Original Research Article

Effect of bio-agents and elicitors on *Alternaria brassicae* of mustard (*Brassica juncea* L.)

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ABSTRACT

Rapeseed-mustard is one of the major oilseed crops cultivated in India. *Alternaria brassicae* is the most destructive pathogen of oilseeds. The present study aims to evaluate the effect of bio-agents and elicitors on *Alternaria brassicae* of mustard *in vitro* using dual culture technique and poison food technique in Completely Randomized Design (CRD). The study was conducted at the Department of Plant Pathology Laboratory, Sam Higginbottom University of Agriculture, Technology And Sciences, Prayagraj. The results revealed that among bio-agents, *Trichoderma viride* + *Pseudomonas fluorescens* (87.70mm) recorded the maximum per cent inhibition of mycelial growth of *Alternaria brassicae*, followed by *Trichoderma viride* (82.60mm). Among elicitors, the maximum per cent inhibition of the pathogen was recorded by salicylic acid at 150 ppm (75.33%), followed by salicylic acid at 100 ppm (58.69%).

Keywords: Alternaria brassicae, bio-agents, elicitors, in vitro, mustard, oilseed crops, salicylic acid, Trichoderma viride

1. INTRODUCTION

Brassica juncea (L.), a member of the Brassicaceae (Cruciferae) family, is generally known as Indian mustard and is used worldwide as an oilseed, vegetable, and condiment [1]. *Alternaria brassicae* (Berk.) Sacc., the fungus that causes alternaria blight disease, is a common pathogen. Most cruciferous crops are affected, including broccoli, cauliflower, field mustard, turnip, Chinese mustard or leaf mustard, Chinese or celery cabbage, cabbage, rape and radish [2]. In India, yield losses of up to 70% in productivity have been documented, with a 14-36% fall in oil content [3]. Bio-agents are microorganisms that are used as biological control agents to protect plants from pests and diseases. *Trichoderma viride* is a fungus and bio fungicide that produces spores asexually by mitosis and may produce a number of enzymes, including cellulases and chitinases, which can degrade cellulose and chitin [4]. *Pseudomonas* spp. promotes disease resistance and has the capacity to develop plant defences [5]. Elicitors are oligomeric chemical substances with low molecular weight that induce plant defence responses at low concentrations. Salicylic acid is a phenolic endogenous growth regulator as well as a signalling molecule that regulates physiological activities in plants such as growth, photosynthesis, and other metabolic functions [6]. Seaweed extracts may confer disease resistance in plants by inducing defence genes, PR proteins, defence enzymes, and the buildup of inhibitory chemicals such as phenols and phytoalexins [7]. However, with increasing environmental contamination and the current public perception of pesticide pollutants in foods, particularly edible oils, the development of alternative, economical and environmentally friendly disease management options is required.

2. MATERIALS AND METHODS

The laboratory experiments on alternaria blight caused by *Alternaria brassicae* (Berk.) Sacc. were conducted in the Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology And Sciences, Prayagraj (Uttar Pradesh). The experiments were conducted in completely randomized design (CRD) with five replications in each treatment.

Isolation of the pathogen from diseased leaf of mustard

The leaf spot and lesions, showing the initial and conspicuous characteristic symptoms of alternaria blight were selected for isolation of the pathogen. These selected infected spots were washed 3-4 times in sterilized distilled water and then surface sterilized by dipping in 1% NaOCl solution for 1 minute, followed by washing with sterilized water 3-4 times. Excess of

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moisture was removed by putting these pieces in between two folds of sterilized blotter paper under aseptic conditions in the inoculation chamber. Surface sterilized leaf spot pieces were then aseptically transferred into 9 cm petri dishes containing Potato Dextrose Agar (PDA) and incubated at 25±2°C for 7 days. Thereafter, growing mycelia from margin of apparently distinct colonies of the leaf spot pieces on the medium were aseptically transferred into another petri plate containing PDA medium, where it was grown for 7 days at 25±2°C in the BOD incubator.

Identification

Examination of the fungal colony characteristics were 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. Then, the microscope was used for the examination of morphology and culture characteristics of fungal structures [8].

