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Efficacy of chemical, botanical and biological for the management of Alternaria leaf spot disease of radish

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Abstract

An experiment was conducted to evaluate the effect of different treatments viz. SAAF (mancozeb + carbendazim) (1%, 0.01% and 0.001%), Acorouscalamus root extract (1%, 0.5% and 0.25%) and Trichodermaharzianum extracted from local soil media and two radish varieties (Mino Early and Pyuthaney Rato) against Alternaria leaf spot disease (Alternariabrassicae) of radish in lab of Department of Plant Pathology AFU, Chitwan, Nepal during 2016. Poison food technique and dual culture technique was used to identify the efficacy of the treatments. The best check of mycelial growth of Alternariabrassicae was done by SAAF @ 0.1% which was followed by SAAF @ 0.01% and 0.001% respectively. Highest growth inhibition of the mycelium i.e. 93.65% was shown by SAAF @ 0.1% at the end of the investigation. The best control (31.94%) of the mycelium growth of the Alternariabrassicae was provided by Acorouscalamus root extract @ 1% on PDA which was statically at par with 0.5% of concentration inhibiting pathogen by 29.41%. Highest growth was observed in control. Result from the dual culture showed that at 4 days of incubation the Trichodermaharzianum. Completely covered the plate (8.9 cm) and Alternariabrassicae was limited within 2.30 cm diameter of colony size.

Keywords: Trichoderma, *Acorouscalamus,* alternaria leaf spot, fungicides.

Introduction

Crucifer vegetables are the important winter season cash crop grown in Nepal. Around 70% of Nepal's total household is involved in vegetable farming[1]. Radish (*Raphanussativus* L.) is one of the top five vegetables produced in Nepal, which covers 7.47% of total vegetable production area of Nepal with an average productivity of 14.45 mt ha^{-1} [2]. Radish crop has easy cultivation practice, wider climatic adaption and extensive use and thus it is popular among the farmers. Also, radish is the most important seed crop in terms of high demand of quality commercial seed[3].

The most common and destructive diseases of Brassicaceae crops worldwide are those caused by four species of Alternaria viz., *A. brassicae* (Berk.) Sacc, *A. brassicicola* (Schwein.) Wiltsh., *A. raphani* Groves and Skolko, and *A. alternata* (Fr.) Keissl which are seed borne pathogen. *A. brassicae* (Berk.) Sacc, *A. brassicicola* (Schwein.) Wiltshire are responsible for a serious grey and dark leaf spot disease on those[4,5]. At least 20% of agricultural spoilage is caused by Alternaria species; most severe losses may reach up to 80% of yield[6].

Among the root crops like radish, turnip, beet root and carrot, maximum infection (10-60%) has been found in radish, and the disease has widely spread in all the growing areas of Nepal[7]. Yield reduction up to 45% in radish has been reported from Kathmandu and Chitwan[8]. Average yield losses in the range of 32-57% due to *Alternaria* have been reported from Nepal[9]. Due to high plant parasitic nature of this pathogen it has become major problem in production of seed as it affect most during pod formation stage of the crop affecting seed quality by reducing seed size, seed discoloration and reduction in oil content[10].

Application of chemical fungicide is the arguably easiest and most effective method for the management of the disease. Chemical fungicide inhibits the spore germination and penetration of the pathogen in host but pathogen can generate resistance against the fungicide if not used in proper dose and interval of time[11,12] and cause environmental pollution[13]. These kinds of health and environment issues have created deception and fear mongering situation towards the use of chemical pesticides and thus there are strict rules and regulation towards the judicial use of pesticide around the globe.

Thus, this study was mainly focused on identifying the efficacy of different concentrations of chemical fungicide and botanical extract, *Trichodermaharzianum* extracted from local soil media in lab condition for the management of Alternaria leaf spot of radish.

Materials and methods

Preparation of the treatments: Isolation and identification of *Trichoderma* sp. from soil: As the soil of organic farm land and virgin soil is comparatively more fertile and has more chances

of containing the *Trichoderma* sp.; soil sample were collected from those location of Rampur, Chitwan. *Trichoderma* selective media was used for isolation of *Trichoderma* sp. from soil[14]. Inoculated plates were incubated at 24-25^{o}C in incubator for 1 week.

The culture plates were examined daily and each colony forming unit (cfu) were observed carefully. Phenotype character and fungal growth condition were observed to identify the Trichoderma species. After identification of the Trichoderma *i.e. Trichodermaharzianum,* multiplication of culture was done on PDA plates by transferring the spores of Trichodermafrom the pure culture maintained on the PDA test tubes in aseptic condition in inoculation chamber. Those plates were kept on incubator at 24-25^{o}C for one week.

