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Environmental Challenges on Leaf Spot and Blight of Sunflower (*Helianthus annuus* L.)

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A B S T R A C T

Sunflower is an important major oilseed crop ranks next to groundnut and soybean at global level. It belongs to the family Asteraceae and mostly infected by several fungal, bacterial and viral diseases among which leaf spot and blight caused by *Alternaria helianthi* was considered as one of the most destructive diseases in the sunflower growing areas. An experiment was studied to determine the disease incidence and intensity of the disease in both Kharif and Rabi season (2018-19) by observing the plant raised in field. Attempt was made to evaluate the effect of different range of pH and temperature on growth of the fungus and calculated under field condition in both Kharif and Rabi season. The results obtained from the present investigation indicate that the incidence of the disease and its intensity was found more during Rabi season than in Kharif under field condition which may be attributed to suitable environment and favourable soil conditions. Maximum dry weight growth of the fungus was observed at pH 6.0 followed by pH 5.0 and temperature at 30^0C-35^0C. The above parameters should be considered while growing sunflower in field condition along with other protection measures to grow disease free sunflower crop with maximum yield and good quality seed.

Introduction

Sunflower (*Helianthus annuus* L.) is an important major oilseed crop ranks next to groundnut and soybean at global level. It is cultivated in India over an area of 21.62 lakh ha with a production of 12.24 lakh tonnes in different states like Karnataka, Maharashtra, Bihar, Andhra Pradesh, Haryana and Tamil Nadu. Karnataka occupies first position accounting 53 per cent of total area and 35 per cent of total production in India. Sunflower is susceptible to a large no. of devastating diseases. Fungal diseases are the main constraints for it's production and sunflower industry worldwide (Bhutta, 1998). Leaf blight disease of sunflower caused by *Alternaria helianthi* (Hansf.), Tubaki &

Nishihara is a serious threat to successful cultivation to the crop worldwide. The crop is affected by biotic and abiotic stresses. Among the several biotic factors, susceptibility to fungal diseases is one of the major constraints for successful sunflower production. The major diseases of sunflower in India are leaf blight (*Alternaria helianthi*), wilt (*Sclerotium rolfsi*) and root rot caused by *Rhizoctonia solani* and *Macrophomina phaseolina.* Gulya and Masirevie (1991) listed 80 pathogens occurring on sunflower. Among this leaf blight caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara has been considered as potentially destructive disease in many parts of the sunflower growing countries around the world (Mahipal, S., Ghemawat (1989)).

The first report of disease was recorded in India by Narain and Saksena (1973) and Kolte and Mukhopadhyay (1973) from Uttar Pradesh and subsequently, by Anil kumar *et al.,* (1974) from Karnataka. In Karnataka, the disease occurred in epidemic form in 1987, with a disease incidence of 95 to 100 % (Hiremath *et al.,* 1990). Yield loss may occur through reduced head diameters, number of seeds per head, and oil content or quality. *A. helianthi* can cause leaf and stem lesions, seedling blight and head rot. It has been reported to reduce seed and oil yield up to 84% and 33%, respectively (Balasubrahmanyam and Kolte, 1980, Kolte, 1985).

The pathogen survives between sunflower crops in and on infested crop debris, as a pathogen of safflower and cocklebur, and on seed. Hot weather and frequent rain during the vegetative growth stage of the crop accelerate the disease intensity. Disease development is favoured by 25-27 ^{0}C temperature with at least 12 hours of wet foliage is reported to spread rapidly during the rainy season (Basappa and Santha Lakshmi Prasad, 2005).

Sunflower is an important major oil seed crop grown in different region in Odisha since a long period of time. The conducive weather conditions prevailing in different parts of Odisha become the prime and favourable factors for development and severity of leaf spot and blight disease of sunflower caused by *Alternaria* sp. Considering the fact effort was made to study the disease under local field condition (Bhubaneswar) both in *Kharif* and Rabi season 2018-2019. Attempt was also made to test the impact of pH and temperature on dry weight growth of the fungus under *in vitro* condition.

