Original Research Article

*In vitro*efficacy of different fungicidesagainst leaf blight of Sunflower

Sunflower is one of the major oilseed crops cultivated in India. Alternaria leaf blight is one of the fungal diseases of Sunflower caused due the pathogen *Alternaria helianthi,* which is one of the significant limitations in the cost-effective cultivation of Sunflower in India. Present investigation on evaluation onefficacy of different fungicides against leaf blight of Sunflower under *in vitro* condition by using Completely Randomized Design was conducted at Department of Plant Pathology, Dr. Sharadchandra Pawar College of Agriculture, Baramati during. Under this study seven fungicides *viz.,* Chlorothalonil 75% WP, Hexaconazole 5% EC, Carbendazim+Mancozeb 75 % WP, Carbendazim 50% WP, Tebuconazole 25.9% EC, Propiconazole 25% EC and Azoxystrobin 23% SC were evaluated against the pathogen *Alternaria helianthi* causing leaf blight in Sunflowerat their recommended concentration and observation on mean radial mycelial growth and percent mycelial growth inhibition of pathogen was calculated. Results revealed that among various fungicides the three fungicides *viz.,* Hexaconazole 5% EC, Tebuconazole 25.9 % EC and Propiconazole 25% EC were found most effective with minimum mean mycelial growth and having maximum and similar (94.44 %) percent growth inhibition of pathogen and rest of the fungicides tested were comparatively less effective against the pathogen *A. helainthi* associated with leaf blight of Sunflower.

ABSTRACT

Key words: *Fungicides, Alternaria helianthi, In vitro, Poisoned food technique, Sunflower*

1. INTRODUCTION

Sunflower (*Helianthus annus* L.) having family Asteraceae (Compositae). Sunflower is cross pollinated crop having chromosome number 2n = 34. Sunflower is C_3, day neutral plant. Sunflower has important characters like, short duration, drought tolerance, sensitive to waterlogging, photo and thermo-insensitivity, a high seed multiplication ratio (l:1000), higher water use efficiency and adaptation to a wide range of soil and climatic conditions are some important distinguishing traits.

Sunflower seeds are characterised with highest calorific value and are very nutritious, having between 40 and 50 % oil content and 20 % protein. Because of cholesterol free character of oil, it is regarded as being of good quality and has been suggested for patients with cardiac problems. It has an adequate amount of calcium, iron and vitamins A, B, E and K as well as 60–73% linoleic acid. (Evertt *et al*. 1987)

Sunflower is among the major oilseed crops cultivated in India and in the year 2021-22, it occupied an area of 2.25 lakh ha with a production of 2.28 lakh tons and productivity of 1011 kg/ ha (Directorate of Economics and Statistics, 2022). Sunflower is grown mainly in Maharashtra, Orissa, Andhra Pradesh, Punjab, Karnataka and Haryana. As per Second Advance Estimates of production of oilseeds and commercial crops for 2022-23, total

production of Sunflower is 2.79 Lakh tonnes. (Second Advance Estimates of Production of Foodgrains for 2022-23)

Sunflower is affected by various diseases caused due to several plant pathogens, mainly fungi, bacteria and viruses. More than 35 diseases, predominantly fungus, have been found to live on sunflowers. Under specific climatic conditions, these pathogens may disrupt the natural physiological functions of the plant, resulting in a considerable decrease in production and oil quality.

Alternaria helianthi is a one of the fungal pathogens responsible to causes leaf blight disease in sunflower.It is estimated that 20 to 80 % losses in yield and heavy defoliation have been observed due to *A. helianthi* causal agent of leaf blight of Sunflower (Venkatarmanamma*et al.* 2023). All above ground parts of plant were infected due to this pathogen.Characteristics symptoms of the disease are the appearance of dark brown to black spot, circular to oval in shape. The leaf spots were encircled by necrotic chlorotic zone with greyish- brown necrotic centre with distinct concentric rings. Initially the leaf spots are appearing on the surface of lower leaves and gradually spread to the middle leaves, upper leaves and stem (*agritech.tnau.ac.in).* The development of pathogen is prevalent when the relative humidity is 78 to 80% and the temp. is between 27 and 290C (Kumar and Singh, 1996).

