International Journal of Plant & Soil Science

Volume 35, Issue 19, Page 1253-1259, 2023; Article no.IJPSS.105041
ISSN: 2320-7035

Effect of Bio-Agents and Fungicides in Management of EAF Spot of Chilli (*Capsicum annuum* L.) Caused by *Alternaria alternata* (Fr.) Keissler

Komal Kumari [a*] and Sunil Zacharia [a++]

[a] Department of Plant Pathology, Naini Agricultural Institute, Sam Higginbottom University of Agricultur, Technology and Sciences, Prayagraj- 211007, U.P., India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2023/v35i193664

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/105041

Original Research Article

Received: 22/06/2023
Accepted: 26/08/2023
Published: 01/09/2023

ABSTRACT

The study entitled "Effect of bio-agents and fungicides in management of leaf spot of chilli (*Capsicum annuum* L.) caused by *Alternaria alternata* (Fr.) Keissler" was carried out during the *rabi* season of 2022-2023 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

[++] *Associate Professor;*
**Corresponding author: E-mail: kk8757463257@gmail.com;*

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).

Keywords: Bioagent; fungicides; disease intensity; growth; yields and chilli.

1. INTRODUCTION

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 [1]. 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. India is a major producer, exporter and consumer of chilli. In India, it is grown throughout the country but principal chilli growing states are Andhra Pradesh, Maharashtra, Karnataka, Tamil Nadu, Orissa, Madhya Pradesh, Rajasthan, West Bengal and Uttar Pradesh. Andhra Pradesh, Maharashtra, Karnataka and Tamil Nadu constitute 75% of the total area of its cultivation and production. In Uttar Pradesh chillies are mostly grown in eastern eastern districts viz., Ballia, Azamgarh, Mirzapur, Basti and Faizabad [1]. The Global area under chilli cultivation is 1.776 million hectare with a production of 7.182 million tones. The India‟s area under chilli cultivation is 411 thousand hectare and total chilli production is 4363 thousand MT [2]. The Chilli fruits are used for imparting pungency both at green stage as well as after maturity. The fruit varies in size from 1-20 cm in length from thin, long to conical and thick fleshed blocky shape. The popularity of chilli is due to its wide range of shape, size and sensory attributes such as colour, pungency and piquancy that make generally insipid bulk nutritive flesh, cereal and vegetable foods more appetizing [3]. Most of the varieties grown in the country are pungent varying from very pungent to mild pungency. In food and beverage industries chilli is being used in the form of oleoresin which permits better distribution of colour, flavour in food. Pungency is due to the presence of capsaicin content [4]. Capsaicin is used in the preparation of balms, whereas the colour extracts (caratenoids pigments) find use as colour additives in food industry and prawn feed industry. The main functional properties of chilli are pungency, antioxidant activity, vitamin C and natural pigments [5]. Green chillies are rich source of Vitamin A and Vitamin E. It is widely used in the curry powder, curry paste, all kinds of pickles and preparing sauce, soups, etc. The quality of dried chilli is assessed by a number of different parameters such as colour, hotness, ascorbic acid content and volatile flavour compounds [6-8]. Fungi, bacteria, viruses, nematodes and abiotic stress are the causal entities for this. Among fungal diseases, leaf spot caused by *Alternaria alternate*(Fr.) Keissler and *Cercosporacapsici*. Heald and Wolf causing damage from seed to seed stage in chilli. As foliar pathogen they are more severe compared to their seed-borne nature in many regions around the world. These pathogens will cause damage to crop from early stage itself. In later stages pathogens cause damage to fruits also, ultimately less yield and reduction in quality of the produce [9] reported that 70-80 per cent chilli fields are affected with *Alternaria* spp. in Shouguang district. Among the major constraints in the production of chilli biotic factors plays an important role. Leaf spot disease caused by *Alternaria alternata* and *Cercosporacapsici* were the very common biotic factors in almost all chilli growing areas around India. As an air-borne or seed-borne nature of pathogen they will cause less to moderate damage to foliage, yield and quality of the produce. Biological control is an important method in the management of plant pathogens. Advantages include reduction of dependence of high-risk chemicals for diseases, control or other ecological and economical benefits [10]. 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 alternata* [11]. *Pseudomonas fluorescens* encompasses a group of common, gram negative, rod shaped, non pathogenic saprophytes bacteria that colonize in soil, water and plant surface environments. Since they are well adapted in soil, *P. fluorescens* strains are

being investigated extensively for use in biocontrol of pathogens in agriculture [12]. Biological control of plant pathogens by antagonistic micro organisms is a potential non-chemical means and is known to be a cheap and effective eco-friendly method for the management of crop diseases [12]. *Pseudomonas fluorescens* is adapted to survival in soil and colonization of plant roots [13] and this applies also to the particular case of biocontrol agents from this species.

2. 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 entitled "Effect of bio-agents and fungicides in management of leaf spot of chilli (*Capsicum annuum* L.) caused by *Alternaria alternata* (Fr.) Keissler" was carried out during the *rabi* season of 2022-2023.

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 $25^0C \pm 2^0C$ in BOD for 5 to 7 days. After 5 days mycelin growth was observed around leaf bits from this colony growth [14,15] 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

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.

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

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.

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 5^0C. The pathogen was sub cultured regular intervals of 1 month to maintain the live culture.

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_6Carbendazim(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.

Plate 3. Microscopic view of *alternaria alternata*

Plate 4. Culture of *Alternaria alternata*

3. 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 [16] whoreported that the fungicide carbendazim was able to inhibit mycelium growth and conidia germination *Alternaria* sp. Pairashi [17] 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 [17] 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. These results are in agreement with those reported by [18] tested relative efficacy of different fungicides against *Alternaria alternata* in vitro. Among fungicides tested Manocozeb (Indofil M-45) 0.25 per cent was found most effective showing 100 per cent inhibition of growth and sporulation of *A. alternata* [19]. But the bioagent such as *Pseudomonas fluorescens and Trichoderma viride* were less as compared to the systemic fungicides. But given highest yield because chemicals attributed to elicitor's effect on physiological processes in plant such as ion uptake, cell elongation, cell divition, enzymatic activation and protein synthesis [20].

4. CONCLUSION

Among the treatments taken up for research Mancozeb (1g/L)+Carbendazim (1g/L as 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 spotofChilli 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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