International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2021; 9(1): 3022-3028

Received: 18-10-2020
Accepted: 29-12-2020

Rajkumar Debsharma
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Saidul Islam
1. Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India
2. Nadia Krishi Vigyan Kendra, Bidhan Chandra Krishi Viswavidyalaya, Gayeshpur, Nadia, West Bengal, India

Debanjana Debnath
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Sunanda Chakraborty
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Sunita Mahapatra
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Corresponding Author:
Sunita Mahapatra
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Efficacy study of some botanical oils against spot blotch disease (*Bipolaris sorokiniana*) of wheat under *In-vitro* condition

Rajkumar Debsharma, Saidul Islam, Debanjana Debnath, Sunanda Chakraborty and Sunita Mahapatra

DOI: https://doi.org/10.22271/chemi.2021.v9.i1ap.11690

Abstract
Bipolaris Sorokiniana (Teleomorph *Cochliobolus sativus*) is the causal agent of spot blotch of wheat. The fungus is one of the most important foliar diseases for wheat growing areas and causes significant yield losses particularly in South Asia's intensive "irrigated wheat-rice" production system. In West Bengal as well as all Eastern India, one of the most concerning disease is spot blotch of wheat. Intensive efforts in many countries are now underway to develop effective management strategies. However, current practices mostly rely on chemical fungicides which are costly as well as environmentally harmful. Naturally available plant protectants, such as essential oils (EOs) which show antimicrobial properties, have low mammalian toxicity and are environment-friendly, could be used as alternatives for chemical fungicides. Five botanical oils [Ginger oil, Eucalyptus oil, Clove oil, Til oil and Neem oil] and one fungicide (Mancozeb 75% WP) were tested against the *Bipolaris sorokiniana* causing spot blotch of wheat, at five different concentrations: 1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm, and 3000 ppm. Among them, Clove oil exhibited strongest fungal toxicity followed by Ginger oil, Eucalyptus oil, Til oil and Neem oil, at all concentrations. The fungistatic ability increased with an increase in concentration of the plant protectants. All the essential oils showed fungistatic activity while maximum inhibition of the pathogen was observed at a concentration of 3000 ppm.

Keywords: Spot blotch, essential oils, *Bipolaris sorokiniana*, *in-vitro*, wheat

1. Introduction
Wheat (*Triticum aestivum* L.), belonging to family Graminaceae is a dominant cereal crop and staple food of millions of people in the world. In the year of 2018-19, wheat production in India reached 101.20 million tonnes which marked an all-time highest crop productivity of 3424 kg/ha (Annual Progress Report, 2018-19) [1]. The importance of foliar blight must be expressed in terms of yield losses but the estimate widely varied according to variety (Nema and Joshi, 1971) [21]. With every one percent increase in disease severity there is a significant crop loss caused by spot blotch of wheat (Devi *et al*., 2018) [6]. Foliar blight is a serious problem in the North Eastern region, while being significantly destructive throughout the wheat growing belts of the country (Tamang *et al*, 2020) [30]. It is apparent from their development that foliar blight may pose a threat to wheat in the near future. *Bipolaris Sorokiniana* (Teleomorph: *Cochliobolus sativus*) is the causal agent of spot blotch or foliar blight of wheat. The fungus is one of the most important foliar disease constraints for wheat crops growing in warmer areas and causes significant yield losses. High temperature and high relative humidity favour the outbreak of the disease, particularly in South Asia's intensive "irrigated wheat-rice" production system. In West Bengal as well as all Eastern India one of the major fungal diseases is foliar blight caused by *Bipolaris sorokiniana* and *Alternaria triticina*, which may attack singly or together causing yield loss exceeding 60% (Prabhu and Singh, 1974) [22]. In view of the high yield losses, breeding for resistance is gaining focus, with having ample genetic variability within the host population as the need of the hour. However, the continuous use of high yielding but disease prone wheat varieties coupled with injudicious use of fungicides aggravates the problem. Different researchers have carried their work on different locations and developed predication equation for disease forecasting (Forrest and Nuttar 1989; Viani *et al*., 2017) [7, 34], management (Singh *et al*. 2007; Mahapatra and Das,

