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In vitro evaluation of some fungicides against *Bipolaris sorokiniana* a causal agent of spot blotch of Barley

Sakshi Kohli, Rajendra Prasad, Sandeep Kumar and Mahipal Singh

Abstract
Plant diseases, particularly foliar diseases, are one of the major issues limiting India's barley production. Many fungal infections impact barley, resulting in significant crop losses each year. Foliar disease-related yield losses in barley are estimated to vary from 10% to 40% globally. The entitled experiment was conducted in Plant Pathology laboratory, School of Agriculture (SOA), Uttaranchal University Dehradun. The experiment was done in completely randomized design by using poisoned food technique. Three fungicides namely carbendazim, tebuconazole+sulphur, polyram were tested against *Bipolaris sorokiniana* a fungal pathogen isolated from spot blotch of barley for radial mycelial growth and percent inhibition growth of fungal mycelia at different concentrations (50, 100, 200, 300ppm). The maximum radial mycelial growth was observed in carbendazim 35.66mm at 50ppm and lowest or no mycelial growth was observed by tebuconazole. The maximum percent growth inhibition of mycelia of fungal pathogen was recorded by tebuconazole i.e 100% at 100,200 and 300ppm. The lowest percent inhibition growth was recorded by carbendazim 22% at 50ppm. Among all the fungicides tebuconazole was found to be most effective against *B. sorokiniana.*

Keywords: barley**,** *Bipolaris sorokiniana*, *In vitro*, spot blotch, poisoned food technique

Introduction
Barley (*Hordeum vulgare* L.) is a cereal grain crop of the grass family Poaceae is known as "Jau" in Hindi. It is a self-pollinating, diploid species with 14 chromosomes. It is widely used for food and fodder. Barley can be grown in the summer or the winter. It can be grown well in tropical and subtropical climates. During the growing season, the temperature should be around 12-15 °C, and at maturity, it should be around 30-32 °C. At any stage of development, the crop is highly susceptible to cold. Any frost during the flowering stage can result in a significant reduction in production. It can survive at high temperatures because of its drought-tolerant capacity. In India, Eastern Uttar Pradesh, Rajasthan, Madhya Pradesh, Haryana, Punjab, Bihar, and Himachal Pradesh are the main growing areas of barley. Barley is grown in all of India's wheat-producing states. It accounts for less than 1% of overall cereal production and area. Poor people eat it as chapati or sattu, and it's also used as cow feed. In the malt industry, barley is used to make fermented beverages. In India, the area under barley has decreased from 3.0 million hectares to 0.64 million hectares, and production has decreased from 3.0 million tonne to 1.14 million tonne over the last two decades. However, because to the emergence of high yielding varieties, average yield increased from 1.2 to 1.8 t ha-1. This is due to farmers' choice for more profitable winter crops such as wheat, mustard, and Bengalgram.

The correct native place of barley contends but possibly originating in Egypt, Ethiopia, the Near East, or Tibet (Duke 1983) [5]. Domesticated barley was believed to have spread from Central Asia to India, Persia, Mesopotamia, Syria, and Egypt (Stachowski, 2018) [14]. The only crop listed lots of times in the Rigveda and other Indian texts as one of the primary cereals in ancient India was barley known as Yava in both Vedic and Classical Sanskrit (Witzel ME, 2016) [15]. Barley cultivation was also discovered in the post-Neolithic Bronze Age Harappan civilization, which existed 5700–3300 years ago. Based on spike morphology, there are two types of barley: two-row and six-row barley and, based on growth habit, there are three types: winter, spring, and facultative (Poehlman, 1994) [11]. Barley was one of the first grains to be cultivated as early as 10,000 years ago, particularly in Eurasia (Zohary D, Hoph M, 2000) [16]. Barley ranked 4th among grains behind maize, rice and wheat in quantity produced (149 million tonnes), led by Russia producing 14% of the world total (FAOSTAT, 2017) [3]. Barley is a drought-tolerant crop with a short growing season (McZee, 1986).

The global barley producing area is around 49.02 million hectares, resulting in total production of 139.81 million metric tonnes, with average productivity of 2.85 metric tonnes per hectare.

