Journal of Advances in Biology & Biotechnology

Volume 27, Issue 9, Page 171-178, 2024; Article no.JABB.121339
ISSN: 2394-1081

In-vitro Evaluation of Efficacy of *Trichoderma harzianum* on the Radial Growth of *Alternaria alternata*

Ahana Sarkar [a++*], Sobita Simon [a#] and Abhilasha A. Lal [a†]

[a] *Department of Plant Pathology, NAI, SHUATS, Prayagraj, U.P., India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i91286

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/121339

Original Research Article

Received: 10/06/2024
Accepted: 17/08/2024
Published: 22/08/2024

ABSTRACT

Stevia rebaudiana Bertoni, a herbaceous perennial known for its natural sweetness, has garnered global recognition and is found in various regions of India. A study was carried out to investigate the leaf spot disease in Stevia, caused by *Alternaria alternata* (FR.) Keissler to find out the most suitable design of dual culture technique. Since the leaves are the primary site for synthesizing sweet glycosides, in Stevia this disease leads to significant losses and ultimately reduces the yield which leads to a serious concern. Due to the harmful effects of chemical fungicides, finding a safer alternative to control the pathogen became a priority. This prompted experiments with bioagents for pathogen control. Bio fungicides derived from *Trichoderma* are increasingly being recognized as successful agricultural applications, with over 50 registered products available worldwide. The present study was conducted in the Laboratory, Department of Plant Pathology, Naini Agricultural

[++] *M. Sc. Scholar;*
[#] *Rtd. Professor;*
[†] *Assistant professor;*
**Corresponding author: E-mail: sarkarahana.99@gmail.com;*

Cite as: *Sarkar, Ahana, Sobita Simon, and Abhilasha A. Lal. 2024. "In-Vitro Evaluation of Efficacy of Trichoderma Harzianum on the Radial Growth of Alternaria Alternata". Journal of Advances in Biology & Biotechnology 27 (9):171-78. https://doi.org/10.9734/jabb/2024/v27i91286.*

Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj (Uttar Pradesh). The dual culture technique was carried out on Completely Randomized Design (CRD) with three replications and six treatments. Examination of fungal colony characteristics was done through microscopic examination. At 7 DAI, maximum mycelial inhibition of 96.70% was recorded in the treatment T_6 (six discs of *Trichoderma harzianum* against one disc of *Alternaria alternata*).

Keywords: Alternaria alternata; bioagent; dual culture; fungal colony; stevia; Trichoderma harzanium.

1. INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni) is a member of the Asteraceae family and is recognized as one of the most significant natural sweetener plants with low-calorie content. India is known as the "Diabetes Capital" for its increasing number of diabetes cases. Stevia emerged as the realm of sweetness to all the diabetic individuals. The sweetness of stevia is due steveoside and rebauside. Maximum steveoside content is present in the leaves of stevia, emerging it as the main economic part. The global stevia market expanded significantly, increasing from ₹61.65 billion in 2022 to ₹67.40 billion in 2023, reflecting a compound annual growth rate of 9.9%. The increasing demand for natural sweeteners has encouraged Indian farmers to cultivate stevia on a large scale [1]. Currently, the nation produces roughly 900 tonnes of dry leaf annually. A major concern is leaf spot disease caused by the infestation of *Alternaria alternata* (FR.) Keissler. Since the leaves are the primary site for synthesizing sweet glycosides, this disease leads to significant losses and ultimately reduces the yield [2]. The new survey conducted over the past five years indicates that Alternaria leaf disease has been prevalent in medicinal plants cultivated in various districts of West Bengal, India [3]. Alternaria diseases primarily impact the leaves, stems, flowers, and fruits of annual plants, particularly vegetables and ornamentals. The pathogenic fungus *Alternaria alternata* can produce endo-polygalacturonase (endo-PG) and pectate lyase (PL) enzymes. These enzymes are responsible for breaking down pectic components of the plant cell wall. Depending on the interaction between the plant species and the microorganism (compatible or incompatible), these pectinases may function as part of the fungal infection mechanism or trigger a hypersensitive response by releasing oligosaccharides that act as elicitors of the plant response [4]. Biological control, which involves using living organisms (antagonists) to reduce pathogen activities, is a highly promising approach for managing plant diseases. Unlike chemical fungicides, biological control does not lead to the development of resistance in pathogens, nor does it contaminate the environment. Additionally, it meets the requirements of profitable markets. Among 25 fungal antagonists studied, the genus *Trichoderma* was identified as having the greatest biocontrol potential [5]. *Trichoderma* is a prominent and widely distributed filamentous fungus found in soil, where it plays a role in decomposing vegetative materials, plant matter, and wood. *Trichoderma* is considered an exceptional biocontrol agent because of its distinctive traits, including its rapid multiplication, wide distribution, and ease of isolation and cultivation [6,7]. The control mechanisms exhibited by *Trichoderma* spp. vary depending on the fungal species and environmental conditions. *Trichoderma* spp. utilize direct biocontrol mechanisms, including mycoparasitism, competition for space and nutrients, production of antimicrobial compounds (antibiosis) and lytic enzymes. They also employ indirect mechanisms, such as induction of systemic resistance, growth promotion, and rhizosphere competence [8]. Various studies have been conducted both *in vitro* and *in vivo* using *Trichoderma harzianum* against *Alternaria alternata.* This study is conducted to know the most suitable design in dual culture technique using *Trichoderma harzianum* against *Alternaria alternata* for maximum mycelial inhibition of the pathogen.