2013; Singh *et al*., 2015) [26, 19, 20, 38], screening of varieties (Kumar *et al*. 2019; Kumar *et al*., 2020; Mahapatra *et al*., 2020) [13, 14] but adequate information is not available regarding the important pathogen and its availability, viable and feasible eco-friendly disease management systems under West Bengal conditions. Application of higher concentrations of chemicals, in an attempt to overcome foliar blight disease, increases the risk of high levels of toxic residue accumulation. Natural plant protectants, such as essential oils (EOs) which show antimicrobial properties, low mammalian toxicity and environment-friendly, could be used as alternatives for chemical fungicides (Burt, 2004) [3]. The botanicals are natural antioxidants which are well known for their antimicrobial and biodegradable properties (Mahapatra and Das, 2013) [19, 20]. They do not leave any residual effect on fresh produce, are environment friendly and hence known as 'reduced risk' pesticides (Tzortzakis, 2007) [31]. In this respect, use of the EOs is very much appropriate comparing to other methods of managing the spot blotch of wheat caused by *Bipolaris sorokiniana.* The use of synthetic fungicides is the commonly used strategy against spot blotch (Mahapatra and Das, 2013; Singh *et al*., 2014) [19, 20]. However, the use of these conventional synthetic chemicals resulted in environmental pollution, soil and ground water toxicity, development of resistant strains of the fungal pathogens, slow biodegradation and effect on non-target organisms including humans. Control of phyto-pathogens using botanicals is one of the best alternatives for chemical fungicides. They are also very cost effective (Kekuda, 2016) [23]. Plant extracts represent a natural and ecological approach for managing diseases and provides many distinctive benefits to farmers, as they degrade quickly, have very short pre-harvesting intervals, and reduce the risk of residual effects on food. So, the objective of this study was to identify the botanicals which possess antifungal properties against *Bipolaris sorokiniana*, as well as to evaluate the most effective concentration in which they can be utilized as an environmentally safe, fast acting and non-polluting alternative to synthetic fungicides.

2. Methods and Materials

The host plant used for the investigation was Wheat (*Triticum aestivum* L.). Infected leafs of wheat were collected from instructional farm, Jaguli (Latitude- 23.09175 and Longitude - 88.55925) under Bidhan Chandra Krishi Viswavidyalaya (BCKV) and then pathogens were isolated from the infected leaves.

2.1 Isolation and the pathogen

The pathogen used for the entire course of the experiments was *Bipolaris sorokiniana*. The infected plant parts (leaves) with typical symptoms collected from the field. The standard tissue isolation procedure was followed to isolate the pathogen. The infected tissue with some green portion was surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for 30 seconds and repeatedly washed separately in sterilized distilled water and then transferred to the sterilized petriplate containing Potato dextrose agar (PDA). The petriplate were incubated at room temperature (27 ± 1 ^{0}C) and observed periodically for growth. Bit of fungal growth developed from the infected tissue were transferred to PDA slants. Then mycelial tip or single spore isolation was done for the purification of the pathogen. Then the pure culture was used for further studies.

2.2 Plant extract used

Table 1: Show the Plant extract

Sl. No.	Common name	Scientific name	Used part	Type of extract
1.	Ginger	*Zingiber officinale*	Rhizome	Methanol extraction
2.	Eucalyptus	*Eucalyptus globulus*	leaf	Oil through hydrodistillation
3.	Clove	*Syzygium aromaticum*	bud	Steam distillation
4.	Til (Sesame)	*Sesamum indicum*	Seed and leaf	Oil through hydrodistillation
5.	Neem	*Azadirachta indica*	Leaf	Oil through hydrodistillation

2.3 Preparation of extract: - Methanol extract

Fresh leaves of previously mentioned plants were collected and sundried. The weighted material of each botanical was filled into soxhlet. The required solvent (Methanol) was filled up five times more than the total quantity of sample material in to the flask of apparatus. The temperature of heating mantle containing the flask with solvent was maintained according to the boiling point of the solvent. The process was carried out for 18-20 hours for each sample. The extract so obtained was transferred to petriplate and the solvent was allowed to evaporate. The desired stock solution of each evaporated botanicals was made by adding more solvent till the plant material was dissolved completely and stored in a refrigerator at 5^{0}C for future use. Further dilution were made by adding distilled water with emulsifier (Tween-20)

2.4 Oil extraction

The oils were extracted by hydro-distillation process Mahapatra and Das (2011) [18]. The herbs and bulbs of those grasses were cut into 2 inch pieces; sun dried for two days, and then put in the distillation container. Distillation was done by direct boiling of the materials in the distillation container. The distilled oil were made moisture free by adding sodium sulphate, then packed in galvanized container and stored in a dark cool place.