Plant diseases, particularly foliar diseases, are one of the major issues limiting India's barley production. Many fungal infections impact barley, resulting in significant crop losses each year. Foliar disease-related yield losses in barley are estimated to vary from 10% to 40% globally, totaling billions of dollars per season (Sharma and Duveiller, 2006) [12]. The pathogen infects a wide range of hosts, and its pathogenicity is varied in nature. When the temperature is between 15 °C and 22 °C during the first two weeks after the development of full ears, spot blotch damage might affect grain output by 10% to 20% (Steffenson, 1997). The disease causes significant yield loss in warm, humid environments, particularly in Uttar Pradesh, Bihar, Jharkhand, West Bengal, Assam, and the plains of India's north-eastern regions. Depending on the environment, the incubation period for disease development is 3-6 days. The most severe losses in grain output are caused by early and heavy infection on flag leaf.

In India, the spot blotch of barley was originally documented in Pusa, Bihar (Butler, 1918). It was later recorded from several parts of the country, including North Bihar and India (Butler, 1929-30). It has been seen in a number of other countries (Dickson, 1956; Mathre, 1982) [4]. Cochliobolus sativus is the teleomorph (sexual stage) of Bipolaris sorokiniana (anamorph), which is responsible for a wide range of crop diseases. The pathogen can infect and produce illness on roots, leaves and stems, and head tissue (common root rot). Leaf spotting (spot blotch) appears as little brown lesions with a diameter of less than 1 cm that can combine into vast elongated blotches of necrotic tissue. Spot blotch is more common in damp weather, on lower leaves, and during the development of the head.

Bipolaris sorokiniana (Sacc.) Shoemaker (syn. *Helminthosporium sativum*, teleomorph: *Cochliobolus sativus*), a hemibiotrophic phytopathogenic fungus is a well-known cause of spot blotch disease in barley and wheat. The fungus survives the winter in barley straw and stubble, as well as in the soil and on seed. In the spring, spores are formed on barley trash and transported by wind and rain. Inoculum on the seed or in the soil can cause infection in barley seedlings. Temperatures over 20°C, as well as damp, humid conditions inside the crop canopy, favor the formation of spot blotches. During the growing season, favorable weather conditions may encourage the formation of new spores and lesions, resulting in rapid disease development. Yield losses due to spot blotch vary from 16 to 33% in barley (Clark, 1979) [2]. The disease could be particularly harmful in the United States' Upper Midwest. When environmental conditions are favorable to disease growth, yield losses of 10-30% are possible (Mathre, D.E, 1997) [9].

The disease is managed by using resistant varieties, clean seed, seed treatments, foliar fungicide and rotation to non-cereal crops. Fungicide applications made at the right time can help to limit disease damage and incidence. There are additional cultural measures that can be taken to prevent the fungus from spreading. At the first evidence of the disease, barley with spot blotch should be treated with registered fungicides. Studies show that four treatments of fungicide over the season have been shown to help control spot blotch and reduce grain loss. Hence, the present experiment was conducted to evaluate the effect of some fungicides against the causal agent of Spot Blotch of Barley under *in vitro* conditions.

Material and Methods

The experiment was carried out at Plant Pathology laboratory of School of Agriculture (SOA), Uttaranchal University, Dehradun (Uttarakhand). Three fungicides were tested against *Bipolaris sorokiniana*. Efficacy of three fungicides (Table no 1) was used at different concentration 50ppm, 100ppm, 200ppm and 300ppm to test the inhibitory effect on the growth of mycelia by using Poisoned food technique (Nene and thapliyal, 1973) [10].

Isolation

The infected diseased leaf samples are taken from practical crop field (PCP) fields of (SOA) Uttaranchal University campus for isolation of pathogen which shows specific symptoms of Spot Blotch. The selected leaves are washed with water to remove the dust from the leaves. The leaves are cut into small pieces, with some healthy portion of the leaf, with the help of a blade or scalpel. The cut leaf pieces are dipped in the surface-sterilized sodium hypochlorite solution for 15-20 seconds before transferred to petri plates thoroughly washed cut leaves pieces with distilled water three times to remove the chemical. Then the leaves are dried with blotting paper. The dried leaf pieces are then transferred into petri plates by using an inoculation needle, with 2-3 pieces placed on each petri plate having PDA media.

