate reliance on synthetic fungicides as their long-term use may lead to fungal resistance, biocontrol assays against threatening fungal diseases hold significant importance as an eco-friendly management approach. Antagonists are gradually becoming hotspots and hold broad applicable prospects. Trichoderma harzianum, T. viride, and T. asperellumwere screened for their biocontrol action against highly pathogenic isolates of F. sambucinum causing dry rot of potato in Pakistan. In vitro dual culture assay, observed after incubation at 27°C for one week, showed that all three tested Trichoderma species considerably inhibited the radial growth of F. sambucinum as compared to the control treatment where no visible fungal mycelial inhibition was recorded. Our results revealed that using antagonists against globally threatening fungal diseases may enhance the quality and production of important cash crops by generating handsome revenue.

Keywords: *Fusarium sambucinum, Trichoderma harzianum, T. viride, T. asperellum, Biocontrol agents*

INTRODUCTION

Dry rot of potato is a devastating fungal disease of potato (*Solanum tuberosum L.*) affecting quality and quantity of crops. It is observed that dry rot of potatoes decline annual worldwide production of potatoes to about 57%. Whereas the yield losses recorded in the field are approximately 23% [1]. Potato is the first non-cereal food crop for human consumption and holds high potential for food security in developing nations [2]. About 1.3 billion people consume fresh potatoes as a staple food (50 kg per person annually) in India and China.

In Pakistan, potatoes cultivated in various areas. In Punjab, about 83 percent of the potato production originates followed by Khyber Pakhtunkhwa 10%, in Baluchistan 6% and in Sindh 1% potato is cultivated [3]. Potatoes are grown over a large area of Pakistan with the production of roughly 4.1 million tons. Potato crop is cultivated mostly in central and northern areas of Pakistan, viz: GB (Gilgit Baltistan), Kalat, Pishin, and Killa Saifullah, respectively. Moreover, it is pertinent to mention

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Received Date: Dcember 30, 2024
Accepted Date: January 04, 2025
Published Date: January 06, 2025

Citation: Shawal Shakeel, Gull-e-laala Khan, Gulshan Irshad, Nayla Haneef, Amna Maqsood, Salman Ghuffar, Nigarish Saghir. Efficacy Evaluation of *Trichoderma Harzianum*, *T. Viride*, & *T. Asperellum*: Eco-Biocontrol Agents Against Dry Rot of Potato in Pakistan. International Journal of Fungi. 2025; 2(1): 46–50p.

that neighboring Pakistani countries like India and Bangladesh are also huge potato producers [4]. The potato crop is affected by various biotic and abiotic factors. Mostly the fungal deteriorating pathogens of Fusarium genera, viz *sambucinum, oxysporum, solani, culmorum* and *avenaceum* cause prominent infections in potatoes [5]. Other microbial infections include early, and late blight of potatoes followed by black scurf and wilting, that result and maximum annual decline of potato crop (Aydin et al., 2016). Among all mentioned rots, the most prominent pathogen causing severe destruction is *Fusarium sambucinum* [6]. *F. sambucinum* exists as chlamydospores for several years in soil debris and infects potato crops. The temperature ranges from 59°F and it requires high humidity. The pathogen enters through wounds or natural openings. The tubers and roots are the main plant parts that are directly affected by dry rot disease [7]. The economically significant part of the potato is a tuber, a site for the storage of carbohydrates, and consists of about 77% water, and 20% indigestible carbohydrate [8].

The disease can temporarily be suppressed by some postharvest applications to the tubers. *Trichoderma* species act as an excellent eco-biocontrol agent, against *Fusarium spp*, in soil debris [9]. Application of Trichoderma as antagonists depends on diverse strains, pathogen species, and host environmental susceptibility [10]. It is pertinent to mention that effective tuber treatments along with other applications prior to planting or during storage may reduce the severity of dry rot disease [11]. Therefore, the main objective of the proposed research was to screen and evaluate the bio-efficacy of different *Trichoderma spp*., against dry rot of potato.

MATERIAL AND METHODOLOGY

The experiment was conducted at the National Agriculture Research Centre (NARC) Islamabad with the objective of combatting the efficacy of biocontrol agents against dry rot of potato. Potato tubers with visible symptoms of dry rot were collected from potato fields at NARC and were carried in polythene bags to the fungal plant pathology lab. Samples were air-dried for 24 hours, surface sterilized with 70% ethanol, and pierced into 5 mm pieces. The pieces were cultured on PDA media and incubated at 25°C in the light for 7 days. The conidia were isolated using the single spore isolation technique. Morphological and microscopic features were also measured according to protocol [12].