2.5 Preparation of plant oil emulsion

Plant oils belong to chemically broader groups of fats and oils, are as such insoluble in water. Keeping in view the objective of the present work, the selected plant oils needed to be dissolved in water which is hindered by the surface tension between oil and water. Hence, a drop of emulsion or surface active was used to reduce to maintain the hydrophobic-lyophilise balance (HLB) in the emulsion. So, all the oils and methanol leaf extracts were emulsified using 0.1% Tween- 20 as an emulsifier. The oils were separately dissolved by mixing 99.9 ml of oil+0.1 ml of Tween-20 to prepare the stock solution (100%) of oil emulsion. This stock solution was taken in a sterilized conical flask and then blended in an electrical blender for thorough mixing. Various concentrations was obtained by adding necessary volume of oil emulsion to sterilized PDA medium in conical flaks (as for eg.0.1% conc. was obtained by adding 0.1 ml of oil emulsion in 99.9 ml of PDA medium)

2.6 Preparation of different concentration of oils

The oils are generally not dissolved in water and for preparing the aqueous solution it needs a surface active agent or spreader like Tween-20. The different oil concentrations were used: 0.1%, 0.15%, 0.2%, 0.25% & 0.3%. These concentrations were prepared by mixing the oils in a conical flask with PDA medium as follows-

1. 0.1%=0.1 ml of oil in 99.9 ml of PDA medium
2. 0.15%=0.15ml of oil in 99.85ml of PDA medium
3. 0.2%=0.2ml of oil in 99.80 ml of PDA medium
4. 0.25%=0.25ml of oil in 99.75ml of PDA medium
5. 0.3%=0.3 ml of oil in 99.70 ml of PDA medium

2.7 Evaluation of fungicide against pathogen

The fungicide Mancozeb 75% WP (Trade name - Indofil M-45) was tested at different concentrations to find out the ED50 value against *Bipolaris sorokiniana* in *In-vitro* condition through the "poisoned food technique". Required quantity of the fungicide was added to PDA so as to get the desired concentration. Each plate was aseptically inoculated at the centre with the fungal mat of 4 mm disc. The freshly growing hyphal mat was cut with a sterile disc cutter/cork-borer from the edge of a 4 day old culture of the pathogen for this experiment. Three replications were maintained for each treatment. PDA without any fungicide served as the control. Inoculated plates were incubated at 27 ± 1^0 C and observations on the mycelial radial growth were recorded after 2 days of inoculation. Final observations were taken for each treatment when the growth of the fungus in control covered the entire petriplate.

2.8 Evaluation of plant extracts against the pathogens

Different concentration of the oil of ginger, eucalyptus, clove, sesame and neem were prepared in aqueous solution using Tween -20 as emulsifier using the earlier method to determine the ED50 value against the pathogen. This experiment was done in *in-vitro* condition through the "poisoned food technique". The percent reduction in growth obtained was plotted against fungicide and botanicals at different concentration on a log probity scale and the ED50 value was determined. The percent inhibition of the fungal growth over control was calculated by using the formula given by (Vincent 1947 *c.f.* Dasgupta 2015) [4].

$$I = \frac{C - T}{T} \times 100$$

I=Percent inhibition
C=Radial growth of fungus in control
T=Radial growth of fungus in treatment

2.9 Statistical procedures adopted:

All the experiments were designed as Completely Randomized Block Design (CRBD). The experimental results were statistically interpreted through calculation of "Analysis of variance'' by a standard method (Gomez and Gomez; 1984) and the significances of different treatments were tested by "Error mean square by Fisher and Snedicor's F-test" at probability level 0.05 for determination of Critical Difference (CD) at 5% level of significance Fisher's and Yate's tables were consulted.