Poisoned food technique

Potato dextrose agar medium was prepared and sterilized by autoclaved. For prepration of different concentration of fungicides, fungicides were weighed with the help of electronic weighing balance. The stock solution of fungicide was prepared for accurate concentrations. Appropriate amount of stock solution were poured into pda media to get the desired amount of concentration of fungicide. The amount of stock solution of fungicides to be used in a PDA medium was calculated by using formula:

$C_1V_1=C_2V_2$

Where,
C_1= Concentration of stock solution (ppm)
C_2= Desired concentration (ppm) of fungicides
V_1= Vol. (ml) of the stock solution to be added
V_2= Measured vol. (ml) of the PDA

About 20 ml of PDA media containing fungicide was poured from conical flask to 90mm petri plates and allowed to solidify. The 5mm of disc of a 10 days old fungus was transferred to centre of the each plates having poisoned medium. A control plate was also maintained with pathogen under same condition on PDA without poisoning media with fungicide. Inoculated plates were incubated at 25psi for 9-10 days. The colony diameter was recorded by measuring radial growth of the fungus at 3 DAI (days after inoculation), 6 DAI, 9 DAI and average diameter was calculated. Three replications of each treatment were prepared by randomized block design. The percent growth inhibition of fungal pathogen was calculated by using formula given by (Vincent, 1947).

I=C-T/C*100

Where,
I= Percent inhibition
C= Growth of fungal mycelia in control
T= Growth of fungal mycelia in treatment

Table 1: List of fungicides with their common and trade name:

Treatment	Common name	Trade name
T_1	Carbendazim 50% WP	Mavestin
T_2	Tebuconazol 10%+Sulphur 65% WG	Haru
T_3	Metiram 70% WP	Polyram

Statistical analysis of data

Data were analyzed by using completely randomized design (CRD) with the help analysis of variance table wherever required. The data was analyzed by one factor analysis using OPSTAT.

Result and Discussion

Three fungicides namely mavestin, haru, polyram were evaluated at different concentrations (50ppm, 100ppm, 200ppm, 300ppm) to test the *in vitro* efficacy of fungicides against *Bipolaris sorokiniana.* The data was recorded till the 9th day after inoculation to observe the radial growth of fungal mycelia and inhibition growth percent of the fungal pathogen. Among these fungicides the maximum mycelial growth was observed by carbendazim 35.66mm at 50ppm and lowest or no growth of fungal mycelia was observed by tebuconazole at 100,200 and 300ppm (Table no-2, Figure-1). The maximum percent growth inhibition was observed in tebuconazole that ranged 57.9%, 100%, 100%, 100% at 50ppm, 100ppm, 200ppm, 300ppm and the lowest percent growth inhibition was observed in mavestin 22.5%, 32.1%, 37.5%, 49.8% at 50ppm, 100ppm, 200ppm, 300ppm (Table no-3).

Table 2: *In vitro* efficacy of fungicides on mycelia growth of *Bipolaris sorokiniana*

Mycelial growth of pathogen in (mm)												
Fungicides	**50PPM**			**100PPM**			**200PPM**			**300PPM**		
	3DAI	**6DAI**	**9DAI**	**3DAI**	**6DAI**	**9DAI**	**3DAI**	**6DAI**	**9DAI**	**3DAI**	**6DAI**	**9DAI**
Carbendazim	19	31.33	35.66	13	29	33.33	13.66	24	31.66	12.33	16.66	26.66
Tebuconazole	12.66	15.66	18.33	0	0	0	0	0	0	0	0	0
Polyram	19.66	25.66	30.33	17	24	27.66	15.66	19	24	14	17.66	21
Control	29	35	47	29	35	47	29	35	47	29	35	47
C.D.	3.53	1.65	1.65	2.13	3.02	1.56	1.23	1.35	1.46	1.10	2.06	4.09
SE(m)	1.06	0.5	0.5	0.64	0.91	0.47	0.37	0.40	0.44	0.33	0.62	1.23

Table 3: Percent inhibition growth of mycelia of *Bipolaris sorokiniana*

Percent Growth Inhibition of fungal mycelia					
Fungicides	**50ppm**	**100ppm**	**200ppm**	**300ppm**	**Mean**
Carbenazim	22.5	32.1	37.5	49.8	35.47
Tebuconazole	57.9	100	100	100	89.47
Polyram	31.83	38.16	47.16	52.56	42.42