Furthermore, the pathogenicity assay was conducted to determine the pathogenic nature of isolated fungal. A minimum of two equal size 4 mm diameter mycelial plugs of 6–7 days pure fungal culture were picked and grown on media (PDA). The specimens (potato tubers) were completely surface sterilized using ethanol (70%) and 4mm mycelial plugs were inserted. The inoculated tubers were placed in the trays and kept in the laboratory at 25°C. Disease symptoms were observed after all odd DPI and were compared with purified dry rot disease culture on PDA. After 9 days, potato tubers were cut down longitudinally to observe internal symptoms. Koch's postulates were confirmed according to the protocol of [13]. Moreover, fungal isolates were preserved in slants for long-term usage.

Dual Culture Assay

Three species of *Trichoderma* viz; *Trichoderma viride, Trichoderma harzianum* and *Trichoderma asperellum* were cultured on PDA media and incubated for 7 days at 25°C in the incubator. A dual culture assay technique was conducted for the management of *F. sambucinum* by placing a 5 mm diameter mycelial discs of the 7-day old culture of *T. viride, T. harzianum,* and *T. asperellum* individually along *F. sambucinum* petri plates. All experiments were repeated in triplicate along with three replications (FSRM11, FSRM18, FSRM29) isolates at eqi-distance from the periphery. Plates were incubated for 7 days at 25°C. Petri plates without antagonists were used as a control treatment. The mean radial mycelial growth was equally measured after all odd DPI.

RESULTS AND DISCUSSION

Fungal colonies were observed with a white velvety appearance above and a pinkish-white color under near the surface of the Petri plates (Figure 1(a–b)). Colonies were observed with a rapid growth of about 4.5 cm in four days with arial mycelium having a white to cream color. Thick wall vegetative

cell chlamydospores were observed with oval to ellipsoid septate hyaline hyphae that were measured 3–7 in diameter in microns (Figure 1(c); Table 1). Furthermore, 4–7 septations were recorded in fungal macro-spores, and after one-week macroconidia were observed, developing from conidiophores with multiple branches. Based on morphological features the fungal rot was identified as *Fusarium sumbucinum* initiating dry rot of potato. Pathogenicity assay was conducted where three isolates of *F. sambucinum* viz; FSRM11, FSRM18, and FSRM29 were revealed as highly pathogenic in nature and were further selected for management experiments.

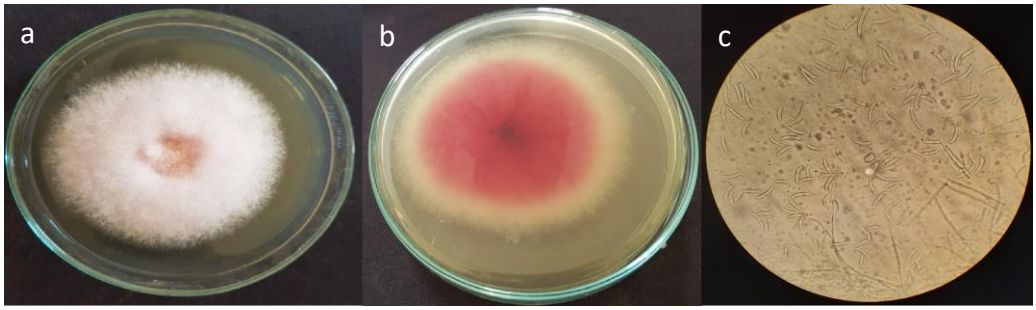

Figure 1(a) and (b). Colony appearance of *F. sambucinum* on PDA, (c) Chlamydospores of *F. sambucinum* observed under Nikon microscope.

Table 1. Morphological features of *Fusarium sambucinum.*

S. N.	FSR	Spores	Shape	Size	Color	Septation	Hyphae	Pathogenic
1.	FSRM11	Chlamydospores	Oval to ellipsoid	3–7 diameter	Whitish to pink color.	4–6 septation.	Hyaline.	Moderately.
2.	FSRM18	Chlamydospores	Oval to ellipsoid	3–7 diameter	Whitish to pink color.	5–7 septation.	Hyaline.	Severely.
3.	FSRM29	Chlamydospores	Oval to ellipsoid	4–6 diameter	Whitish to pink color.	4–7 septation.	Hyaline.	Highly pathogenic.

Dual Culture Assay

All three *Trichoderma* species act as an excellent antagonist against *F. sambucinum* isolates (FSRM11, FSRM18, FSRM29) when cross-planted in a dotted pattern on the PDA medium. Furthermore, it was observed that *T. harzianum* inhibited mycelial growth of *F. sambucinum* isolatesviz; 91.61%, 93.20% and 94.1% followed by *T. viride*inhibiting radial growth of fungus 86.22%, 86.9%, 93.91%, and *T. asperellum* as an antagonist was recorded suppressing fungal mycelium viz; 83.91%, 93.22%, and 85.91%, respectively (Figure 2). Whereas no visible growth inhibition was recorded in the control treatment. The most efficient antagonists for *F. sambucinum* FSRM29 were *T. harzianum* and *T. viride*. The antagonists for *F. sambucinum*FSRM18 were *T.viride* and *T.asperellum*whereas of *F. sambucinum* FSRM11 were *T. harzianum* and *T. viride*, respectively. *Trichoderma* isolates showed hyper parasitic nature and inhibited the mycelial growth of fungal colonies by fungal colonies by growing over them completely by showing strong resistance.