Morphological characterization

Identification of *Alternaria* is done by morphological characteristics like size and shape of conidia and cultural characters like colony outline, shape, colour and texture. The genus could be organized into six groups based upon common characteristics of conidia length, width and septations, with each group designated by a typical species. Three sections for the genus based upon the formation of conidia in long chains (Longicatenatae), short chains (Brevicatenatae), or singly (Noncatenatae). *Alternaria* species are either parasites on living plants or saprophytes on organic substrate. The host range of pathogenic *Alternaria* is very broad. It is easy to recognize *Alternaria* species by the morphology of their large conidia. They are catenate, formed in chains or solitary, typically ovoid to obclavate, often beaked, pale brown to black, multi-celled and muriform. Based on morphological characteristics, the causal fungus was identified as *Alternaria brassicae* (Berk.) Sacc. Colonies of *A. brassicae* were amphigenous effused rather pale olive, hairy and immersed mycelium. Conidia were produced in chains of up to 4 with average 3-8 transverse septa and 1-8 longitudinal septa, pale olive and the beak about 1/3 to ½ the length of the conidia [9].

Purification of culture

The culture of *Alternaria brassicae* was purified by single spore technique and maintained by periodic sub-culturing on PDA petri plates and slants. These were incubated at 25±2°C temperature.

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Culture of *Trichoderma viride* :

In a clean and sterilized test tube, 1 g of the product was mixed in 9 ml of sterilized distilled water to make a 10^{-1} dilution (1:10). It was thoroughly shaken and 1 ml of the suspension was mixed with 9 ml of sterile water in a tube to make a 10^{-2} dilution (1:100). To achieve a 10^{-6} dilution, four more serial dilutions were performed in the same manner. 1 ml of this suspension was transferred to sterile petri plates, followed by 15-20 ml of sterilized, melted and cooled PDA medium. The plates were gently rotated to allow it to solidify. For five to seven days, the perti plates were incubated in a BOD incubator at 25±2°C. The growth of a typical *Trichoderma viride* colony was observed.

Culture of *Pseudomonas fluorescens*

In a clean and sterilized test tube, 1 g of the product was mixed in 9 ml of sterilized distilled water to make a 10^{-1} dilution (1:10). It was thoroughly shaken and 1 ml of the suspension was mixed with 9 ml of sterilized water in a tube to make a 10^{-2} dilution (1:100). Six more serial dilutions were performed in the same manner to achieve a 10^{-8} dilution. 15 ml of sterilized, molten, and cooled King's B medium was added to 1 ml of this suspension in sterile petri plates. The plates were gently rotated to allow it to solidify. The petri plates were incubated in BOD incubator at 30±2°C for two days. The development of typical *Pseudomonas fluorescens* colony was observed.

Evaluation of bio-agents on *Alternaria brassicae* by dual culture technique

Trichoderma viride was evaluated *in vitro* on *A. brassicae* by applying dual culture technique [10]. Seven days old cultures of the test bio-agent and test fungus (*A. brassicae*) grown on potato dextrose agar medium was used for the study. Twenty ml of PDA was poured into sterile petri plates. Culture growth of the test fungus and the bio-agent were cut out with the well sterilized corkborers with a disc of size 5mm in diameter. Then the test fungus and bio-agent were placed aseptically with the help of two culture discs at equidistance and opposite with each other on solidified PDA medium in petri plates and one control was maintained wherein only test fungus was grown and these plates were incubated at 25±1°C.

Pseudomonas fluorescens was evaluated by streaking at opposite side to test pathogen. One control was maintained wherein only test fungus was grown. The plates were incubated at 28±2°C for four days. *Trichoderma viride* + *Pseudomonas fluorescens* was evaluated by streaking *P. fluorescens* grown on King’s B medium on one side of a petri plate containing

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PDA. The other side of the petri plate was inoculated with 5mm disc of *T. viride* (7 days old) and at the centre seven days old culture of test fungus was placed. Then one control was maintained wherein only the test fungus was grown and these plates were incubated at 28±2°C for four days.

