**Effect of bio-agents and fungicides in management of leaf spot of chilli (*Capsicum annuum* L.) caused by *Alternaria alternata* (Fr.) Keissler**


## ABSTRACT

The study was carried out during the *rabi* season of 2022 at the Central Research Field, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology & Sciences, Prayagraj. The experiment was carried out in Randomized Block Design (RBD). Among the treatments taken up for research Mancozeb (1g/L)+Carbendazim (1g/L for foliar spray) was found most effective against *Alternaria alternata*. The minimum disease intensity (11.21, 14.42 and 18.97) was obtained in Mancozeb (1g/L)+Carbendazim (1g/L as foliar spray). It can be concluded that foliar spray treatment of chilli with Mancozeb (1g/L)+Carbendazim (1g/L as foliar spray) recorded higher plant height (61.63), number of leaves per plant (184.60), number of branches per plant (12.07), number of fruit per plant (55.93), fruit weight (g) (4.61), fruit yield (g) per plant (258.70), fruit yield (g) per plot (1293.49) and fruit yield (t $ha^{-1}$) (6.47).



**Key word:-** Bioagent, Fungicides, disease intensity, growth, yields and chilli.


## INTRODUCTION

"Chilli is an important vegetable and spice crop and it belongs to the family 1lternaria. Capsicum annuum L. and Capsicum frutescence L. are two important species cultivated in several tropical and sub tropical climates both for green and ripen dry fruit" (Abhinav *et al.*, 2021). "Chilli(*Capsicum annuum* L.) belongs to family Solanaceae, which is emerging as one of the commercial vegetable crops at the global level, and is probably most important vegetable after Tomato. Chilli finds its place in spice as well as condiments. Chilli fruits are rich sources of vitamin C, vitamin A and E" (Singh *et al.*, 2004). "Pungency of chilli is due to a crystalline acrid volatile alkaloid called capsaicin, present in the placenta of fruit. It is also a good source of chilli oleoresin, which is the total flavour extract of dried and ground chillies. The natural colour extracts of chilli are also finding their increased value in place of artificial colours in the food items" (Katheek *et al.*, 2018). "Worldwide chilli is grown on an area of 20.20 Mha with production of 37.62 Mt respectively. India is leading the world in chilli production with 13.76 (36.57%) million tonnes per annum" (Geetha and Selvarani, 2017). "The chilli crop suffers due to a number of fungal, bacterial and viral diseases, which render its production into stake" (Mukherji and Bhasin, 1986; Singh, 2003; Agrios, 2004). "Among the various fungal diseases leaf spot, fruit rot incited by Alternaria alternata (Fr.) Keissler is becoming a limiting factor and posing a major problem in Kanpur and adjoining areas" (Narain et al., 2000). "The pathogen has been reported to cause seed, seedling, leaf and fruit diseases" (Sreekantiah et al., 1973; Mehrotra, 1980; Alam et al., 1981; Singh, 2003). "Post harvest decay of fruits and seeds has also been recorded due to this pathogen" (Leyendecker, 1954 a; Mathur and Agnihotri, 1961; Spalding and King, 1981). "The use of *Trichoderma* as biological control agent is being considered because of its antagonistic properties against pathogenic microorganisms and its beneficial effect to the environment. This alternative method may help reduce pesticide use. In spite of several studies on the antifungal effect of the biocontrol agents, *T. harzianum* and *T. viride* were reported to be effective in controlling the *Alternaria alternate"* (Rajathilagam and Kannabiran, 2001).

*"Pseudomonas fluorescens* is adapted to survival in soil and colonization of plant roots" (Kiely *et al*., 2006) and this applies also to the particular case of biocontrol agents from this species. "Biocontrol strains have noticeably been observed at the root surface, (i.e. the rhizoplane) often forming microcolonies or discontinued biofilms in the grooves between epidermal cells. Certain strains are also capable of endophytic colonization. Within root tissues, they are mostly found in the intercellular spaces of the epidermis and the cortex" (Duijff *et al*., 1997; Pavithra et al., 2021). "Many biocontrol agents from *P. fluorescens* and closely related species are well characterized for their ability to produce antimicrobial compounds, including 2,4- diacetylphloroglucinol (DAPG), phenazines, hydrogen cyanide and surfactants" (Haas and De´fago, 2005).