2. MATERIALAND METHODS

2.1 Experimental Site

The experiment was conducted in the Laboratory, Department of Plant Pathology at Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj (Uttar Pradesh). The dual culture technique was carried out on Completely Randomized Design (CRD) with three replications and six treatments.

2.2 Isolation of Pathogen

"For isolating and culturing of pathogen, Potato Dextrose Agar (PDA) medium was used. Diseased portion of the leaf was cut under aseptic conditions into small bits into a sterile dish with the aid of scissors which was flamed over a spirit lamp flame and surface sterilized in 0.1% sodium hypochlorite. The cut diseased and surface sterilized bits with 70% ethanol was placed on Petri dishes poured with solidified potato dextrose agar (PDA). The inoculated plates were incubated at room temperature until visible growths are seen on the plates. The fungal colonies growing in the incubated plates was sub-cultured into fresh medium until pure cultures are obtained" [9]. "Sub-culturing was done at regular intervals, by using single spore method purification of the culture was made" [10]. The pure culture was maintained in slants and stored at 4°C temperature in the refrigerator.

2.3 Identification

"Examination of the fungal colony characteristics was done through microscopic examination. Using a sterile needle, a small portion of the culture was taken and placed on a sterile glass slide. It was stained using lactophenol and cotton blue and were identified using the key of" [11,12].

2.4 Morphological Characteristics

Conidiophores were simple, light brown, variable in length ranging from 17.10 to 61.56 µm and mostly 2-3 septate rarely 4-5 septate. Conidia were found light to dark brown in colour, uniform with 0-2 longitudinal septa and 1-6 transverse septa, and variable in shape and size, mostly oval shape with rudimentary beak and in size measuring about 10.26-77.52 x 4.56-14.82 µm. Based on the morphological characters, the organism was identified as *Alternaria Alternata* [11,12] (Plate 1).

2.5 Biocontrol Agent *Trichoderma harzianum*

The bio control agent *Trichoderma harzianum* was obtained from Laboratory, Department of Plant Pathology, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, U.P. *Trichoderma harzianum* was sub-cultured *in vitro* for antagonism test against *Alternaria alternata* (Plate 2).

2.6 Methods

Dual culture was performed to check the antagonism ability of *Trichoderma harzianum* against *Alternaria alternata* for mycelial inhibition and overgrowth. This was done following six different designs of dual culture technique to observe the efficiency of *T. harzianum* against *A. alternata* and control plates were also set. *T. harzianum* and *A. alternata* was subcultured onto PDA for 7 days. The margin of the colony was cut with sterile cork borer and was placed in 90 mm diameter Petri plate containing PDA in different designs [13].