Materials and Methods

Study of disease symptoms under field condition, Isolation of the pathogen and its identification

The pathogen *Alternaria helianthi* was isolated from the infected leaf sample showing typical symptoms of the disease. The disease samples were washed with distilled water to remove the foreign materials. A small disease portion (4-5 mm) of leaf along with healthy portion from the periphery were cut into pieces and surface sterilized with sodium hypochlorite (0.1%) solution for 2 minutes followed by subsequent washing in sterile distilled water for 3 times to remove residues of sodium hypochlorite from cut pieces. Then the diseased cut pieces were aseptically transferred in the center of PDA plates as well as PDA slant. They were incubated inside a B.O.D incubator at temperature 25±1^{0}C for 7-10 days. The pure culture of the fungus was obtained by further growing the culture and following hyphal tip culture under aseptic conditions (Rangaswamy, 1972). The fungus was identified by observing the morphological and cultural characteristics in PDA slant as well as plate.

Weather parameters during Kharif and Rabi 2018-19

Meteorological parameters in response to disease incidence

In order to study the relationship of meteorological parameters on the natural occurrence of disease (leaf spot and blight) by *Alternaria helianthi* in sunflower plant. The observations were recorded from July to October 2018 as Kharif crop and December (2018) to March 2019 as Rabi crop under field condition at experimental plot of instructional farm, SOADU, Bhubaneswar. The weather parameters like maximum and minimum temperature (^{0}C), maximum and minimum RH (%), rainfall (mm) and bright sunshine hours (hr) were co-related with sunflower leaf spot/blight disease incidence. A standard scale (0-9) basing on the intensity of the disease appearance on sunflower leaves was recorded through visual estimation. Percent disease incidence was calculated by using the formulae as mentioned below

$$\text{Incidence (\%)} = \frac{\text{Number of leaves infected}}{\text{Total number of leaves examined}} \times 100$$

$$\text{PDI} = \frac{\text{Total numerical rating}}{\text{Total number of units examined}} \times 100$$

For recording the disease incidence 100 number of sunflower plants were grown in instructional farm (4×4m) in each season (Kharif and Rabi). The percentage of plant infected was calculated basing on their number and the disease intensity was recorded in (0-9) scale as per the formula already given (Mayee and Dater, 1986).

Standard meteorological parameters were obtained from the Meteorological observatory, Department of Agro-Meteorology, SOADU, Bhubaneswar.

In vitro evaluation of pH on growth of fungus

Alternaria helianthi was isolated from the infected sunflower plant and maintained as pure culture on PDA plate. 10 days old culture was used for testing the growth of the fungus at different pH. The PD broth to be used for the growth of test fungus adjusted in different ranges of pH starting from 2.0 to 8.0 with help of Philips precision pH meter by adding 0.1 N acid (HCL) and 0.1N alkali (NaOH). Three replication made for each treatment (10 days old).The fresh growth of the fungus maintained in PDA plate (7mm) was cut and inoculated in PD broth maintained at different pH ranges.

The final growth was obtained in the PD broth at different pH finally filtered through whatman filter paper and which was properly dried in hot air oven at temperature 60-80^0 c continuously for 2-3 days. The final dry weight of the fungal growth with filter paper was taken and deducted from the weight of the dry filter paper used for the experiment. The net weight was recorded and mean dry weight growth of the fungus was calculated for each range of pH.

Effect of temperature on growth of fungus under *in-vitro* condition

The effect of temperature on dry weight growth of the fungus was done in different ranges like 10, 15, 20, 25, 30, 35 and 40^{0}C. 7 days old fresh culture of the fungus (7mm) was used and inoculated to sterilized conical flask containing PD broth.

After inoculations the flasks were kept in B.O.D incubator adjusted in different range of temperature like 10, 15, 20, 25, 30, 35 and 40^{0}C. For each range of temperature three replications were maintained. The growth was obtained in the PD broth at different ranges of temperature finally filtered and properly dried

in hot air oven at temperature 60-80^0 c continuously for 2-3 days. The final dry weight of the fungal growth was recorded and deducted from the weight of dry filter paper used for the experiment. The net weight was recorded and mean dry weight growth of the fungus was calculated.

Results and Discussion

Symptomatology

Sunflower plant were found to be affected by leaf spot and blight disease in instructional farm during the year 2018-19 in *Kharif* and *Rabi* season.

The disease symptoms appeared in form of dark brown to black spot measuring 0.2 to 0.5 mm in diameter. The spots appeared circular, oval, and sometimes oblong and irregular in shape surrounded by yellowish halo.