Evaluation of elicitors and fungicide on the colony growth of test fungus

For the evaluation of antifungal activity of the elicitors and fungicide using the poisoned food technique according to [11]. salicylic acid @ 50 ppm, 100 ppm and 150 ppm, *Ascophyllum nodosum* @ 1%, 2% and 3% and ridomil gold (treated check) @ 0.2% as dissolved in 100 ml of sterilized molten PDA prior to inoculation of *Alternaria brassicae*. PDA plates without elicitors but inoculated with *Alternaria brassicae* was served as control. Five replications were maintained for all the treatments and plates were incubated in BOD incubator at a temperature of 25±1°C. The colony diameter of the fungus was measured on 7th day of incubation and compared with the colony growth of the fungus in control. Per cent inhibition of mycelial growth was calculated using the following formula.

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

where,

I = per cent inhibition,

C = Growth (mm) of test fungus in untreated control plates,

T = Growth (mm) of test fungus in treated plates.

3. RESULTS AND DISCUSSION

In present investigation two bio-control agents, *viz.*, *Trichoderma viride* and *Pseudomonas fluorescens* were screened for their antagonistic potential on *Alternaria brassicae* by dual culture technique and elicitors by poison food technique. The results are shown in (Tables 1 and 2, figures 1 and 2, plates 1 (a), (b), and (c), and plate 2) below.

Effect of bio-agents on *Alternaria brassicae* by dual culture technique

Data presented in Table 1 depicted in figure 1 and plate 1 (a), (b) and (c) indicate that bio-agents (*Trichoderma viride*, *Pseudomonas fluorescens* and *Trichoderma viride* + *Pseudomonas fluorescens*) significantly inhibited the growth of *Alternaria brassicae*. Among the bio-agents, *Trichoderma viride* + *Pseudomonas fluorescens* (1:1:1) was most effective, showing maximum mycelial growth inhibition per cent of *A. brassicae* (87.7%),

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which was superior among the treatments, followed by *Trichoderma viride* suppressing radial growth 82.6%, *Pseudomonas fluorescens* (1:1) ratio suppressing radial growth 54.4% after 7 days of inoculation. All the treatments are statistically significant over control. Among the treatments, all are statistically significant over other treatments.

Concurrent with present findings [12,13] reported "the highest level of inhibition (80.68%) and (76.84%) of the *Alternaria brassicae* respectively, that microscopic observations on hyphal interactions between antagonists and *A. brassicae* revealed lysis and protoplasmic disruption of hyphae of test fungus at many locations". These results suggests that the antifungal compound has a strong inhibitory effect on *Alternaria brassicae* and could potentially be used as a natural alternative to synthetic fungicides in agricultural practices.

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Table 1. Effect of bio-agents on *Alternaria brassicae* by dual culture technique

S.No.	Treatments	Radial growth of pathogen (mm)	Per cent inhibition
1.	Control	90	-
2.	*Trichoderma viride*	15.6	82.6
3.	*Pseudomonas fluorescens*	42	54.4
4.	*T. viride* + *P. fluorescens*	11	87.7
	S.Em (±)	0.87	-
	C.D. (*P* = 0.05)	2.87	-