**MATERIALS AND METHODS**

The experiment was conducted in the research laboratory of Department of Plant Pathology and Central Research Farm, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad. The present investigation was carried out during the *rabi* season of 2022.

**1. Isolation**

Leaves were collected from infected chilli plant bearing characteristics symptoms of concentric rings of *Alternaria alternata*. These leaves symptoms after mounting on solid were examined under microscope to confirms the presence of *Alternria* spp. These selected infected leaf parts was cut into small pieces of 2 to 3mm dimension in a manner so that pieces may have some green portion also. Such leaf bits were washed 3 times in sterilized distilled water and then surface sterilized with0.1% mercuric chloride solution for 30sec. Excess of moisture was removed by putting these pieces in between two folds of sterilized blotting paper under aseptic conditions in the inoculation chamber. Five leaves bits were transferred on PDA medium contained in petri plates aseptically with the help of sterilized forceps. These ptri plates were incubated at $25^0$C+ $2^0$C in BOD for 5 to 7 days. After 5 days mycelin growth was observed around leaf bits from this colony growth (Ahmad *et al*., 2013), a portion from the periphery having single hyphal tip were separated and transferred to other petri plates having medium to get pure culture and identification of the pathogen was confirmed by observing the morphological feature of colony, spore characteristic and referring the relevant literature.

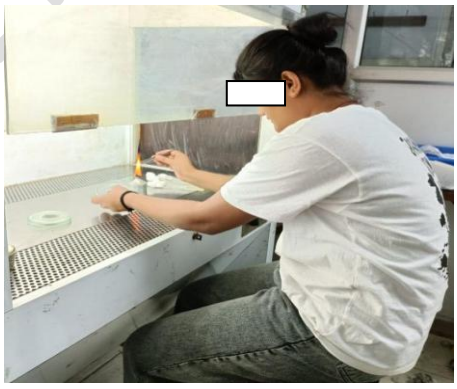

**Plate 1: Isolation of the pathogen**

**2. Identification of pathogen:**

The fungi initiation of disease symptom is from basal leaves in the form of small, yellow, circular patches which becomes necrotic, having 2-5 concentric rings in centre of the spots on

leaves and light brown in colour (1-7 mm in diameter). Sometimes these spots coalesce with each other and occupy large blighted area. In later stage, spots become larger in size with distinct concentric rings and dark brown to black necrotic lesions on the stem, leaves, twigs and fruits and also become shown symptoms whole plant parts and after shown typical blight symptoms and its produced a bull's eye appearance of concentric rings. Usually, the spots formed by the Alternaria are surrounded by a chlorotic halo. Fungal colonizes the in xylem of the host plant, and as a result, blockage and breakdown of the xylem lead to wilt disease symptoms such as, leaf wilting, yellowing and eventually the death of the plant.

**(a)** **(b)**

**Plate 2: Severely infected chilli leaf with leaf spot (*Alternaria alternata*)**

**3. Morphological characterization**

The culture of the fungal colony was initially the hyphae were hyaline slender, radiating and septet.In advanced age of culture was white, cottony with profuse aerial mycelium which gradually turned grey colour. Aged culture appeared completely greyish with aerial mycelium and distinct concentric rings was formed on medium. Conidiophores were short to long, simple or branched, crect simple cylindrical, golden to brown coloured with 2-9 transverses and 0-2 longitudinal septa. Conidia were born in long chains , they were thick walled, straight or curved body of conidium ellipsoidal tapering to the beaked and brown in colour.With the above characteristics, the pathogen was identified as *alternaria alternata* in accordance to the report of Ellis. The pathogen city of the fungus was established by following Koch 's postulates.

**(a)** **(b)**

**Plate 3: Microscopic view of *alternaria alternata***

**Maintenance and preservation of culture:**

The stock culture of the *Alternaria* spp. Associated with chilli plants was mour on PDA slant and preserved in refrigerator at $5^0$C. The pathogen was sub cultured regular intervals of 1 month to maintain the live culture.