The symptoms gradually started spreading from the lower leaves to upper leaves and subsequently to stem, branches and flower head. In advance stages of the disease the spots enlarged coalesced resulting the symptoms of blightening. The severity of the disease results stunting of plant growth, defoliation and poor head formation with infected seed (Plate-1). Although the disease symptoms of leaf spot and blight found more or less similar both in Kharif and Rabi but the intensity of the disease was found higher in Rabi (Plate-2 and Plate-3).

The symptoms of leaf spot and blight affecting sunflower was observed in India and abroad in different times by many scientist. The initial stage of the disease symptoms and subsequent development of disease resulting it's severity was also observed by scientist like Tubaki & Nishihara, 1969; Narain and saksena, 1973; Kolte and Mukhopadhyay, 1973; Anil Kumar *et al.*, 1974 and Vikas *et al.*, 2010).

Disease incidence in Kharif and Rabi

Sunflower plants were raised in instructional farm (IAS) to observe and compare the disease incidence of leaf spot/blight in both Kharif and Rabi season. The result on disease incidence on leaf spot/blight (Table-2) showed that all the plants got infected by the disease in both Kharif and Rabi (2018-19).

The disease incidence in Kharif season found within 5-30% which was found more in Rabi varying from 15-40% (Plate-2 and Plate-3). While studying the disease intensity of the infected plant it was observed that the plants raised in Kharif season showed lower disease intensity (40-60%) which was found higher in Rabi i.e. 60-80% (Plate-3).

The symptoms of leaf spot/blight observed in sunflower plant both in Kharif and Rabi were apparently similar only except variation in disease intensity.

The higher percentage of plant infection and disease intensity in Rabi season may be attributed to high inoculum load in soil and air and the favorable climatic conditions like temperature, relative humidity and soil moisture prevailing during that period of time.

The optimum temperature (max. 32.5^{0}C, min. 25^{0}C), Relative humidity (74-94) and Rainfall (420 mm) and adequate soil moisture (Table-3 and Table-4) during Rabi season probably favored higher disease incidence in sunflower. Present finding was supported by scientist Mayee (1994) and Mayee and Wankhede (1997).

Table.1 Showing scale and disease intensity of *A. helianthi*

Numerical rating	Description
0	No symptoms on leaf
1	Small, irregular brown spots covering 1% or less of the leaf area
3	Small, irregular, brown spots with concentric rings covering 1-10% of the leaf area.
5	Lesions enlarging, irregular, brown with concentric rings covering 11-25% of the leaf area.
7	Lesions coalesce to form irregular brown patches with concentric rings covering 26-50% of the leaf area. Lesions also on stem and petioles.
9	Lesions coalescing to form irregular, dark brown patches with concentric rings covering 51% or more of the leaf area. Lesions seen on the stem and petiole

Table.2 Disease incidence and degree of disease intensity of Leaf spot/blight of sunflower both in Kharif and Rabi 2018-19

Treatments	Total no. of plants	Total no. of plantinfected(%)	Disease intensity (%) of the infected plants
Plant sown in kharif	100	10%	+
		38%	++
		52%	+++
Plant sown in Rabi	100	12%	+
		25%	++
		63%	++++

Table.3 Standard meteorological parameters during Kharif 2018

Month	Year	Temperature (°c)		Rainfall(mm)	Relative Humidity	
		Max.	Min.		7hr	14hr
July	2018	32.5	26.0	639.2	94	79
Aug	2018	32.2	25.9	414.2	93	77
Sept	2018	32.6	25.2	390.4	94	74
Oct	2018	32.5	22.8	236.8	94	60

Table.4 Standard meteorological parameters during Rabi 2018-19

Month	Year	Temperature(°c)		Rainfall(mm)	Relative Humidity	
		Max.	Min.		7hr	14hr
Dec	2018	27.2	14.1	11.1	89	45
Jan	2019	28.5	13.0	0.0	95	38
Feb	2019	32.9	17.0	24.6	94	35
Mar	2019	35.2	23.2	7.1	92	46