Isolation and identification of *Alternaria* sp.: Leaves of radish infected with Alternaria leaf spot were collected from the infected plot and washed properly in tap water. The leaves were cut with scissors into small pieces (1-1.5 cm) containing both diseased and healthy part. The cut pieces were sterilized by dipping in sodium hypochlorite (1%) for a minute then washed with sterilized water thrice. After washing, those cut pieces were placed on three layered wetted filter paper in petridish and incubated at 25°C. After, 7 days of incubation the growth of *Alternariabrassicae* was observed under stereomicroscope. Single conidia of *Alternariabrassicae* were picked up with sterile needle and transferred to PDA medium to obtain pure culture.

Fungal species were identified on the basis of morphological characters of the conidia. The pure culture of the isolate of *Alternariabrassicae* from PDA plates was transferred to PDA slants in test tubes and stored in refrigerator below 5^{o}C for further research work**.**

Poisoned food technique: The efficacy of seven treatments (three concentration of SAAF i.e. SAAF @ 0.1%, 0.01%, 0.001% on PDA and three concentration of *Acorouscalamus* root extract @ 1%, 0.5%, 0.25 % on PDA including control against *Alternariabrassicae* was evaluated by poisoned food technique in *in vitro* condition. Five millimeter diameter of *Alternariabrassicae* of one week old culture was cut by cork borer and picked up with the help of inoculating needle and placed on to the center of the plate. The plates were incubated at 26±28^{o}C room temperature for ten days and the mycelium growth was measured at 2nd, 4th, 6th, 8th, 10th day. Inhibition of the mycelium was calculated as below:

$$\text{Mycelial inhibition\%} = \frac{\text{mycelial growth in control} - \text{mycelial growth in treatment}}{\text{mycelial growth in control}} \times 100$$

Dual culture technique: *In-vitro* biological activity of *Trichoderma* sp. on *Alternariabrassicae* was investigated by double cultures on the potato dextrose agar media. Trial was set up in four replications. The plates were incubated at 26±28^{o}C temperature for ten days and the mycelium growth was measured at 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th day respectively.

Statistical data analysis: The data were tabulated in Excel data sheet for analysis. The data with more than 30% coefficient of variation was transformed. The data was processed to fit into R-studio and analyses were conducted using R 3.0.3 (R Core Team, 2013) and the Agricolae v1.1-8 package (de Mendiburu, 2014). The data entry was done to develop ANOVA table and different treatments were compared through Duncan's multiple range test. All the figures and graphs were prepared by using Microsoft excel 2013.

Results and discussion

Effect of different concentration of SAAF on mycelial growth of *Alternariabrassicae:* The effect of SAAF (Carbendazim + Mancozeb) incorporated PDA on the growth of *Alternariabrassicae* in different incubation period at temperature (24-26^{o}C) is presented in Table-1. Results showed that the different concentration of SAAF had significant effect in checking the growth of *Alternariabrassicae* as compared to control when incubated for longer period of time, up to 10 days. In 2nd day 0.01% SAAF and 0.001% SAAF on PDA had no significant difference between them. In 4th day 0.1% SAAF and 0.01% SAAF on PDA had no significant difference between them. But in 6th, 8th, 10th day all the treatments were significant with each other. After 4th day 0.1% SAAF on PDA completely checked the growth of *Alternariabrassicae* as concentration of SAAF increased the growth of *Alternariabrassicae* decreased in all dates of incubation. The growth of *Alternariabrassicae* on PDA (control) was 1.47 and 8.67 cm in diameter and in 0.1% SAAF was 0.52 and 0.55 cm in diameter when incubated for 2 days and 10 days respectively. From 2nd day to 8th day maximum growth inhibition (64.62%-93.65%) of the *Alternariabrassicae* was done by SAAF @ 0.1% on PDA (Figure-1).