* Average of five replications

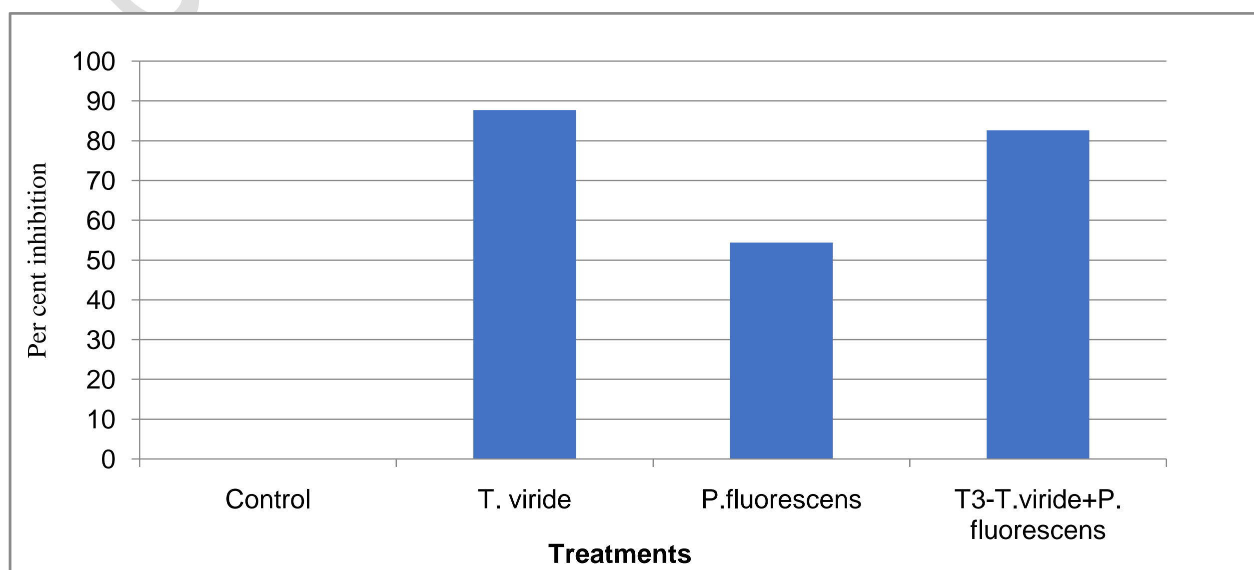
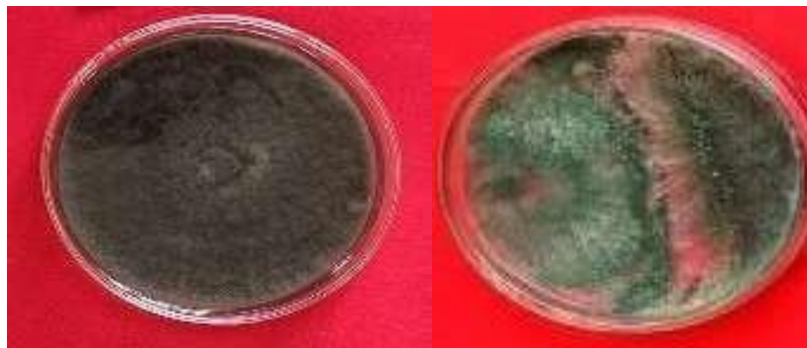


Figure 1. Effect of bio-agents on *Alternaria brassicae* by dual culture technique



T_0 – Control (168 hrs)

@ 168 hrs

Plate 1 (a) Effect of *T. viride* on the radial growth (mm) of *Alternaria brassicae*

T_0 – Control (168 hrs)

@ 168 hrs

Plate 1(b) Effect of *P. fluorescens* on the radial growth (mm) of *Alternaria brassicae*

T_0 – Control (168 hrs)

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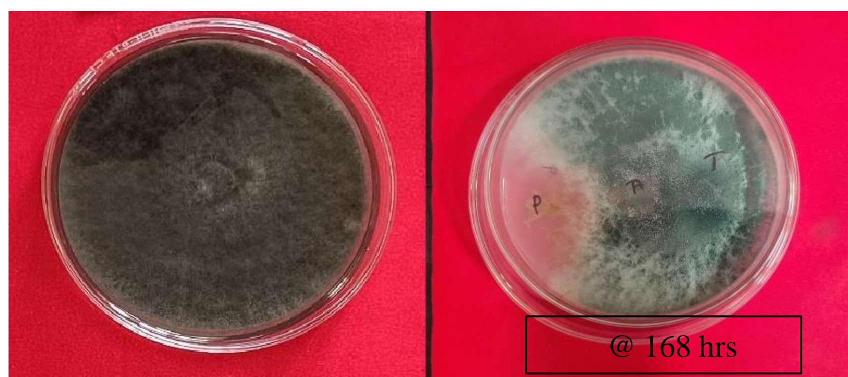

Plate 1 (c) Effect of *T. viride* + *P. fluorescens* on the radial growth (mm) of *Alternaria brassicae*

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Effect of elicitors on the mycelial growth of *Alternaria brassicae*