2.6.1 Designs of Dual culture plates

In first design, one disc of *A. alternata* was set against one disc of *T. harzianum*. In second design, two discs of *T. harzianum* were set on the periphery of both sides of the Petri dish and one disc *A. alternata* was set at the center of the Petri dish. In third design, three discs of *T. harzianum* were set on the periphery of the Petri dish surrounding one disc of *A. alternata* on the center of the Petri dish. In fourth design, four discs of *T. harzianum* were set on the periphery

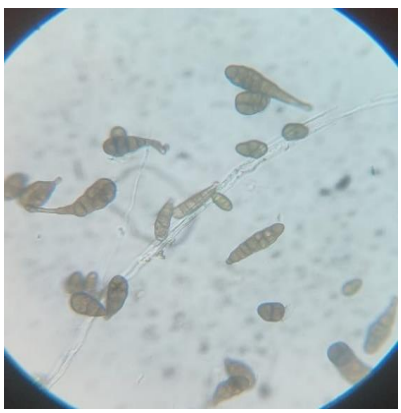


Plate 1. Conidia of *Alternaria alternata* (45X)

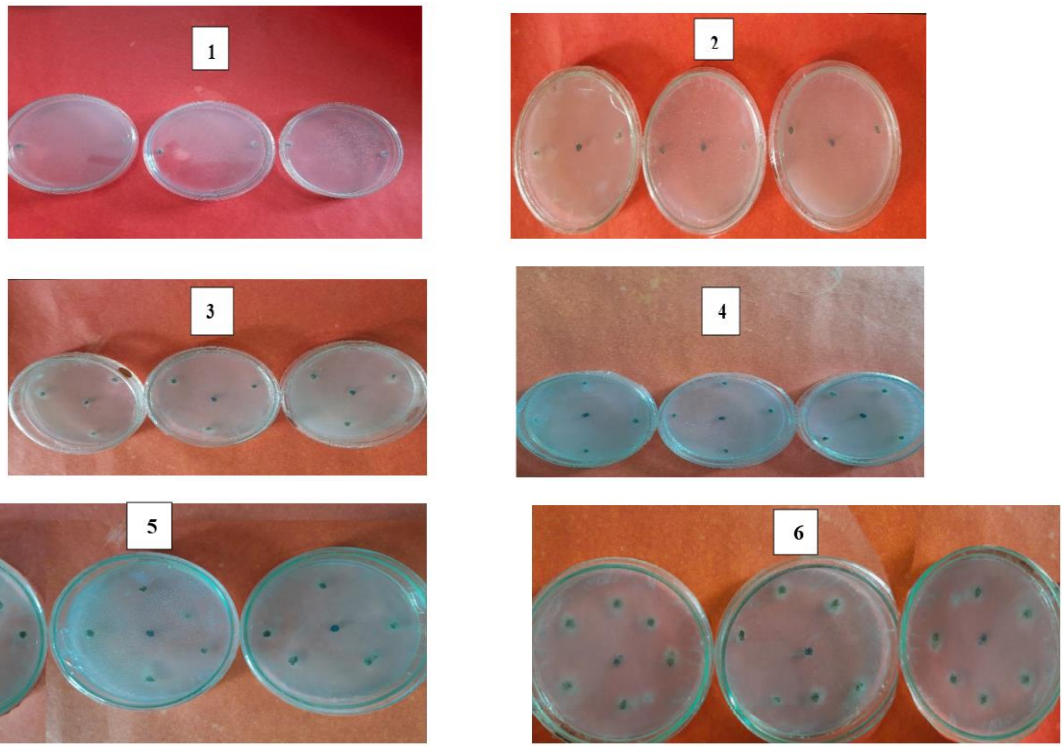
Plate 2. Pure culture of *Trichoderma harzianum*

of the Petri dish surrounding one disc of *A. alternata* on the center of the Petri dish. In fifth design, five discs of *T. harzianum* were set on the periphery of the Petri dish surrounding one disc of *A. alternata* on the center of the Petri dish. In sixth design, six discs of *T. harzianum* were set on the periphery of Petri dish surrounding one disc of *A. alternata* on the center of the Petri dish. In control plate, only one disc of *A. alternata* was set on the center of the Petri dish (Plate 3).

Each design of dual culture technique was replicated three times. All the plates are incubated at 28±1°C and antagonistic activity was tested 7 days after incubation by measuring the radius of the *A. alternata* colony (T) in the treatment plates and the radius of the *A. alternata* colony in the control plate (C) at regular intervals. The ability of *T. harzianum* to overgrow the colony of *A. alternata* was observed and compared with the control treatment.

