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In vitro Evaluation of Fungicides and Bioagents against *Alternaria raphani* Inciting Alternaria Blight of Radish

Monika Gusain and Sanjeev Ravi*

Department of Plant Pathology, College of Horticulture, VCSG UUHF, Bharsar, (Pauri Garhwal) Uttarakhand, India

**Corresponding author*

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A B S T R A C T

The effect of fungicides and bioagents on Alternaria blight of radish caused by *Alternaria raphani*. Effect of six fungicides *viz.* Mancozeb, Carbendazim, Ridomil-MZ, Copper oxychloride, Propiconazole and Hexaconazoleat (100, 250, 500, 750 and 1000 ppm) were evaluated to see their efficacy. The observations were recorded Hexaconazole (100%) maximum mycelium growth inhibition while Copper oxychloride found minimum mycelium growth inhibition *i.e.* (21.57, 28.88, 36.13, 45.55 and 60.17%) at all five concentrations. Among three bioagents *T.harzianum* was found (60.71%) maximum per cent mycelium inhibition while minimum was observed in *B. cereus* (14.69%).

Introduction

Radish (*Raphanus sativus* L.) is a most important edible root vegetable of the family Brassicaceae, which grown throughout the year, being mostly eaten raw as a crunchy salad and leaves also used. In general, radish contains carbohydrates, vitamins, proteins and dietary fiber. While it also appears in continental cuisine. It grown as companion as well as catch crops in India. Alternaria leaf blight of oilseed brassicas is known to be incited by three species namely *Alternaria brassicae* (Berk.) Sacc., *Alternaria brassicicola* (Schw.) Wiltshire., *Alternaria raphani* Groves and Skolko (Jasalavich*et al.*, 1995; Saharan and Mehta, 2002). Alternaria blight is the most devastating causing yield loss of 35-38% (Kolte *et al.*, 1987). Meenu and Hundal (2004) reported that seed yield losses due to Alternaria blight in radish is about (46.48%) and varied from year to year.

The radish crop is severely affected by Alternaria blight caused by *Alternaria raphani* during both seed and root crop production. All foliage is full of small, circular spots and very destructive at siliqua formation stage where all floral part, pods peduncle and seeds become black (Mangala *et al.,* 2006). Tu *et al.,* (2015) they found against *A. brassicae i.e.* (60.84, 100, 100 and 100%) at different concentrations *viz.* (100, 250, 500 and 1000 ppm) in Hexaconazole. Khalse *et al.,* (2017) found that against Alternaria leaf spot of cabbage caused by *Alternaria brassiace*. The bioagents that maximum inhibition of mycelial growth was recorded in T_1- *Trichoderma harzianum* (65.21%) followed by T_2- *Pseudomonas fluorescens* (62.41%). In present investigation applied fungicides and bio-control agents in a compatible manner for effective management of *Alternaria raphani.*

Materials and Methods

The present investigation was conducted in laboratory of the Department of Plant Pathology, College of Horticulture, VCSGUUHF, Bharsar (Pauri Garhwal) Uttarakhand. Alternaria specimen diseased portion along with healthy part of the specimen were cut into small pieces with the help of sterilized scissors and then surface sterilized by immersing in 0.1% mercuric chloride ($HgCl_2$) or (70%) ethanol for about 30 seconds. After that, the specimens were washed thoroughly at least three changes of sterilized water and specimen were transferred into Petri plates containing PDA medium and incubated at 25±1ºC for 96 hours. Pathogenic isolates from respective host plant species were isolated from the moist chambers as well as on PDA Petri plates. All the isolates were cultured under sterilized conditions in a laminar air flow and incubated at 25±1ºC for 4 days till proper growth Shoaib *et al.,* (2017). Culture was purified from single colony appearing on PDA after observed under microscope and maintained on PDA slants at 4ºC in a refrigerator for further use. The shape and size of conidia/spore arrangement was studied.

***In vitro* efficacy of fungicides:** Six fungicides *viz.* Mancozeb, Carbendazim, Ridomil MZ, Copper oxy chloride(CoC), Propiconazole and Hexaconazole at five concentrations *i.e.*100, 250, 500, 750 and 1000 ppm were evaluated using the poisoned food technique (Nene and Thapliyal, 1993).Inoculated plates were incubated at 25±1º C for 96 hours. Average radial mycelia growth in (mm) was calculated, (r) is radius of diameter, per cent mycelium growth inhibition was calculated by formula and mentioned in below.

***In vitro* efficacy of bioagents:** Three bio agents *viz. Bacillus cereus, Trichoderma harzianum*and *Pseudomonas fluorescens* were evaluated using dual culture technique (Faheem *et al.,* 2010). The bioagents and the test fungus were inoculated both sides on a single Petri plates containing solidified PDA with five replications for each treatment. Control was also run along with the other treatments. Inoculated plates were incubated at 25±1º C for 96 hours. The radial growth of the colony of bioagents and the pathogen measured in two directions and average radial mycelial growth was recorded. Per cent mycelium growth inhibition was calculated by using the following formula given by Vincent (1947).

$$\text{Per cent mycelia inhibition} = \frac{C - T}{C} \times 100$$

Where,

C= radial growth in control
T= radial growth in treatment

The data obtained and analyzed by using standard statistical procedure in the simple completely randomized design (CRD) with the help of OPSTAT.