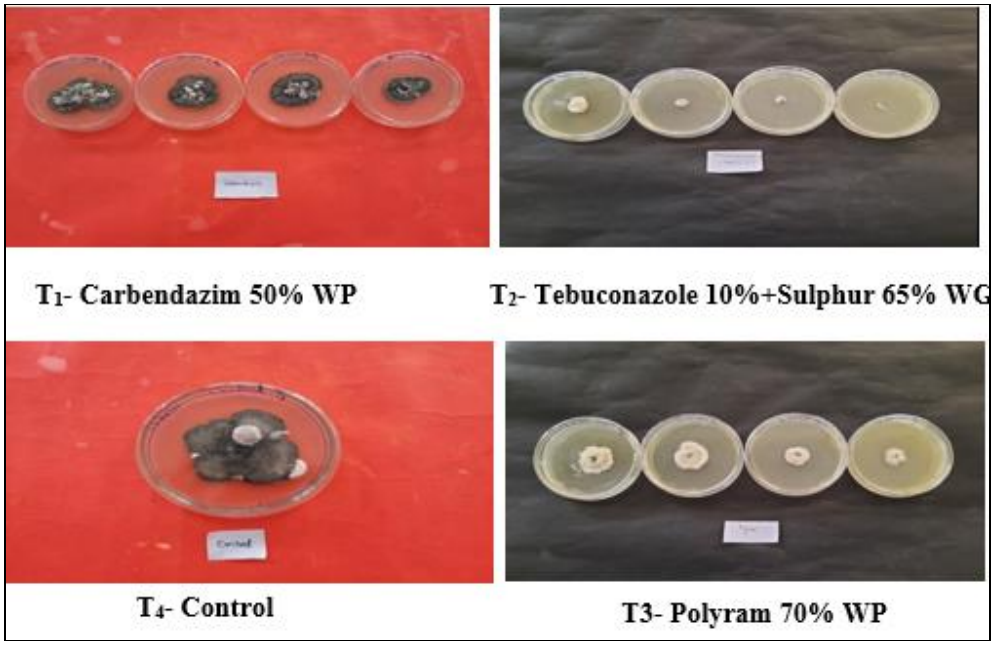

Fig 1: *In vitro* efficacy of fungicides against *B. sorokiniana*

The result stated that the fungicides tested against the pathogen inhibited the mycelial growth of *Bipolaris sorokiniana* as compared to control. As the concentration of fungicides increases, the efficacy for contolling the mycelial growth of pathogen was also increased. Tebuconazol and polyram were proved to be effective against Spot Blotch. No mycelial growth was observed at the lowest concentration of tebuconazole+sulphur fungicide. As per results agreed with Chauhan *et al*. (2001), Hasan *et al*. (2012) [7], Kavita *et al*. (2015) [8], Jaysena *et al*. (2002), Samia *et al* who reported that percentage of inhibition was increased as the concentration of fungicide increases and also that propiconazole and tebuconazole is the most effective against *Bipolaris sorokiniana* by inhibiting 100% fungal mycelial growth at the

lowest concentration and the other fungicides like carbendazim, mancozeb and copper oxychloride slowed mycelial growth after 7 days and fully inhibit the mycelial growth after 12 days at higher concentration. The present results stated that at 50ppm concentration of tebuconazole inhibit 57.9% growth of fungal mycelia so it is necessary to check the growth of fungicides at less than 50ppm concentration.

Conclusion

In vitro evaluation of some fungicides was tested for their efficacy against Bipolaris sorokiniana a causal agent of Spot Blotch of barley by using Poisoned food technique. The highest percent inhibition growth of pathogen was noticed in T_2 (Tebuconazole+sulphur) with 89.47% mean percent and second best treatment was T_3 (Polyram) with 42.42%. The least percent growth inhibition was observed in T_1 (Carbendazim) with 35.47%. Based on evaluation, it can be concluded that increase in concentration also increases the percent growth inhibition of a pathogen. The results obtained from this study play a vital role to check the fungicides efficacy against the fungal pathogen of spot blotch of Barley (*Bipolaris sorokiniana*) under laboratory conditions. Furthermore investigations are done along with other treatments to confirm the results and also to generate more information for controlling *B. sorokiniana*.

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