The growth inhibition percentage (GI%) for each treatment was calculated according to Arora and Upadhay [14] as follows:

GI%= [(C-T)/C] x 100 where, GI%= percent of growth inhibition over control, C= radius growth of control (mm), T= radius growth of *Trichoderma harzianum* (mm).



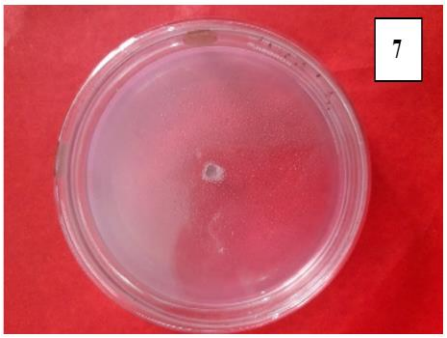


Plate 3. Different designs of dual culture plates containing *Trichoderma harzianum* and *Alternaria alternata* in PDA; 1- one disc of *A. alternata* was set against one disc of *T. harzianum*, 2- two discs of *T. harzianum* were set on the periphery surrounding one disc of *A. alternata* on the centre of the Petri dish, 3- three discs of *T. harzianum* were set on the periphery surrounding one disc of *A. alternata* on the centre of the Petri dish, 4- four discs of *T. harzianum* were set on the periphery surrounding one disc of *A. alternata* on the centre of the Petri dish, 5- five discs of *T. harzianum* were set on the periphery surrounding one disc of *A. alternata* on the centre of the Petri dish, 6- six discs of *T. harzianum* were set on the periphery surrounding one disc of *A. alternata* on the centre of the Petri dish, 7- control plates of *A. alternata*

3. RESULTS AND DISCUSSION

Dual culture was carried out with different number of discs of *Trichoderma harzianum* against *Alternaria alternata* to check the efficacy of biocontrol before applying it in the field.

Table 1. Radial growth and mycelial inhibition in dual culture technique using *Trichoderma harzianum* against *Alternaria alternata*

Treatment Notation	Treatment details	Radial growth (mm)	Mycelial inhibition (%)
		7 DAI	
T_0	Only one disc of *A. alternata*	50.33[a]	-
T_1	One disc of *T. harzianum* against one disc of *A. alternata*	18.50[b]	63.24
T_2	Two disc of *T. harzianum* against one disc of *A. alternata*	9.50[c]	81.12
T_3	Three disc of *T. harzianum* against one disc of *A. alternata*	7.25[d]	85.59
T_4	Four disc of *T. harzianum* against one disc of *A. alternata*	5.00[e]	90.06
T_5	Five disc of *T. harzianum* against one disc of *A. alternata*	3.67[e]	92.70
T_6	Six disc of *T. harzianum* against one disc of *A. alternata*	1.66[f]	96.70
$CD_{0.05}$		**1.43**	

**Here the alphabets a, b, c, d, e, f denotes significant data at 5% level of significance*

All six designs have shown reduced growth of *Alternaria alternata* in the dual culture plates when compared with the control plate. Among the six designs of our experiment, the sixth design where six discs of *Trichoderma harzianum* was set on the periphery of the Petri dish surrounding *Alternaria alternata* on the center have shown maximum mycelial inhibition of 96.70%, where the radial growth of *Alternaria alternata* was 1.66 mm followed by fifth design where five discs of *Trichoderma harzianum* were set on the periphery of the Petri dish surrounding one disc of *Alternaria alternata* on the center of the Petridish where the radial growth of *Alternaria alternata* was 3.67 mm, fourth design where four discs of *Trichoderma harzianum* were set on the periphery of the Petri dish surrounding one disc of *Alternaria alternata* on the center of the Petri dish where the radial growth of *Alternaria alternata* was 5mm. The first, second and third design recorded radial growth of *Alternaria alternata* of 7.25 mm, 9.50 mm, 18.50 mm respectively. In control condition, radial growth of *Alternaria alternata* was 50.33 mm

after 7 days of incubation (Table 1). Mycelial overgrowth of *Trichoderma harzianum* was also observed in the dual culture plates after 7 days of incubation (Plate 4).