It is important to work out an effective plant extract and fungicide, which would be helpful in controlling this disease. In the current study, both plant extract and fungicides have been tested under *in vitro* conditions against pathogen *A. helianthi* causing blight in Sunflower.

2. MATERIALS AND METHODS

2.1 Isolation and identification of the pathogen

The disease affected plant parts was collected from infected Sunflower plants which shows typical symptoms of disease in the fields of Malegaon, Baramati region. Affected Sunflower leaves washed with tap water to remove dirt, air dried and affected parts were cut into small pieces with sharp sterilized blade, keeping some healthy portion along with diseased parts. These pieces were disinfected with 0.1% aqueous solution of NaOClfor two minutes and rinsed into three changes of sterilized water and then aseptically transferred to sterilized plates poured with Potato Dextrose Agar medium in Laminar-air-flow cabinet. Inoculated plates were then incubated in BOD incubator at 25 ± 1°C temperature for 5 to 6 days in inverted position and sub culture was done until the pure isolate of Alternaria blight was obtained and identified. Identification was made depending on the visual morphological characteristics of the fungus from the culture growth pattern. (Pathare, 2019)

2.2 Pathogenicity test

Pathogenicity was demonstrated by using the “Koch's postulates”. The pathogenicity of the test fungi was assessed on three-week-old susceptible Sunflower hybrid (KBSH-44) seedlings by applying a foliar spray of an aqueous spore suspension, prepared from a 10-day-old pure culture. The seedlings were placed in a moist chamber for 24 hours, after which they were inoculated with a 10-day-old culture of the test fungal pathogen. Seedlings which are uninoculated with the test fungal pathogen were sprayed with sterilized water and it was serve as the control. After inoculation of test fungus, each seedling was covered with a polythene bag for twenty-four hours. Following this incubation period, the polythene bags were removed to allow for disease development. The Sunflower seedlings were continuously monitored for the emergence and appearance of leaf blight symptoms. After symptoms development on plant parts, the pathogen was re-isolated from the infected parts and compared with the original culture to confirm its identity and pathogenicity.

2.3 *In vitro* evaluation of fungicides

Seven different fungicides *viz.* Chlorothalonil 75%WP, Hexaconazole 5% EC, Carbendazim + Mancozeb 75 %WP, Carbendazim 50% WP, Tebuconazole 25.9% EC,

Propiconazole 25% EC and Azoxystrobin 23% SC were evaluated under *in vitro* condition against the *Alternaria helianthi* pathogen causing blight in Sunflower by using Poison Food Technique (Nene and Thapliyal, 1993). In this study Potato Dextrose Agar was used as a basal culture medium.

Adequate concentrations of each chemical were prepared and mixed into sterilized and cooled Potato Dextrose Agar medium in conical flasks (250 ml cap.). 20 ml of each treated medium were transferred into 90 mm sterilized petri plates and each plate was inoculated separately with an actively growing 5 mm mycelial disc of *A. helianthi* under aseptic condition. Control plate containing PDA medium without any fungicide treatment was maintained. Three replicates were conducted for each treatment. The plates were then incubated at 25 ± 2°C Once the untreated control plates were completely covered by the mycelial growth of the test pathogen, the mean radial mycelial growth was calculated in all the treatment plates. Using the formula (Arora and Upadhyay, 1978) the percentage inhibition of mycelial growth compared to the control was calculated.

$$\text{Per cent growth inhibition} = \frac{\text{Colony growth in Control plate} - \text{Colony growth in treated plate}}{\text{Colony growth in control plate}} \times 100$$