**(a) Pure culture** **(b) Sub-culture**

**Plate 4: Culture of *Alternaria alternata***

Research field situated at 25°27'North latitude 80°50 'East longitudes and at an altitude of 98m above sea level. The climate is typically semi-arid and sub-tropical. The maximum temperature reaches up to 48°C in summer and drops down to 2.5°C in winter. The experiment was laid out in a single randomized block design (RBD) with seven treatments including untreated control and treated control, each replicated three times. $T_0$ Control (untreated check), $T_1$ *Pseudomonas flurescens*(2g/L as seedling treatment), $T_2$ *Trichoderma viride*(2g/L as seedling treatment), $T_3$ *Pseudomonas fluorescens*(2g/L as seedling treatment) +carbendazim(0.5g/L as foliar spray), $T_4$ Mancozeb(2g/L as folair spray), $T_5$ Mancozeb(1g/L)+Carbendazim(1g/L as foliar spray) and $T_6$Carbendazim(2g/L as foliar Spray). Standard disease rating scale (0-9 scale) for assessing PDI of *Alternaria alternata* of chilli 0-No symptoms on plant.; 1- Small spots on leaves, less than 1 per cent of leaf area diseased; 3- Medium six spots on leaves covering 1-10 per cent infected area; 5- Spots big; coalescing covering 11-25 per cent of leaf area.; 7- Spots large; coalescing covering 26-50 per cent of leaf area; 9- Spots on leaves covering above 51 per cent of leaf area.

## RESULTS AND DISCUSSION

The data presented in table 1 represents the response of the treatments used against *Alternaria alternata* in chilli at 75 DAT days after transplanting under field condition. The minimum disease intensity % at75 DAT was recorded in treatment $T_5$ Mancozeb (1g/L)+Carbendazim (1g/L as foliar spray) followed by $T_4$ Mancozeb (2g/L as foliar spray) and $T_6$ Carbendazim (2g/L as foliar Spray) and the highest was recorded in treatment Control (untreated check) (50.12). All the treatments are significant over control. The data on plant heigh, number of leaves and number of branches per plant of chilli at 90 DAT is presented in table 1. The response of selected treatments used against *Alternaria alternate* leaf spot of chilli under field condition perusal of the data indicated that all the treatments were significantly superior over control. The maximum plant height (cm) obtained at 90 DAT was recorded in $T_5$ Mancozeb (1g/L)+Carbendazim (1g/L as foliar spray) followed by $T_4$ Mancozeb (2g/L as folair spray) and $T_6$ Carbendazim (2g/L as foliar spray) as compared to $T_0$ control untreated check. All the treatments were found statistically significant over $T_0$ control. Among the treatments ($T_1$ and $T_0$) were significant over all the other treatments and among the treatments ($T_5$ and $T_4$), ($T_3$, $T_6$, and $T_2$) are statistically non-significant with each other. The maximum number of leaves per plant obtained at 90 DAT was recorded in $T_5$ Mancozeb

(1g/L)+Carbendazim (1g/L as foliar spray) followed by $T_4$ Mancozeb (2g/L as folair spray) and $T_6$ Carbendazim (2g/L as foliar spray) as compared to $T_0$ control untreated check. All the treatments were found statistically significant over $T_0$ control. Among the treatments ($T_5$, $T_4$, $T_3$, $T_2$, $T_1$, and $T_0$) were significant over all the other treatments and among the treatments ($T_6$ and $T_3$) are statistically non-significant with each other. The maximum number of branches per plant obtained at 90 DAT was recorded in $T_5$ Mancozeb (1g/L)+Carbendazim (1g/L as foliar spray) followed by $T_4$ Mancozeb (2g/L as foliar spray) and $T_6$ Carbendazim (2g/L as foliar spray) as compared to $T_0$ control untreated check. All the treatments were found statistically significant over $T_0$ control. Among the treatments ($T_6$, $T_5$, $T_4$, $T_1$ and $T_0$) were significant over all the other treatments and among the treatments ($T_6$ and $T_3$), ($T_3$ and $T_2$), ($T_2$ and $T_1$) are statistically non-significant with each other.