From the result it was clear that best check of mycelial growth of *Alternariabrassicae* was done by SAAF @ 0.1% which was followed by SAAF @ 0.01% and 0.001% respectively. Highest growth inhibition of the mycelium i.e. 93.65% was shown by SAAF @ 0.1% at the end of the investigation (Figure-1). This result was supported by Sadana and Didwania[15], the highest reduction in disease was achieved by applying mancozeb (1500 ppm) that caused 86.4 percent inhibition of mycelial growth. The fungicides (Captan, Cobox and Dithane M-45) @ 200 mg l^{-1} significantly ($P<0.05$) reduced the colony diameters of *A. solani* compared with the control (no fungicide) treatments. Dithane M-45 was found to be more effective than Captan and Cobox[16]. Mancozeb followed by Captafol were reported as potential fungicides against *A. solani* infecting tomato plants[17,18]. Similar to our study, Kumar *et al.*[19] tested 17 fungicides *in vitro* against *A. brassicae*, cause of Alternaria blight of radish and recommended Iprodione, Mancozeb, Achook, Riodmil MZ, Ziram and Captanto to control the pathogen. The inhibition of the pathogen might be due to the fungitoxic effect of the chemical SAAF (mancozeb + carbendazim).

Table-1: *In-vitro:* Effect of different concentration of SAAF (Carbendazim + Mancozeb) on mycelial growth of *Alternariabrassicae.*

Treatments	Mean colony diameter (cm)				
	2nd day	4th day	6th day	8th day	10th day
SAAF @ 0.1% on PDA	0.52[c]	0.52[c]	0.55[d]	0.55[d]	0.55[d]
SAAF @ 0.01%on PDA	0.67[b]	0.95[c]	1.05[c]	1.05[c]	1.07[c]
SAAF @ 0.001% on PDA	0.72[b]	1.20[b]	1.90[b]	2.00[b]	2.02[b]
Control	1.47[a]	2.15[a]	3.90[a]	5.95[a]	8.67[a]
LSD (=0.05)	0.07	0.15	0.20	0.18	0.22
SEm (±)	0.02	0.05	0.06	0.05	0.07
Coefficient of variation (%)	5.9	8.5	7.3	5	4.7
Grand mean	0.85	1.26	1.85	2.38	3.08

Figures in column with same letter are not significantly different (p=0.05). LSD = Least significant difference. SEm= standard error of mean.

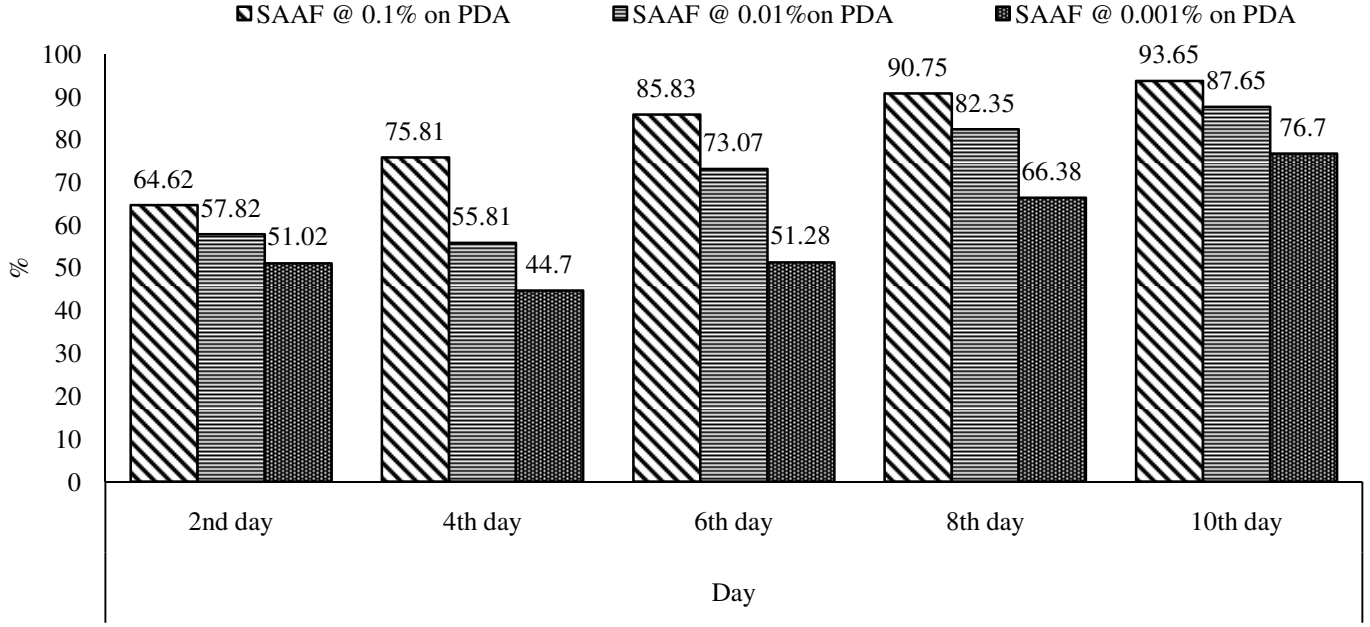

Figure-1: Growth inhibition of the mycelia of *Alternariabrassicae* under different concentration of SAAF (mancozeb + carbendazim).