Table.5 Effect of pH on dry weight growth of the fungus *A. helianthi*

Treatments	pH	Mean dry weight growth(mg)
T1	2.0	4.00
T2	3.0	116.00
T3	4.0	125.00
T4	5.0	176.33
T5	6.0	228.00
T6	7.0	134.67
T7	8.0	132.67
	SE(m)+	1.25
	CD (0.05)	3.86

Table.6 Effect of temperature on dry weight growth of the fungus *A. helianthi*

Treatments	Temperature(°C)	Mean dry weight growth(mg)
T_1	10	366.00
T_2	15	682.00
T_3	20	893.33
T_4	25	1272.00
T_5	30	1558.00
T_6	35	1522.33
T_7	40	1015.00
	SE(m)±	1.35
	CD (0.05)	4.17

Fig.1 Effect of pH on dry weight growth of the *A. helianthi*

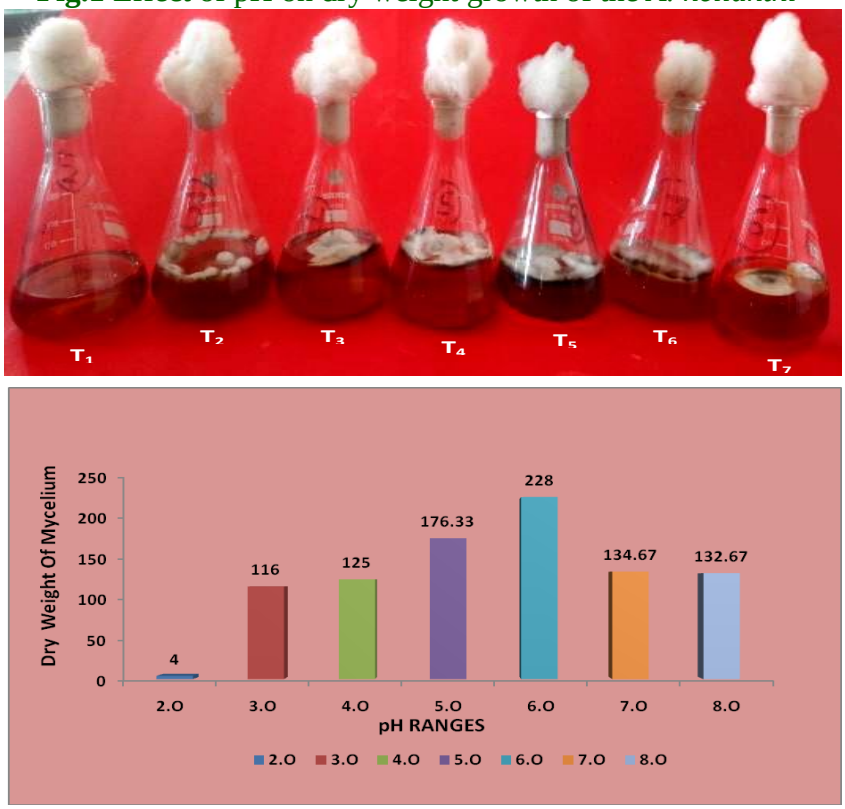
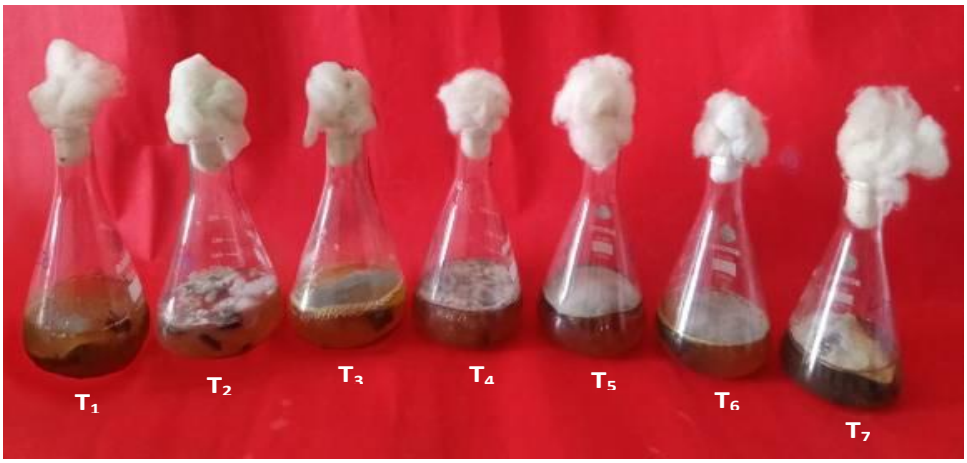