The probable reasons for such findings may be due to the secretion of extracellular cell degrading enzymes and production of secondary metabolites by *Trichoderma harzianum* which inhibited the growth of the *Alternaria alternata* Zade et al. [15]. The results of the present findings agree with Nafiza et al. [13] who carried out dual culture with different number of discs of

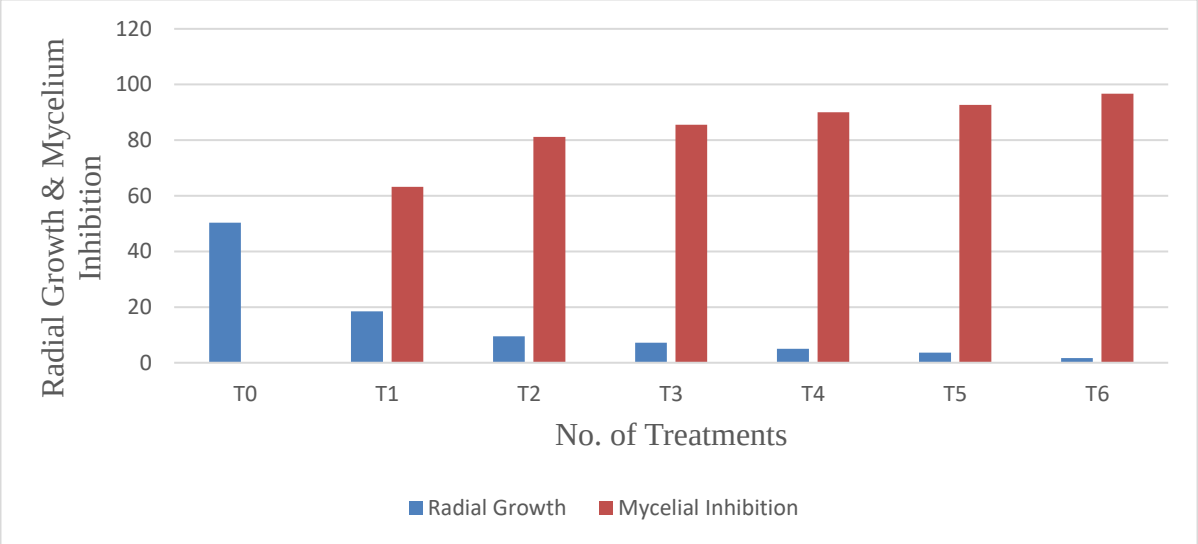


Fig. 1. Graphical representation of efficacy of *Trichoderma harzianum* against *Alternaria alternata* in dual culture technique at 7 DAI

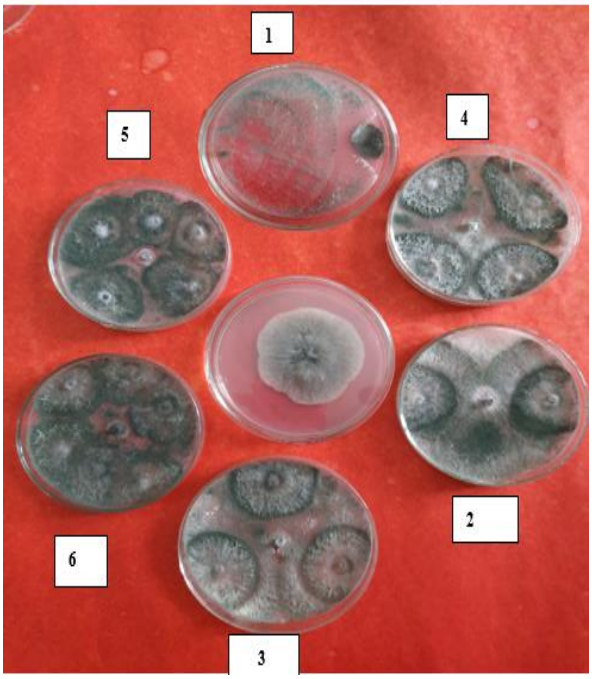


Plate 4. Plates after 7 DAI (1- one disc of Aa with Th, 2- one disc of Aa with two discs of Th, 3- one disc of Aa with three discs of Th, 4- one disc of Aa with four discs of Th, 5- one disc of Aa with five discs of Th and 6- one disc of Aa with six discs of Th
**Th- Trichoderma harzianum, Aa- Alternaria alternata*

Trichoderma harzianum against *Magnaporthe oryzae* and recorded maximum inhibition of pathogen in the plate with maximum number of discs of *T. harzianum* set against the pathogen. Similar findings were also recorded by Khalique et al. [16] who also observed maximum inhibition of *A. alternata* when *T. harzianum* was applied from four sides keeping one disc of pathogen at the centre.