Effect of different concentration of botanical root extract (*Acorouscalamus*) on the mycelial growth of *Alternariabrassicae:* The *Acorouscalamus* root extract incorporated PDA had significant (p=0.05) effect on the growth of the *Alternariabrassicae* at temperature 24-26°C as compared to control in all concentrations and at different duration of growth except in the lowest dose (0.25%) in 6th day but after 6th day it was also significant with all other treatments. Lowest colony growth (5.90) and highest inhibition (31.94) was recorded from 1% extract on PDA in comparison to other treatments and control (Table-2 and Figure-2).

In the present investigation the best control (31.94%) of the mycelium growth of the *Alternariabrassicae* was provided by *Acorouscalamus* root extract @ 1% on PDA (Table-2) which was statically at par with 0.5% of concentration inhibiting

pathogen by 29.41%. Highest growth was observed in control. Thus all concentration (0.1%, 0.5% and 0.25%) of *Acorouscalamus* was found effective in controlling the *Alternariabrassicae* this result was supported by Chijamo and Daiho[20] they reported *Acorouscalamus* was found effective in inhibiting the mycelial growth of *Curvularialunata* at all concentration (5%, 7.5% and 10%) with inhibition of 29.61%, 45.21% and 42.74% respectively. Maximum *in vitro* inhibition of mycelial growth and spore germination of *A. solani* was observed with (5-10%) dried root extracts of *Acorus calamus*[21]. Antifungal activity of aqueous *Acoruscalamus*@ 1000 mg/ml against *Fusariumoxysporum f. sp. Lycopersici*showed inhibition of 91.39%[22]. This reduction or inhibition in growth of the pathogen might be due to the chemical content (alpha-asarone or beta-asarone) present in the *Acorouscalamus* root extract, which was toxic to the pathogen and reducing its growth significantly.

Table-2: Effect of different concentration of botanical root extract (*Acorouscalamus*) on the mycelial growth of *Alternariabrassicae.*

Treatments *Acorouscalamus* root extract	Mean colony diameter (cm)				
	2nd day	4th day	6th day	8th day	10th day
1% on PDA	0.95^{c}	1.85^{c}	3.42^{b}	4.97^{c}	5.90^{c}
0.5% on PDA	1.17^{b}	1.90^{c}	3.52^{b}	5.05^{c}	6.12^{bc}
0.25% on PDA	1.22^{b}	2.05^{b}	3.77^{a}	5.27^{b}	6.30^{b}
Control	1.47^{a}	2.17^{a}	3.90^{a}	5.92^{a}	8.67^{a}
LSD (=0.05)	0.19	0.11	0.14	0.13	0.29
SEm (±)	0.06	0.03	0.04	0.04	0.09
Coefficient of variation (%)	10.5	3.8	2.5	1.7	2.8
Grand mean	1.20	1.99	3.65	5.30	6.75

Figures in column with same letter are not significantly different (p=0.05). LSD= Least significant difference. SEm= standard error of mean.