Results and Discussion

Effect of fungicides

The data recorded and presented in (Fig-1) among all the fungicides Hexaconazole was found most effective with maximum mycelium inhibition (100%) at all concentration which was followed by Propiconazole (75.86, 100, 100, 100 and 100%), Mancozeb *i.e*. (40.14, 52.86,58.13, 100 and 100%), Carbendazim and Ridomil-MZ. Copper oxychloride among all fungicides least effective at all concentrations *i.e*. (21.57, 28.88, 36.13, 45.55 and 60.17%).

Similar observations also found by Tu *et al.*, (2015) against *A. brassicaei.e.* (60.84, 100, 100 and 100%) at different concentrations *viz.*(100, 250, 500 and 1000 ppm)in Hexaconazole. Jakatimath *et al.,* (2017) evaluated fungicides against fruit rot of brinjal (*A.alternata*) and found that increased in all the fungicides when concentrations were increased. Difenoconazole and Tebuconazole were recorded 100.00% inhibition at all the concentration tested and hence recorded mean inhibition of 100.00 %. Propiconazole (91.25%) and Carbendazim (85.41%) were the next most effective fungicides when mean per cent inhibition was considered. Panwar *et al.,* (2013) tested seven fungicides *i.e.* mancozeb, tebuconazole, myclobutanil, tricyclazole, metalaxyl + mancozeb, carbendazim and hexaconazole at (0.05, 0.10 and 0.20%) concentrations, among these three fungicides *viz.*, tebuconazole, myclobutanil and hexaconazole completely inhibited growth of test pathogen at all concentrations.

Fig.1 Effect of fungicides on per cent mycelium growth inhibition of (*A.raphani)* at different concentration

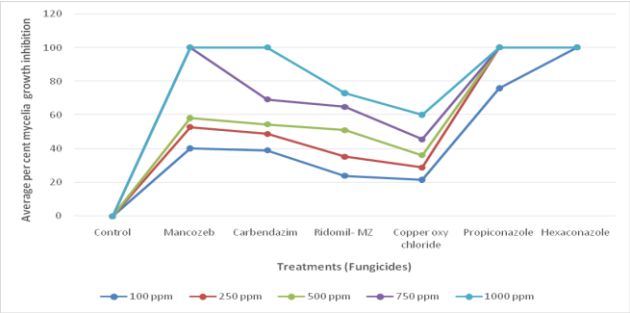
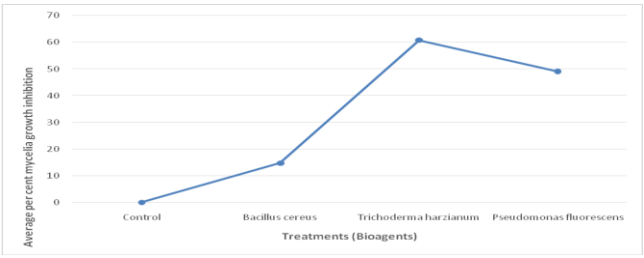


Fig.2 Effect of bioagents on per cent mycelium growth inhibition of (*A. raphani).*



Effect of biocontrol agents

Effect of three bio-agents were observed and presented in (Fig. 2). *Trichoderma harzianum* was found (60.17%) maximum per cent mycelium growth inhibition followed by *Pseudomonas fluorescens* (49.07%) and least inhibition was recorded in *Bacillus cereus* (14.69%)**.** Waghe *et al.,* (2015) observed against Alternaria blight, *A.helianthi* (Hansf) of sunflower; fungal bioagents *T. harzianum* was found most effective and recorded maximum mycelial growth inhibition (72.22%) followed by *T. viride* (70.27%). Bacterial antagonist *P. fluorescens* was found comparatively least effective with 48.60% inhibition of the test pathogen. Khalse *et al.,* (2017) found that against Alternaria leaf spot of cabbage caused by *Alternaria brassiace*.

The bioagents, maximum inhibition of mycelial growth was recorded in T_1- *Trichoderma harzianum* (65.21%) followed by T_2- *Pseudomonas fluorescens* (62.41%). Vivekan and *et al.,* (2018) tested among the biocontrol agents *viz. T. harzianum* was found more effective with 50.89% inhibition than *P. fluorescens* against chilli anthracnose, *C.capsici.* Abbo *et al.,* (2014) Effect of Bacillus spp. (*B. subtilis, B. megaterium, B. pumilus*and *B. cereus)* against early blight *(A. alternata)* and reported that the promising effect of the four bacteria species tested in reducing disease incidence and severity in comparison with the control.

In conclusion the tested six fungicides at different concentration on the growth of *A. raphani.* Among the fungicides Hexaconazole was found 100% maximum mycelium growth inhibition while Copper oxychloride was found minimum mycelium growth inhibition. In bioagents *Trichoderma harzianum* was found most effective while least effective was found in *Bacillus cereus*.

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