Original Research Article

Management of Alternaria Leaf Spot in Cabbage Through Bio-Inoculation of *Trichoderma* Spp. and Fluorescent Pseudomonas

Abstract

Alternaria leaf spot caused by *Alternaria sp. (A. brassicae and A.brassicicola)* has been reported from all the continents of the world and is one among the common diseases of cabbage. Alternaria blight is one of the most dominant one that causes average yield loss in the range of 32-57% . Bio-inoculated seedlings
exhibited significantly higher vigour index. Seedling vigour was found to be increased in single or com bination of *Trichoderma* and *Pseudomonas fluorescence* applied through seed treatment. Higher root and shoot dry biomass observed when *Trichoderma* applied as seed treatment. The rate of chlorophyll degradation was less in two treatments *P. fluorescence* applied through seed treatment and combined inoculation of *Trichoderma* and *P. fluorescence* applied through seed treatment. Low A UDPC was recorded, were *Trichoderma* and *P. fluorescence* applied combined through seed treatment.

Keywords: Alternaria leafspot, *Trichoderma*, *P. fluorescence,* Vigour index, AUDPC.

Introduction

Cabbage is one of the most popular crucifer vegetable grown in world. India is one of theimportant cabbages growing country in Asia. India is next to China in cabbage production Besides, good technology and certified seeds, the desirable production is not achieved becauseof damages caused by insect pests and diseases. In India about 20% of the crop yield is lost due to insect pests and disease per year, which approximately amo unt to Rs. 1500 crores but incase of outbreaks, loses, increased upto 50-90% (Singh et al, 2001). Alternaria black leaf spot disease is onethe most destructive disease of cabbage and brassicas worldwide (Meah *et al.* ,2022). A complex of Alternariaspecies (A. brassicicola (Schw.) Wiltsh., A. brassicae (Berk.) Sacc., A. alternata (Fr.)Kreissler and A. raphani Groves and S kolko) are responsible for considerable yield losses (Meah *et al.,* 2022).The pathogens are greatly influenced by weather with the highest disease incidence repo rted in mild, wet seasons and in areas with relatively high rainfall The pathogen appears on leavesand stems of cabbage seedlings and adult plants. It can also affect the siliquae causing a severereduction in the amount and the quality of head or seed production. Cabbage can be affected inall stages of growth, thus typical symptoms include black necrotic lesions surrounded by chlorotic areas on leaves, stems and siliquae (Mac et al., 1989) .A. brassicae and A. brassicicola can affect host species at all stages of growth, includingseeds. On seedlings symptoms include dark stem lesions immediately after germination, thatresult in damping-off, or stunted seedlings [Valkonen, *et al.* 1990)]. In addition to destruction of a seed crop, the pathogens can live within the seed, spread the disease to other fiel ds, and cause a loss oseedlings [Rangel *et al.,* 1945)

Materials and methods

Bio-inoculation of *Trichoderma sp* and *Pseudomonas fluorescence* to identify the Effect of bio-inoculation on physical health of cabbage seedlings under pot condition were tested during 2022-23 rabi crop season at Department of Plant Pathology, Rama University, Kanpur Five treatment s viz, seed treatment, soil application, seed treatment + soil application, seed treatment + foliar a pplication and foliar application by using *Trichoderma harzianum*, *Pseudomonas fluorescence* and *Trichoderma harzianum* + *Pseudomonas fluorescence.*

Bio agents

Pure culture of *Trichoderma harzianum* (UBT18) and *Pseudomonas fluorescence* (VPF-1) was obtained from, Department of Plant Pathology, Rama University, Kanpur. *Pseudomonas fluorescence* strain VPF-1 was mass multiplied on King´s B broth IN 250 ML Erlenmeyer flasks, incubated at 28 ºC for 48 hrs. in shaker incubator. Cell suspension of bacterial strain was adjusted to concentration of 106 CFU/ml and used along with talc and Carboxy methyl cellulose. *Trichoderma harzianum* (UBT18) was mass multiplied

on potato dextrose broth, after full mycelia growth obtained media mixed with talc and Carboxy methyl cellulose.

Preparation of pathogen inoculums

Alternaria sp. was isolated from leaf spot affected cabbage leaf. The fungal inoculums were tak en from petridishes and transferred to potato dextrose broth (PDB) and was kept for 7 days of incubation. After the full growth of pathogen the mycelial mat was harvested and ho mogenized. Conidial concentration was determined using a haemocytometer and adjusted to 5 x 105 conidia per mL. Plants were labeled and spray inoculated with a conidial suspension of pat hogen using 1000 mL hand held atomizer directed at the central part of the upper leaf side. Approximately 0.3 mL conidial suspension per leaf was applied to the plants.