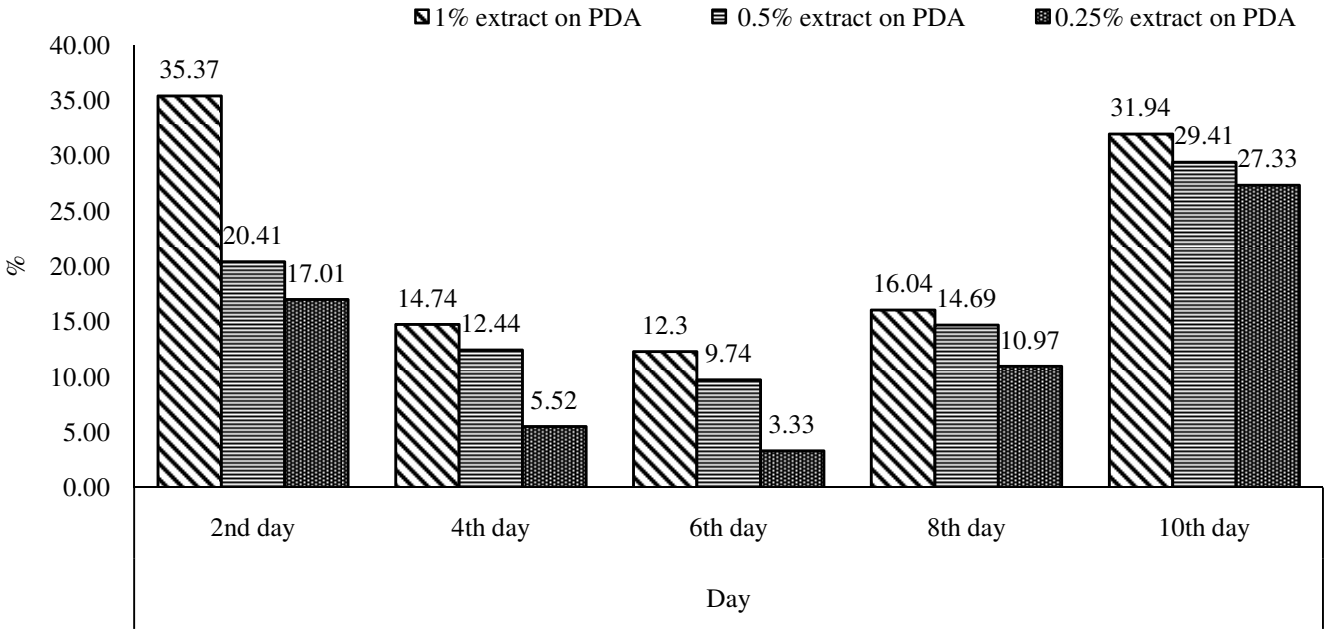

Figure-2: Growth inhibition of the mycelia of *Alternariabrassicae* under different concentration of *Acorouscalamus* root extract.

Effect of *Trichoderma* sp. on the development of *Alternariabrassicae:* The growth of *Alternariabrassicae* was restricted by the *Trichoderma* harzianum as shown in the result from dual culture experiment. Hyper-parasitism is the most considered and the most direct form of antagonism[23]. Trichoderma over grew on the pathogen suppressing and inhibiting its growth which could be clearly seen (Table-3). After 4 days of incubation the *Trichodermaharzianum* completely covered the plate (8.9 cm) but *Alternariabrassicae* was limited within 2.30 cm diameter of colony size. Khan and Khan[24] reported the growth of *A. brassicae* and *A. brassicicola* was restricted by *T. viride* and overgrew on pathogenic fungus. There was distinct change in colour at the zone of contact, which became light greenish yellow in case of *A. brassicae* as well as *A. brassicicola*. Efficacy of their conidial suspension and culture filtrates was evaluated. In dual culture test, *T. viride* caused maximum inhibition of radial growth of *A. brassicae* (74%) and *A. brassicicola* (77%). Harmanet al[25] reported that the rhizosphere competent strain of *T. harzianum*, Th-22, provided control that was both spatially and temporally distant from the point of application. *In vitro*, Percentage of growth inhibition of *Helminthosporium* by *T. harzanium* (1 and 2) was found to be 79.18 and 69.03%[26]. All the six isolates of *Trichoderma* sp. significantly ($p<0.05$) reduced the colony diameters of *A. solani* as compared to control[16b]. Direct antagonism by hyper-parasitism could be reason behind the reduction in incidence of the *Alternariabrassicae*.

Table-3: Effect of *Trichodermaharzianum* on the development of *Alternariabrassicae.*

Diameter (cm)		Days			
		1	2	3	4
Fragments on half medium	*Alternaria-brassicae*	1.10	1.69	1.90	2.30
	Trichoder-mahrzianum	1.89	5.79	7.77	8.9
Check	*Alternaria-brassicae*	1.11	1.47	1.90	2.45
	Trichoder-maharzianum	1.90	5.91	7.91	9

Conclusion

From the present study it is clear that among the three concentrations of SAAF best check of mycelial growth of *Alternariabrassicae* was done by SAAF @ 0.1%. However lower concentration of SAAF 0.01 % and 0.001 % was also significantly different from the control. Thus, lower concentration of SAAF 0.001 % could be used for the management of disease as it will be less deleterious for environment and health hazards. The higher concentration (1%) of *Acorouscalamus* root extract was found to be most effective among the three concentrations. Similarly *Trichoder-maharzanium* isolate prepared from local soil media was also efficient in control of the pathogen. In conclusion for complete check of the pathogen chemical fungicide was found to be best but for ecofriendly approach *Acorouscalamus* 1% root extract and *Trichodermaharzanium* could be used. However, further studies on efficacy of different isolates of Trichoderma can be done.

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