The data presented in table 2 represent that the maximum number of fruit per plant was recorded in $T_5$ Mancozeb (1g/L)+Carbendazim (1g/L as foliar spray) followed by $T_4$ Mancozeb (2g/L as folair spray) and $T_6$ Carbendazim (2g/L as foliar spray) as compared to $T_0$ control untreated check. All the treatments were found statistically significant over $T_0$ control. Among the treatments ($T_6$, $T_5$, $T_2$, $T_1$ and $T_0$) were significant over all the other treatments and among the treatments ($T_4$ and $T_6$), ($T_6$ and $T_3$) are statistically non-significant with each other. The maximum fruit weight (g) was recorded in $T_5$ Mancozeb (1g/L)+Carbendazim (1g/L as foliar spray) followed by $T_4$ Mancozeb (2g/L as folair spray) and $T_6$ Carbendazim (2g/L as foliar spray) as compared to $T_0$ control untreated check. All the treatments were found statistically significant over $T_0$ control. Among the treatments ($T_6$ and $T_0$) were significant over all the other treatments and among the treatments ($T_5$ and $T_4$), ($T_3$, $T_2$ and $T_1$) are statistically non-significant with each other. Among all the treatments the maximum fruit yield (g) per plant was recorded in $T_5$ Mancozeb (1g/L)+Carbendazim (1g/L as foliar spray) followed by $T_4$ Mancozeb (2g/L as folair spray) and $T_6$ Carbendazim (2g/L as foliar spray) as compared to $T_0$ control untreated check. All the treatments were found statistically significant over $T_0$ control. Among the treatments ($T_6$, $T_1$, and $T_0$) were significant over all the other treatments and among the treatments ($T_5$ and $T_4$), ($T_3$ and $T_2$) ($T_2$ and $T_1$) are statistically non-significant with each other. Among all the treatments the maximum fruit yield (g) per plot was recorded in $T_5$ Mancozeb (1g/L)+Carbendazim (1g/L as foliar spray) followed by $T_4$ Mancozeb (2g/L as folair spray) and $T_6$ Carbendazim (2g/L as foliar spray) as compared to $T_0$ control untreated check. All the treatments were found statistically significant over $T_0$ control. Among the treatments ($T_6$, $T_3$, and $T_0$) were significant over all the other treatments and among the treatments ($T_6$ and $T_5$), ($T_4$ and $T_3$) ($T_3$ and $T_4$) are statistically non-significant with each other.Among all the treatments the maximum fruit yield (t $ha^{-1}$) was recorded in $T_5$ Mancozeb (1g/L)+Carbendazim (1g/L as foliar spray) followed by $T_4$ Mancozeb (2g/L as folair spray) and $T_6$ Carbendazim (2g/L as foliar spray) as compared to $T_0$ control untreated check. All the treatments were found statistically significant over $T_0$ control. Among the treatments ($T_6$, $T_3$, and $T_0$) were significant over all the other treatments and among the treatments ($T_5$ and $T_4$), ($T_3$ and $T_2$), ($T_2$ and $T_1$) are statistically non-significant with each other. Chilli is one of the most important commercial vegetable and spice crops of India. The crop is subjected to attack by a number of diseases, of which Alternaria leaf spots caused by *Alternaria alternata* are becoming major limiting factors in cultivation of chilli. The information regarding the pathogens as well as disease on this crop is very less. To bridge this gap, the present investigation on disease survey, isolation and identification of the pathogen, pathogenicity test, cultural and physiological studies, *in-vitro* evaluation of bio-agents and chemical of best treatments from *in-vitro* was studied in field conditions against the leaf spot pathogens. Results showed that tested fungicides could inhibit the conidial germination of *Alternaria* spp. The perfect findings are in line with the findings of (**Koka *et al.*, 2021)** who reported that the fungicide carbendazim was able to

inhibit mycelium growth and conidia germination *Alternaria* sp. The evaluation of fungicides against *A. alternata* revealed that mancozeb (0.2 %) showed maximum per cent inhibition of pathogen in poisoned food technique. The efficacy of mancozeb against *Alternaria* spp. was reported by several workers, **(Maheswari and Singh, 1998; Kannan and Subbaraja, 1999; Muthulakshmi, 1990; Babu, 1994; Mohan, 1996 and Sumathi, 1997).** The reason may be the fungicidal compound may affect the sterol biosynthesis in fungal metabolism **(Vidyasekaran, 1998). Dar *et al.* (2013)** evaluated nine fungicides namely: carbendazim, hexaconazole, thiophonate methyl, triadimefan, metalaxyl, mancozeb, captan, copper oxychloride and chlorothalonil. Pairashi *et al.* (2007) who reported that spraying of carbendazim 50% WP (0.05%) immediately after appearance of the disease followed by another spray at 10-12 days interval recorded minimum disease incidence of frog eye leaf spot of tobacco. Pairashi et al. (2007) who reported that field evaluation of P. fluroscens (2 g/lit) in the control of frog eye leaf spot of tobacco recorded minimum disease incidence.