3. Result and Discussion

3.1 Sensitivity and efficacy of essential oils against *Bipolaris sorokiniana* in *in-vitro* condition

To find out the efficacy of some leaf and other plant part extracts derived through hydro distillation and methanol extraction technique, an experiment was carried out against pathogen, *Bipolaris sorokiniana* in *in-vitro* condition. Different concentration (1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm and 3000 ppm) of different essential oils were used and compared with the untreated control regarding the radial growth of the pathogen and its inhibition percentage. The experimental design was conducted in complete randomized block design (CRBD) with three replications. Different scientists have reported that the essential oils exhibited antimicrobial, allelopathic, anti-oxidant and bio regulatory properties (Vaughn and Spencer, 1991) [32]. The different essential oils and their effective doses for inhibition of the *Bipolaris sorokiniana* are discussed below

3.2 Efficacy of Ginger oil on inhibition percentage of *Bipolaris sorokiniana* in different days after inoculation

The results (Table-2) showed that with the increasing concentration of Ginger oil the % inhibition also gradually increased in all concentrations. The lowest % inhibition was 31.56% noticed in lowest concentration i.e., 1000 ppm at 10 DAI and highest % inhibition was 51.59% highest concentration i.e., 3000 ppm. So % inhibition of radial growth produced with increasing concentration there was a significant increased in inhibition percentage. Maximum inhibition percentage was noticed at 2 DAI and minimum inhibition at 10 DAI, indicating that the oil possesses fungistatic activity (Plate.1). Crude extract of ginger (15%) reduced the growth of different fungus including the *Bipolaris sorokiniana* (Hasan *et al.*, 2012; Hasan, 2013) [11]. It was also observed that % inhibition between concentration and DAI was statistically significant.

Plate 1: Experiment by different concentration of Ginger oil against *Bipolaris sorokiniana*

3.3 Efficacy of Clove oil on inhibition percentage of *Bipolaris sorokiniana* at different days after inoculation

It was observed that with the increasing concentration of Clove oil the % inhibition also gradually increased in case of all concentrations. The lowest % inhibition was 15.49% lowest concentration i.e., 1000 ppm at 10 DAI and highest % inhibition 44.78% highest concentration i.e., 3000 ppm. So % inhibition of radial growth with increasing concentration of clove oil there was a significant increased in inhibition percentage. Maximum inhibition percentage was noticed at 2 DAI and minimum inhibition at 10 DAI under all concentrations which indicated that the oil has fungistatic activity. It was also observed that % inhibition between concentrations and DAI was statistically significant (Table 2; Plate.2).

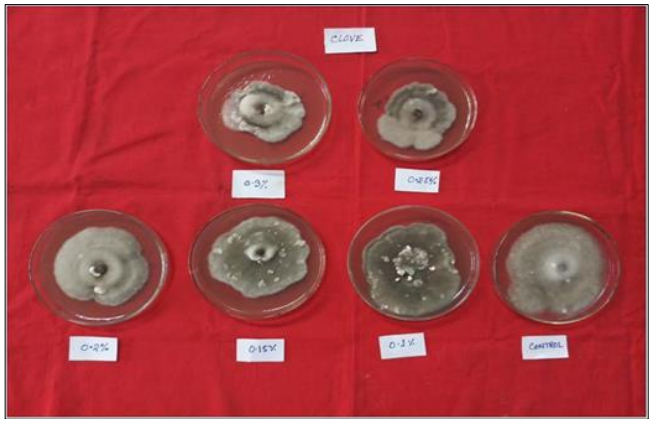


Plate 2: Experiment by different concentration of Clove oil against *Bipolaris sorokiniana*

3.4 Efficacy of Eucalyptus oil on inhibition percentage of *Bipolaris sorokiniana* in different concentrations at different days after inoculation

It was noticed that (Table 2; Plate.3) maximum inhibition was noticed in case of 3000 ppm concentration (29.96%) at 2 DAI followed by 2500 ppm and 2000 ppm (26.13%). It was observed that with the increasing concentration of Eucalyptus oil the % inhibition was decreased. The lowest % inhibition was (24.14 cm) in 1000 ppm at 10 DAI and highest inhibition percentage was 49.91% in 3000 ppm in 10 DAI. Similarly, with increasing concentration there was a significant increase in inhibition percentage and with increased in days after inoculation there was a significant decreased in inhibition percentage. Maximum inhibition percentage was noticed in 2DAI and minimum inhibition in 10DAI. The oil has indicated that fungistatis activity. So at 10DAI, maximum inhibition % was recorded in 3000 ppm (49.9%) followed by 2500 (40.06%) and 2000 (37.54%) and this differences were statistically significant.