Fig.2 Effect of Temperature on dry weight growth of the *A. Helianthi*



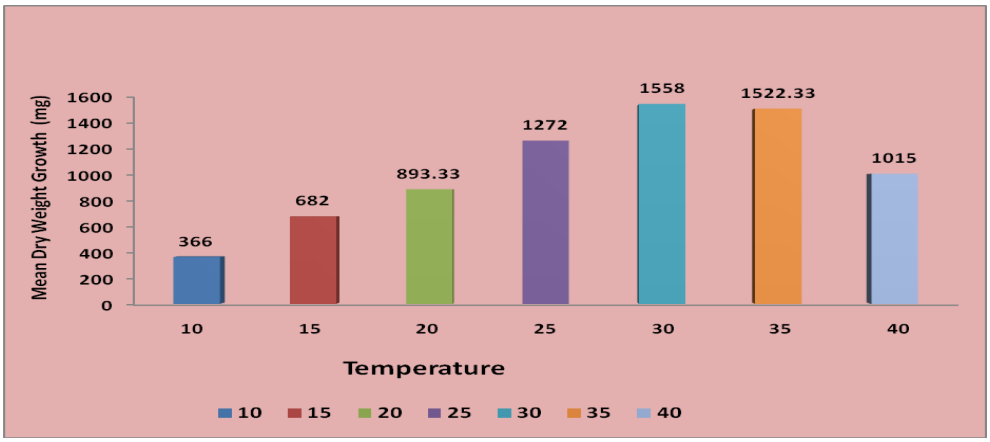


Plate.1 Leaf and head infection showing symptoms under field condition

Plate.2 Disease symptom of leaf spot/blight of sunflower in *Kharif*

Plate.3 Disease symptom of leaf spot/blight of sunflower in *Rabi*

Effect of pH

The experimental finding of effect of pH on dry weight growth of the fungus it was observed that neutral pH 6.0 produced maximum growth (228mg) followed by pH 5.0 (Table-5) (Plate-4).While the growth was declined in higher pH (alkaline) i.e. 7.0 and 8.0 and it was found still less in acidic range i.e. 2.0, 3.0 and 4.0 and minimum in pH 2.0 (4mg) (Fig.-1). The observation of effect of pH on growth of *A. helianthi* was also studied by many scientist and supported the present finding [Jash *et al.*(2003), Ramjegathesh and Ebenezar (2012)].

Effect of temperature on dry weight growth of *A. helianthi*

The experimental result (Table-6) (Plate-5) revealed that highest dry weight growth of fungus *A. helianthi* was obtained at temperature 30°C showing 1558.0 mg followed by temperature 35^{0}C showing the dry weight growth 1522.33 mg and they were significantly superior to other treatment. The dry weight growth of the fungus comparatively less in 25^{0}C i.e. 1272 mg and the same trend was observed minimum (366.0 mg) at 10^{0}C which gradually enhanced with increasing range of temperature and observed maximum within 30-35^{0}C but beyond that it again started declining (Fig.-2). The experimental result was found in agreement with the finding of many scientists like Prathibha *et al.,* (2008) and Hubbali *et al.,* (2010).

The salient findings of the present study clearly indicates incidence of leaf spot and

blight disease symptoms developed in sunflower plant remained more or less similar both in Kharif and Rabi (2018-19). However the disease intensity was found higher in Rabi which may be attributed to the climatic condition and soil moisture and inoculum load in crop rhizosphere.

Considering the rate of multiplication and growth of the test fungus under *in-vitro* condition, the result of the present experiment reveals that maximum dry weight of the fungus obtained in neutral pH *i.e.* 5.0-6.0 which was declined in either acidic or alkaline range. Most favourable range of temperature encouraging maximum growth of fungus under *in-vitro* condition was found within 30-35^{0}C. So climatic condition with above favourable range of temperature and soil pH may affect the crop with higher disease incidence lowering yield and seed quality. Therefore, attention should be paid while taking the sunflower crop in a particular area considering the above environmental factors in order to increasing the yield and quality of the crop.

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