Mode of application

Seeds were treated with *Trichoderma harzianum* and *Pseudomonas fluorescence* at the rate of 5 gram per kg, of seed, applied 30 min. before sowing. *Trichoderma harzianum* and *Pseudomonas fluorescence* were applied i n soil at the rate of 12.5 kg per ha. Foliar spray of *Trichoderma harzianum* and *Pseudomonas fluorescence*, at 1% concentration of talc based formulation done 5 days before pathogen inoculation.

Plant growth promotion

Plant growth promotion activity of *Trichoderma harzianum* and *Pseudomonas fluorescence* we re assessed based on the seedling vigour index was calculated by using the formula described by Abdul Baki and Anderson

Vigour Index = mean root length + mean shoot length x germination (%)

Chlorophyll estimation

Chlorophyll content estimated by using Konica Minolta SPAD-
502 PLUS at transplanting stage and before challenge inoculation

Shoot and root dry weight

Seedlings at the transplanting stage kept at hot air over for 7 days and measured the shoot and root dry biomass.

Percent disease index

Percent disease index (PDI) was calculated for each plot by summing the scores of twenty leave s and analyzing using rating scale. The value was expressed as percentage using the following f ormula:

No. of leaves examined x maximum rating score

Scale (0-6) used for rating:

Rating Symptoms of Alternaria blight on leaves16

0=No infection

1=Up to 5% leaf area covered

3= 5-10% leaf area covered

5=11-25% leaf area covered

7= 26-50% leaf area covered

9 =More than 50% leaf area covered

AUDPC

Area under Disease Progress Curve (AUDPC) was calculated by using the following formula

Si = Disease severity at the end of time I, k = Number of successive evaluation of blight severity

d = Interval between two observations

Result and Discussion

Effect on Vigour Index

Vigour index is one of the most important indices represents the plant health. In the present investigation the results on vigour index revealed that irrespective of delivery system i.e. through seed treatment or soil applica tion or combination of both bio-inoculated seedlings add significantly higher vigour index (1520.00-1813.33) in comparison to untreated check, on the other hand single or combination of *T. harzianum* and *P. fluorescence* applied through seed treatment resulted comparatively higher vigour ind ex. Seed treatment along with soil application did not provided better results as compared to seed treatm ent alone. Although many research findings reported that seed treatment is the most suitable way to intr oduce biocontrol agents to the spermosphere rather than soil application but however seed along with so il application also exhibit incomplete rhizhosphere colonization which reflects through higher vigour in dex but it may not always be true due to spatial structure of population leading to repeated interaction be tween the mutualists (Hamilton *et al* 1964).

Results

on dry biomass of root and shoot of cabbage seedlings at transplanting stage have been presented in tabl e

1 which revealed that shoot and root dry biomass increase significantly over control. Significantly high er shoot biomass was observed when *Trichoderma* was applied thorough seed treatment (0.075 mg/seed ling) which was significantly at par with *Trichoderma* applied through seed treatment and soil applicati on, *P. fluorescence* through seed treatment and soil application and combined application of *Trichoder ma* and *P. fluorescence* seed treatment (0.05-

0.069 mg/seedling). Later three was only significantly at par with *Trichoderma* applied through soil app lication and combined inoculation of *Trichoderma* and *P. fluorescence* through seed treatment and soil application (0.060 and

0.059 mg/ seedling respectively). The result was also in corroboration with the previous results of vigo ur index, seed treatment was comparatively better than applied through seed treatment and soil applicati on in combination. Root dry weight also follow almost the same trend, were *Trichoderma* through seed treatment resulted significantly higher root dry biomass (0.016 m/seedling) and it was significantly at par with *Trichoderma* application with seed and soil (0.014 mg/seedling). The increase in shoot and root dry biomass over control was highest in *Trichoderma* treated seedlings through seed treatment (56.25 and 77.78 % respectively) followed by *Trichoderma* applied combining through seed treatment and soil application (43.75 and 56.56% respectively). Higher the root shoot ratio reveal better increase in root surface area and better establishment of crop in soil. The present result indicated that better root biomass in comparison to shoot was produced by the seedlin gs treated with combined inoculation of *Trichoderma* and *P. fluorescence* through seed treatment and s oil application (R:S-

0.220) which was followed by *Trichoderma* applied through soil application (0.217) and *Trichoderma* applied through seed treatment (0.213). Enhancement of shoot dry weight from 16-48% and root dry weight from 82-137% when inoculated with Fluorescent Pseudomonads [22].