Plate 3: Experiment by different concentration of Eucalyptus oil against *Bipolaris sorokiniana*

3.5 Efficacy of Neem oil in inhibition percentage of *Bipolaris sorokiniana* in different concentrations at different days after inoculation

In the study of % inhibition, 24.88% inhibition was noticed in case of 3000ppm concentration at 2 DAI. Similar % inhibition (22.75) was observed at 2500ppm and 2000ppm. It was noticed that with the increasing concentration of Neem oil the % inhibition also gradually increased in all concentration but with the increase in DAI the rate of % inhibition decreased. The lowest % inhibition was (5.78cm) noticed in case of 10DAI. So the study of percent inhibition of radial growth with increasing concentration there was a significant increased in inhibition percentage and with increased in days after inoculation there was a significant decreased in inhibition percentage. Maximum inhibition percentage was noticed in 2DAI and minimum inhibition in 10DAI. It indicated that the oil has fungistatis activity. So at 10DAI, maximum inhibition % was recorded in 3000ppm (35.22%) followed by 2500ppm (21.91%) and 2000ppm (15.09%) and this difference was statistically significant (Table 2; Plate.4).

Plat 4: Experiment by different concentration of Neem oil against *Bipolaris sorokiniana*

3.6 Efficacy of Til (Sesame) oil on inhibition percentage of *Bipolaris sorokiniana* in different concentrations at different days after inoculation

It was analyzed that (Table 2; Plate.5) minimum % inhibition was noticed in 3000ppm & 2500ppm concentration in 2DAI (31.7% and 24.31% respectively). It was observed that with the increasing concentration of Til oil % inhibition also gradually increased in all concentration but with the increased DAI the rate of % inhibition decreased. The lowest % inhibition was noticed in 1000ppm (8.97cm) in 10DAI. It was also observed that % inhibition between concentration and DAI was statistically significant. In case of % inhibition of radial growth with increasing concentration there was a significant increased in inhibition percentage and with increased in days after inoculation there was a significant decrease in inhibition percentage. Maximum inhibition percentage was noticed at 2DAI and minimum inhibition at 10DAI, indicating that the oil possessed fungistatic activity. So at 10DAI, maximum inhibition % was recorded in 3000 ppm (36.42%) followed by 2500 ppm (30.28%) and 2000 ppm (26.42%) and the differences were statistically significant.

Plate 5: Experiment by different concentration of Til oil against *Bipolaris sorokiniana*

3.7 Efficacy of Mancozeb 75% WP on inhibition percent of *Bipolaris sorokiniana* in different concentration at different DAI: *Bipolaris sorokiniana* produced highest % inhibition percentage at 3000 ppm (92.21%) followed by 2500 ppm (84.45%) at 2 DAI. With increasing the days after inoculation, the inhibition percentage was reduced in non-significant manner. Maximum inhibition percentage was recorded at 3000 ppm (86.79%) and minimum inhibition in 1000 ppm (70.95%) and their difference was statistically significant. In every concentration, increased inhibition of mycelial growth was statistically significant in comparison to untreated control (Table 2).

Table 2: Effect of five botanical oils on inhibition percentage of radial growth of *Bipolaris sorokiniana* in different days after inoculation