## CONCLUSION

Among the treatments taken up for research Mancozeb (1g/L)+Carbendazim (1g/L for foliar spray) was found most effective against *Alternaria alternata*. The minimum disease intensity (11.21, 14.42 and 18.97) was obtained in Mancozeb (1g/L)+Carbendazim (1g/L as foliar spray). It can be concluded that foliar spray treatment of chilli with Mancozeb (1g/L)+Carbendazim (1g/L as foliar spray) recorded higher plant height (61.63), number of leaves per plant (184.60), number of branches per plant (12.07), number of fruit per plant (55.93), fruit weight (g) (4.61), fruit yield (g) per plant (258.70), fruit yield (g) per plot (1293.49) and fruit yield (t $ha^{-1}$) (6.47). Now a days, for the management of leaf spot of Chilli is use of bio-agents and chemical. Chemical treatment take fast action on disease that is harmful to human health but reduce disease and increase yield of crop, where bio-agent take slow action on disease but not hazardous for environment.

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**Table 1 Percentage of Disease intensity and Growth parameters of among various Treatments**

| S. No. | Treatments | Disease intensity (%) & Growth parameters | | | |
|---|---|---|---|---|---|
| | | **Disease intensity (%)75 DAT** | **Plant height (cm)** | **Number of leaves per plant** | **Number of branches per plant** |
| $T_0$ | Control (untreated check) | 50.12 | 43.51d | 147.53f | 6.67f |
| $T_1$ | *Pseudomonas flurescens* (2g/L as seedling treatment) | 44.69 | 51.39c | 155.13e | 8.87e |
| $T_2$ | *Trichoderma viride* (2g/L as seedling treatment) | 38.56 | 53.70b | 160.73d | 9.07de |
| $T_3$ | *Pseudomonas fluorescens* (2g/L as seedling treatment) carbendazim (0.5g/L as foliar spray) | 31.90 | 54.54b | 171.27c | 9.87cd |
| $T_4$ | Mancozeb (2g/L as foliar spray) | 22.34 | 59.96a | 180.13b | 11.07b |
| $T_5$ | Mancozeb (1g/L)+Carbendazim (1g/L as foliar spray) | 18.97 | 61.63a | 184.60a | 12.07a |
| $T_6$ | Carbendazim (2g/L as foliar spray) | 28.47 | 55.78b | 174.40c | 10.13c |
| | **S.Ed. (+)** | **1.06** | **0.97** | **4.36** | **0.38** |
| | **C.D. (0.5%)** | **2.32** | **2.11** | **2.00** | **0.83** |

*Average of three replications

*Data followed by same letter in a column are non-significant to each other at 5% level

**Table 2 Number of different treatments on variance of Yield attributes**

| S. No. | Treatments | Yield attributes | | | | |
|---|---|---|---|---|---|---|
| | | **Number of fruit per plant** | **Fruit weight (g)** | **Fruit yield (g) per plant** | **Fruit yield (g) per plot** | **Fruit yield (t $ha^{-1}$)** |
| $T_0$ | Control (untreated check) | 34.93f | 2.46d | 86.04e | 430.21e | 2.15e |
| $T_1$ | *Pseudomonas flurescens* (2g/L as seedling treatment) | 43.60e | 3.30c | 144.01d | 720.04d | 3.60d |
| $T_2$ | *Trichoderma viride* (2g/L as seedling treatment) | 46.53d | 3.33c | 155.26cd | 776.28cd | 3.88cd |
| $T_3$ | *Pseudomonas fluorescens* (2g/L as seedling treatment) carbendazim (0.5g/L as foliar spray) | 49.73c | 3.56c | 177.37c | 886.87c | 4.43c |
| $T_4$ | Mancozeb (2g/L as foliar spray) | 53.20b | 4.45a | 237.40a | 1186.98a | 5.93a |
| $T_5$ | Mancozeb (1g/L)+Carbendazim (1g/L as foliar spray) | 55.93a | 4.61a | 258.70a | 1293.49a | 6.47a |
| $T_6$ | Carbendazim (2g/L as foliar spray) | 51.00bc | 4.13b | 210.89b | 1054.44b | 5.27b |
| | **S.Ed. (+)** | **2.44** | **0.30** | **22.44** | **112.22** | **0.25** |
| | **C.D. (0.5%)** | **1.12** | **0.13** | **10.30** | **51.50** | **0.56** |

*Average of three replications

*Data followed by same letter in a column are non-significant to each other at 5% level