Variation in total chlorophyll in cabbage under influence of bio-control agents at 20 DAS and 45 DAS.

Variation in total chlorophyll in cabbage under influence of bio-control agents

Results on total chlorophyll as expressed in SPAD -502 readings at transplanting stage at 20 days after sowing and at pathogen inoculation time at 45 days after sowing, have been elucidated in fig.1 showed no significant variation within the bio-

agent treated seedlings irrespective of delivery system and the total chlorophyll was ranged between (36 .4-

37.6) at transplanting state. Although these were significantly higher in comparison to control (36.2). C omparatively higher variation was found at the time of inoculation among the bio-treated plants had significantly higher total chlorophyll was measured in the plants developed with *P. fluorescence* applied through seed treatment and combined in oculation of *Trichoderma* and *P. fluorescence* applied through seed treatment. The rate of chlorophyll degradation was less in those two treatments. Aging of plants increases oxidative stress in chloroplasts and results in reduction of total chlorophyll in leaves [3].

Table 1: Effectofbio-inoculationondrybiomassofcabbageseedlings

Treatment	Shoot dry wt. (mg/seedling)	Root dry wt. (mg /seedling)	Root: shoot	% increase in sh oot dry wt. over control	% increase in ro ot dry wt. over c ontrol
Tr-ST	0.075A	0.016A	0.213	56.25	77.78
Tr-SA	0.060BC	0.013BC	0.217	25.00	44.44
Tr-ST+SA	0.069AB	0.014AB	0.203	43.75	55.56
Pf-ST	0.067AB	0.014B	0.209	39.58	55.56
Pf-SA	0.061B	0.011CD	0.180	27.08	22.22
Pf-ST+SA	0.065AB	0.013BC	0.200	35.42	44.44
Tr+Pf-ST	0.066AB	0.014B	0.212	37.50	55.56
Tr+Pf-SA	0.063AB	0.013BC	0.206	31.25	44.44
Tr+Pf-ST+SA	0.059BC	0.013BC	0.220	22.92	44.44
Control	0.048C		0.009D		0.188
SEm± CD(P=0.05)		0.00428 0.01219		0.00077 0.0021931	

Conclusion

Bio-
inoculated seedlings exhibited significantly higher vigour index. Single or combination of T. ha rzianum and P. fluorescence applied through seed treatment resulted comparatively higher seed ling vigour. Seed treatment along with soil application did not provide better result as compared to seed treatment alone.

Higher shoot biomass was observed when Trichoderma was applied through seed treatment. Root dry biomass also followed the same trend. Increase in shoot and root dry biomass over control was highest in Trichoderma treated seedlings applied through seed treatment.

Higher total chlorophyll was measured in the plants developed with P. fluorescence applied through seed treatment and combined inoculation of Trichoderma and P. fluorescence applied through seed treatment. The rate of chlorophyll degradation was less in those two treatments.

Significant variation in disease development was found then after and it continued up to terminal stage of disease record. Fourteen days after challenge inoculation, lowest disease severity was recorded in the plants raised by combined inoculation of Trichoderma and P. fluorescence applied through seed treatment. Soil application did not show any significant effect in reduction of disease severity at the terminal stage disease record i.e. 28 days of challenge inoculation. Lowest severity was recorded when both Trichoderma and P. fluorescence were applied through seed treatment and foliar application. In control plants 40.74% disease severity was recorded which indicated that as high as 30.90% reduction in disease severity could be achieved through biocontrol agent application. Low AUDPC was recorded were Trichoderma and P. fluorescence applied combined through seed treatment. In control plants the calculated AUDPC was significantly higher.

Before challenge inoculation the highest protein concentration was measured in plants raised with combined inoculation of Trichoderma and Pseudomonas fluorescence applied through seed treatment and foliar application (5.49 mg/g of fresh wt.). After challenge inoculation highest increase was observed in the plants raised with P. fluorescence applied through seed treatment and soil application (116.72%) followed by Trichoderma applied through foliar application (107.17%). In general P. fluorescence was found to be more potential protein inducer compared to Trichoderma.

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