The data presented in (Table 2, depicted in figure 2 and plate II) indicate that elicitors (salicylic acid and *A. nodosum*) significantly inhibited the mycelial growth of *Alternaria brassicae*. Among the elicitors, maximum per cent inhibition was recorded in the treatment salicylic acid @ 150 ppm (75.33%), followed by salicylic acid @ 100 ppm (58.69%), salicylic acid @ 50 ppm (32.44%), *Ascophyllum nodosum* @ 3% (11.77%), *Ascophyllum nodosum* @ 2% (9.11%) and *Ascophyllum nodosum* @ 1% (3.33%) as compared to ridomil gold (treated check) @ 0.2% (100%) and control (0%). All the treatments are statistically significant over control. Among the treatments, T_1, T_2, T_3, T_4 and T_7 are statistically significant with other treatments, and the treatments (T_6 and T_5) are statistically non-significant with each other. Concurrent with the present findings [14,15] observed a dose-dependent relationship between salicylic acid concentration and the inhibition of mycelial growth. These results suggest that higher concentrations of salicylic acid may lead to even greater inhibition of fungal growth.

Table 2. Effect of elicitors on the mycelial growth of *Alternaria brassicae*

Tr.No.	Treatments	Radial growth of pathogen (mm)	Per cent inhibition

T_1	Control (untreated check)	90.00	0
T_2	Salicylic acid @ 50 ppm	60.80	32.44
T_3	Salicylic acid @ 100 ppm	36.00	58.69
T_4	Salicylic acid @ 150 ppm	22.20	75.33
T_5	*Ascophyllum nodosum* @ 1%	87.00	3.33
T_6	*Ascophyllum nodosum* @ 2%	81.80[a]	9.11
T_7	*Ascophyllum nodosum* @ 3%	79.40[a]	11.77
T_8	Ridomil (treated check) @ 0.2%	0	100
	S.Em (±)	0.85	-
	C.D. (*P* = 0.05)	2.46	-

* Average of five replications

* Values in the same column followed with similar alphabet are non-significant to each other at (*P*=0.05).