3. Results and Discussion

3.1 Isolation and identification of pathogen causing leaf blight of sunflower.

Pure culture of the pathogen *Alternaria helianthi* causing leaf blight in Sunflower was derived from the leaf blight infected leaves of sunflower plant. Isolation of the pathogen was carried out aseptically in the laboratory from diseased leaves of Sunflower showing characteristics leaf blight symptom using tissue isolation method on PDA medium. Initially the whitish and abundant proliferation of the fungus mycelium was observed, later which convert into grey to dark brown in colour which acquired whole petri dish within 10 days after inoculation. By using hyphal tip isolation method pathogen was purified. Sub culturing of fungus was done after the pure culture of *Alternaria* blight was obtained and identified. Identification was made depending on the visual morphological characteristics *viz.,* based on spore morphology and colony character of the fungus under the microscopic observation. Pathogen was identified as mycelium had transverse septation, conidiophores were cylindrical to elongated in shape, scattered or grouped together, ranging in colour from pale grey to yellow. After identification the fungus then purified and stored as PDA slants at a temperature of 4°C for future study.

This showed that this fungus is similar to the one isolated, identified and described by Narasimha Rao and Rajagopalan (1977) who observed mycelial colonies of *Alternaria helianthi* on PDA medium was dark brown, profusely branched and frequently septate structures. The conidiophores were cylindrical to elongated, often branched, septate.

Abhilash *et al.*(2018), Prathibha (2005), Nagrale*et al.* (2013) and Pathare*et al.* (2019) isolated the pathogen *Alternaria helianthi* and identified as *A. helianthi* based on spore morphology and colony character.

3.2 Pathogenicity of pathogen associated with leaf blight sunflower

The pathogenicity of *Alternaria helianthi* was assessed using Koch's postulates under polyhouse condition. Three-week-old susceptible sunflower hybrid KBSH-44 plants were inoculated by applying spore suspensions prepared in distilled water onto their foliage using an atomizer. Immediately after inoculation, the plants were covered with polythene bag to maintain relative humidity more than 80 % for 24 to 48 hours. Following this incubation period, polythene bag was removed and keep plants back to the polyhouse for disease development. Prominent brown spots surrounded with yellow halo appeared on the youngest leaves, displaying characteristics symptoms of *Alternaria helianthi* as observed in

the field. In between, the control plants that were sprayed using only distilled water showed no symptoms and remained healthy.

After symptoms development on artificially inoculated plant parts, the pathogen was re-isolated from the artificially inoculated leaves on PDA medium. After incubation period, the fungus was observed for microscopic and morphological observations. It was found that similar observations were recorded with the original culture which was isolated from naturally infected leaves of Sunflower plants. Consequently, the pathogen was recognized as *Alternaria helianthi*, thus confirming the pathogenicity of the test pathogen by applying Koch's postulates.

Similar observation was reported by Waghe *et al.*(2015) pathogenicity of test fungus was conducted on Sunflower hybrid KBSH-44 by applying Koch's postulates. Additionally, the observation on pathogenicity test of *A. helianthi* by using Koch's postulates were observed by Prathibha (2005), Abhilash *et al.*(2018), Pathare*et al.* (2019), Lakshmi Prasad *et al.* (2020).

3.3 Efficacy of different fungicides against *Alternaria helianthi* under *in vitro* condition

The results are presented in (Table 1, Plate 1 and Fig 1), revealed that the three fungicides *viz.,* Hexaconazole 5% EC, Tebuconazole 25.9 % EC and Propiconazole 25% EC reported maximum and similar percent growth inhibition (94.44 %) with having similar mean mycelial growth 5.00 mm which were at with each other and observed as superior among all fungicides tested. It was followed by Carbendazim 12% + mancozeb 63% (75 %WP) which recorded (83.15 %) percent growth inhibition with mean mycelial growth 15.16 mm, Azoxystrobin 23% SC shows (62.96 %) growth inhibition of test fungus with mean colony diameter 33.33 mm, Chlorothalonil 75% WP recorded (54.26 %) growth inhibition with 41.16 mm mean colony diameter. Carbendazim 50 % WP evolved as least effective fungicide as it reported minimum percent growth inhibition (40.18 %) with maximum mean colony diameter 53.83 mm as compared with untreated control.