In another study, the efficacy of *Trichoderma* was evaluated as a biocontrol agent and results revealed that Trichoderma reduced the dry rot disease in potato tubers. Similarly, our results are quite like [14]. Who reported that *T. harzianum* and *T. viride*inhibited mycelial growth of *F. sambucinum* effectively. Various Trichoderma species viz; *T. atrovirde, T. longibrachiaum, T. virens,* and *T. hazianum* act efficiently against *F. sambucinum* where *T.virens* and growing over them completely by showing strong resistance.

Our study emphasizes an important role in investigating the use of biological control agents as bio-pesticides and bioherbicides against phytopathogenic fungi by improving and promoting crop yield. In the present study, the most efficient antagonists of *Trichoderma* against *F. sambucinum*isolate FSRM29

were *T. harzianum,* and *T. viride*. The antagonists for *F. sambucinum*isolate FSRM18 were *T. viride* and *T.asperellum* whereas of *F. sambucinum* FSRM11 were *T. harzianum* and *T. viride*, respectively. Trichoderma isolates showed hyper parasitic nature and inhibited the mycelial growth of *T.hazianum*were highly effective [15]. Enough studies agree on the potential role of *Trichoderma* as a biocontrol agent against potato dry rot caused by *Fusarium sambucinum*, and it is pertinent to mention that various *Trichoderma* species having diverse virulence, showed 100% efficacy against potato dry rot disease [16]. Moreover, different *in vitro* and *in vivo* studies support the research that antagonism is the main mechanism for the biological control of disease [17]. The use of fungal biocontrol agents to control potato dry rot disease holds significant eco-friendly importance as post-harvest conditions are an ideal point for the efficacy of biocontrol agents [18]. In this present study, applied antagonists showed great inhibition activity against potato dry disease.

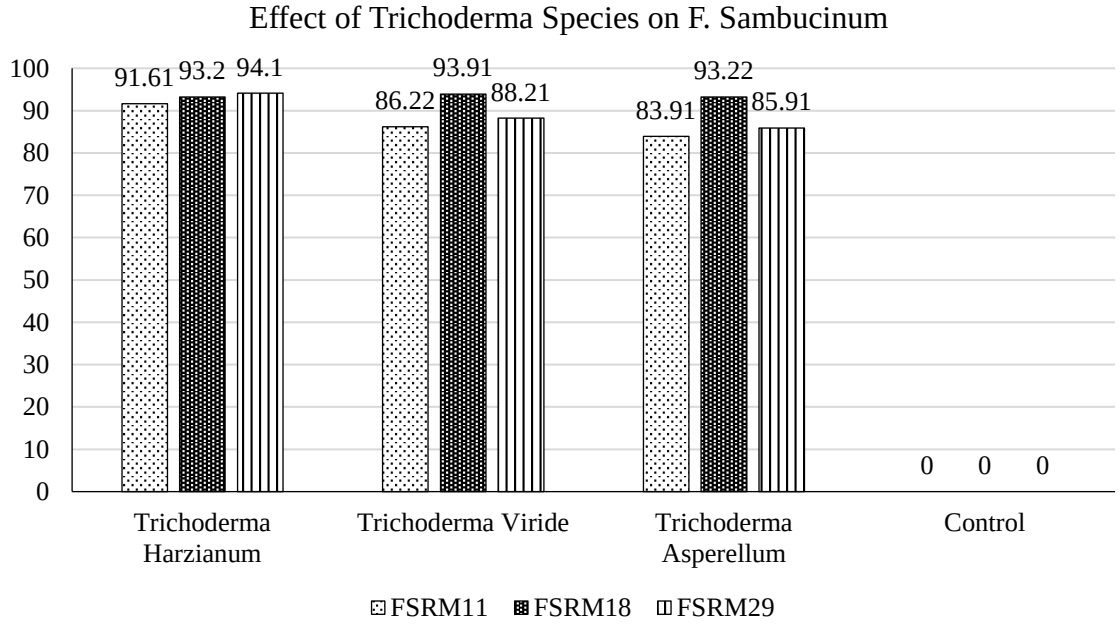

Figure 2. Effect of *Trichoderma* species on *F. Sambucinum*.

CONCLUSIONS

Potato dry rot initiated by *F. sambucinum* is a devastating field and a postharvest fungal rot declining the quality and quantity of potato crops globally. To mitigate reliance on synthetic fungicides antagonism by biocontrol agents holds significant attraction among scientists. Application of *Trichoderma harzianum, T. viride,* and *T. asperellum* suppressed the mycelial growth of potato dry rot fungus in the present study and emphasizes future use of these biological agents as eco-friendly, economical, and natural biocides.

Conflict of Interest

The authors declare no potential conflict of interest.

Authors' Contribution

All authors contribute equally to this research.

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