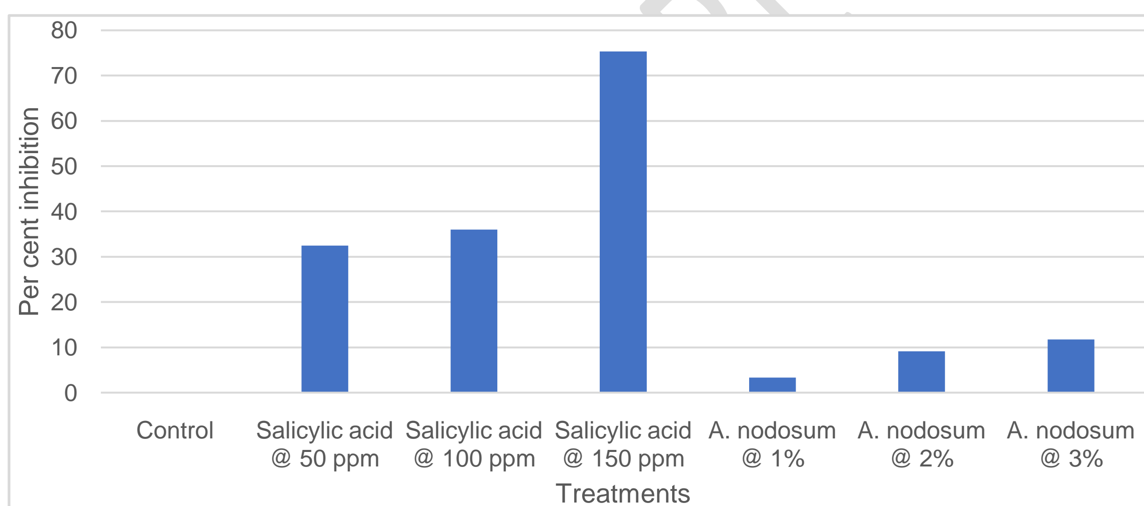

Figure 2. Effect of elicitors on the mycelial growth of *Alternaria brassicae*

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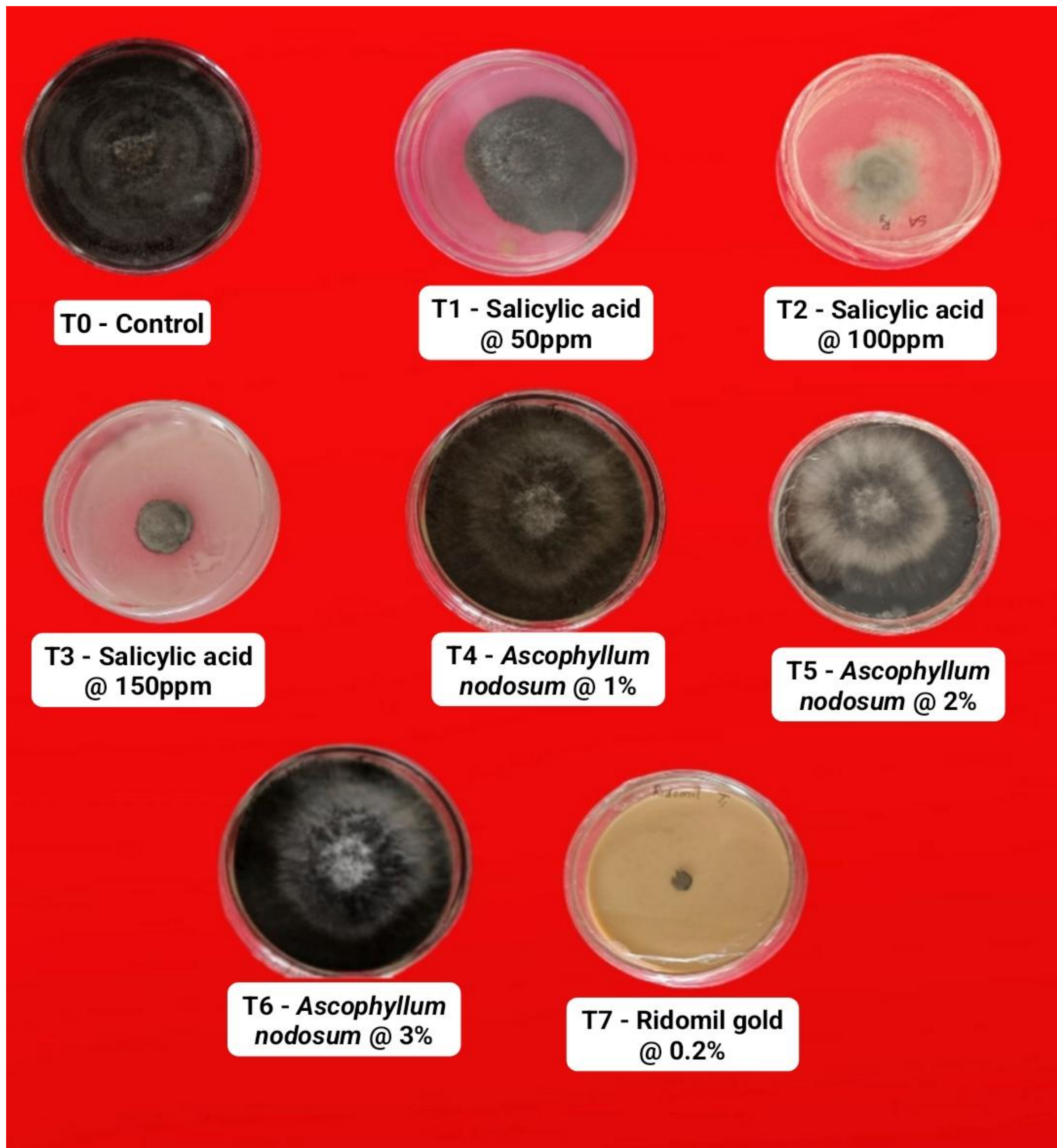

Plate 2 Effect of elicitors on the mycelial growth of *Alternaria brassicae*

4. CONCLUSION

Based on the aforesaid data the lab experiments on antagonistic microbes like *T. viride* + *Pseudomonas fluorescens* effectively inhibited *Alternaria brassicae* in mustard. These bio-control agents have unique antifungal properties, obtaining similar results to conventional chemical fungicides. Among the elicitors used, salicylic acid effectively inhibited the pathogen. This highlights the importance of bio-agents and elicitors, and their efficacy in managing pathogens. Further studies are needed to identify dominant strains of

microorganisms for specific geographical areas, reducing the demand for chemical substitutes that are harmful over time.

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