Akbari and Parakhia (2007) estimatedthe efficiency of various systematic fungicides against the *Alternaria alternata.* Results revealed that hexaconazole 5 EC and propiconazole 25 EC, showed similar effectivity which completely supressed the growth of pathogen. Saqib *et al.* (2020) reported that hexaconazole exhibited most effective as it achieves 100% inhibition of *A. helianthi.*

Table1. Efficacy ofdifferent fungicides against *Alternaria helianthi* under *in vitro* condition

Tr. No.	Treatments	Recommanded Conc (%)	Colony Dia of the fungus*(mm)	% Growth Inhibition*
T_1	Chlorothalonil 75% WP	0.15	41.16	54.26
T_2	Hexaconazole 5% EC	0.2	5.00	94.44
T_3	Carbendazim 12% + Mancozeb 63% (75 %WP)	0.2	15.16	83.15
T_4	Carbendazim 50 % WP	0.2	53.83	40.18
T_5	Tebuconazole 25.9 % EC	0.1	5.00	94.44
T_6	Propiconazole 25% EC	0.1	5.00	94.44
T_7	Azoxystrobin 23% SC	0.1	33.33	62.96
T_8	Control	-	90.00	00.00
	SE(m)±		**0.50**	
	C.D. (0.01)		**2.07**	

*: Mean of three replications, Conc: Concentration, Dia: Diameter

Fig. 1. Efficacy of different fungicides against *Alternaria helianthi* under *in vitro* condition

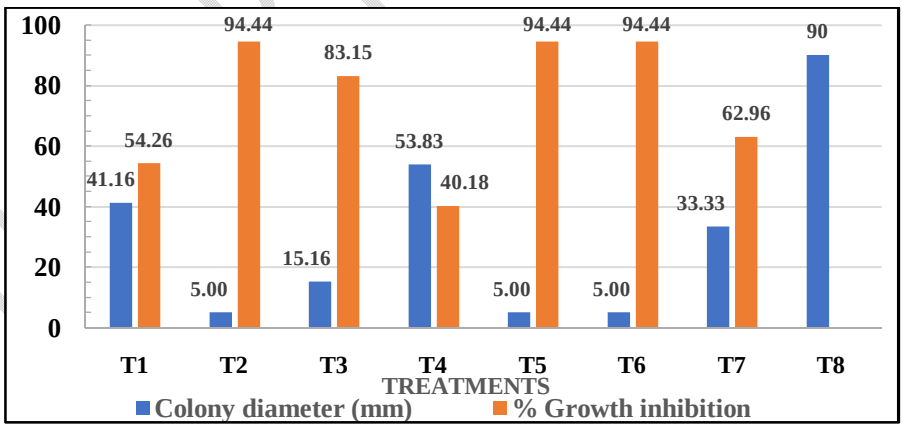

Plate 1: Efficacy ofdifferent fungicides against *A. helianthi* under *in vitro* condition

4. CONCLUSION

Alternaria helianthi is opportunistic foliar fungal pathogen that cause destruction of host tissue through the reduction of photosynthetic potential by inciting spots and blights in linseed. The disease also cause reduction in the oil content and quality of the fiber of crop. The research on the evaluation of efficacy of different fungicides against *Alternaria helianthi* under *in vitro* condition. Since the disease is air borne, hence the most appropriate method of the disease management is the use of suitable fungicides and phytoextracts. Under *in vitro* study of fungicides, three fungicides *viz.,* Hexaconazole 5% EC @ 0.2 %, Tebuconazole 25.9 % EC @ 0.1 % and Propiconazole 25% EC @ 0.1 % reported to be most effective against the pathogenand could be useful for the management of the disease.

DISCLAIMER

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts. COMPETING INTERESTS: Authors have declared that no competing interests exist.

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