4. CONCLUSION

Six different designs of dual culture technique with *Trichoderma harzianum* against *Alternaria alternata* was used. *In vitro* analysis revealed that the sixth design where six discs of *Trichoderma harzianum* was set on the periphery of the Petri dish surrounding *Alternaria alternata* on the center have shown maximum mycelial inhibition of 96.70%. All the designs of dual culture technique were found effective *in vitro* test against *Alternaria alternata.*

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dhange PR, Srinatha TN, Kandpal K. Stevia farming: A moneymaking venture for farmers. Indian Farming. 2023;73(12): 19-22.
2. Nayak AM, Dhange PR, Farooqkhan, Ismayil MSM. Fungal bioagents and botanicals efficacy against *Alternaria alternata* responsible for leaf blight disease of *Stevia rebaudiana*. International Journal of Plant & Soil Science. 2023;35(22):254-260.
3. Chirutala, N, and Simon, S. Use of bio-resources in the management of *Alternaria alternata* and *Rhizoctonia solani* of stevia (*Stevia rebaudiana*); 2021.
4. Roco A, Castañeda P, Pérez LM. Oligosaccharides released by pectinase treatment of Citrus limon seedlings are elicitors of the plant response. Phytochemistry. 1993;33:1301-1306.
5. Ferreira FV, Musumeci MA. Trichoderma as biological control agent: Scope and prospects to improve efficacy. World Journal of Microbiology and Biotechnology. 2021;37:90.
6. Hariharan G, Rifnas LM, Prasannath K. Role of *Trichoderma spp.* in biocontrol of plant diseases. In Microbial biocontrol: Food security and post harvest management. Springer, Cham. 2022;39-78.
   Available:https://doi.org/10.1007/978-3-030-87289-2_3
7. Rashid Z, Ahngar TA, Nazir A, Dar ZA, Khuroo NS, Majeed S, et al. Research technology of Stevia. Cutting-edge Research in Agricultural Sciences. 2021;6.
8. Kumar R, Sharma S, Sharma M. Growth and yield of natural-sweetener plant stevia as affected by pinching. Indian Journal of Plant Physiology; 2014.
9. Rahman MA, Rahman MA, Moni ZR, Rahman MA. Evaluation of bio-control efficacy of Trichoderma strains against *Alternaria alternata* causing leaf blight of Ashwagandha [*Withania somnifera* (L.) Dunal]. Journal of Forest and Environmental Science. 2020;36(3):207-218.
10. Toussoun TA, Nelson PE. A pictorial guide to the identification of Fusarium species (2nd ed.). University Park, PA: Pennsylvania State University Press; 1976.
11. Keissler KV. Zur Kenntnis der Pilzflora Krains. Beihefte Zum Botanischen Centralblatt. 1912;29:395-440.
12. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi. Burgess Publication Company. Minnesota, USA; 1972.
13. Nafiza Z, Aminuzzaman FM, Laila L, Ashrafi ML. Comparative *in vitro* efficacy assessment methods of a bioagent *Trichoderma harzianum* THR 4 against rice blast pathogen Magnaporthe oryzae oryzae. Open Acess Library Journal. 2021;8: e7510.
14. Arora DK, Upadhay RK. Effect of fungicide staling growth substances on colony interaction. Plant and soil. 1978;49:685-690.
15. Zade SB, Ingle YV, Ingle RW. Evaluation of fungicides, botanicals and bio agents against Alternaria alternata incitant of leaf spot of soybean. Journal of Pharmacognosy and Phytochemistry. 2018;7(5):1687-1690.

16. Khaliq A, Khan H, Maria N, Khan N, Zada L, Ali M, Zahoor M, Samiullah. *In vitro* efficacy of *Trichoderma harzianum* Rifai against various isolates of *Alternaria sp*. Journal of Entomology and Zoology Studies. 2017;5(1):442-445.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/121339