Concen.	Ginger oil					Clove oil					Eucalyptus oil				
(PPM)	2DAI	4DAI	6DAI	8DAI	10DAI	2DAI	4DAI	6DAI	8DAI	10DAI	2DAI	4DAI	6DAI	8DAI	10DAI
1000	32.25 (34.55)*	22.31 (27.83)	35.53 (36.41)	43.89 (41.48)	31.56 (34.17)	41.23 (39.94)*	32.16 (34.44)	41.28 (39.84)	43.11 (41.02)	15.49 (23.17)	25.66 (30.23)*	17.09 (23.53)	21.38 (20.89)	23.00 (21.10)	24.14 (29.30)
1500	35.31 (36.44)	30.18 (33.14)	38.90 (38.56)	44.23 (41.68)	36.77 (37.32)	43.52 (41.27)	41.27 (39.94)	46.55 (42.99)	50.05 (45.03)	25.53 (30.31)	25.90 (30.49)	19.48 (26.09)	22.69 (30.63)	27.93 (31.51)	29.17 (32.50)
2000	36.45 (37.13)	30.54 (33.54)	47.01 (43.27)	50.11 (45.06)	42.00 (40.39)	45.54 (42.42)	50.06 (45.03)	53.83 (47.20)	58.85 (50.10)	36.67 (37.26)	26.13 (30.67)	21.87 (27.97)	24.00 (33.53)	35.66 (31.46)	37.54 (37.72)
2500	39.55 (38.95)	36.42 (37.10)	48.63 (44.21)	52.23 (46.28)	44.64 (41.92)	47.73 (43.68)	55.24 (48.02)	56.98 (49.02)	58.85 (50.10)	42.14 (40.48)	26.13 (30.54)	23.49 (28.86)	24.81 (39.17)	40.70 (39.58)	40.06 (39.20)
3000	44.01 (41.55)	48.37 (44.05)	55.26 (48.02)	58.01(49.62)	51.59 (45.91)	54.03 (47.32)	58.18 (49.71)	58.84 (50.11)	60.53 (51.09)	44.78 (40.00)	29.96 (33.05)	28.02 (31.93)	28.99 (41.83)	48.37 (44.00)	49.91 (44.92)
		DAI	Concentration		DAI ×C	DAI	Concentration		DAI×C		DAI	Concentration		DAI×C	
SEm±		0.864	0.864		1.932	0.762	0.762		1.705		1.584	1.586		3.543	
CD at 5%		1.737	1.737		3.885	1.533	1.533		3.428		3.186	3.186		7.123	
CD at 1%		2.318	2.318		5.182	2.045	2.045		4.573		4.250	4.250		9.503	

*DAI = Days after inoculation; Figure in Values in parenthesis arc sine transformed values

Table 2: Cont……

Concen.	Neem oil					Til oil					Mancozeb 75% WP				
(PPM)	2DAI	4DAI	6DAI	8DAI	10DAI	2DAI	4DAI	6DAI	8DAI	10DAI	2DAI	4DAI	6DAI	8DAI	10DAI
1000	18.30 (25.19)*	9.29 (16.75)	10.60 (18.44)	4.26 (11.88)	5.78 (13.68)	6.11 (13.356)*	17.05 (24.31)	17.26 (23.53)	23.94 (29.17)	8.97 (15.90)	58.73 (50.07)*	68.59 (55.99)	67.48 (55.24)	71.10 (57.49)	70.95 (57.39)
1500	20.47 (26.64)	11.61 (18.98)	10.92 (17.71)	10.45 (18.35)	13.75 (21.52)	13.43 (20.86)	19.58 (26.24)	22.21 (27.69)	26.22 (30.75)	15.30 (22.72)	75.34 (6025)	78.98 (62.71)	81.44 (64.52)	80.34 (63.67)	78.85 (62.64)
2000	22.73 (28.31)	13.90 (21.10)	12.39 (19.60)	13.86 (21.26)	15.09 (22.73)	22.23 (27.90)	24.16 (29.42)	23.32 (28.45)	33.13 (35.14)	26.42 (30.89)	78.85 (62.66)	83.63 (66.18)	82.61 (65.41)	82.75 (65.47)	80.74 (64.02)
2500	22.75 (28.34)	16.27 (23.79)	21.45 (27.20)	20.14 (26.58)	21.91 (27.89)	24.31 (29.24)	25.78 (30.48)	27.13 (31.26)	38.37 (38.26)	30.28 (33.38)	84.45 (66.94)	85.66 (67.81)	86.81 (68.76)	86.37 (68.34)	84.91 (67.16)
3000	24.88 (29.45)	23.22 (28.44)	34.71 (36.08)	34.07 (35.58)	35.22 (36.23)	31.71 (34.23)	29.02 (32.56)	37.09 (37.51)	38.53 (38.29)	36.42 (37.08)	92.21 (73.85)	91.14 (72.73)	90.76 (72.32)	89.57 (71.16)	86.79 (68.71)
		DAI	Concentration		DAI ×C	DAI	Concentration		DAI×C		DAI	Concentration		DAI×C	
SEm±		1.532	1.532		3.425	1.248	1.248		2.790		8.7	5.1		2.4	
CD at 5%		3.08	3.08		6.886	2.509	2.509		5.610		20.1	11.7		5.5	
CD at 1%		4.108	4.108		9.186	3.347	3.347		9.484		29.3	11.0		8.0	

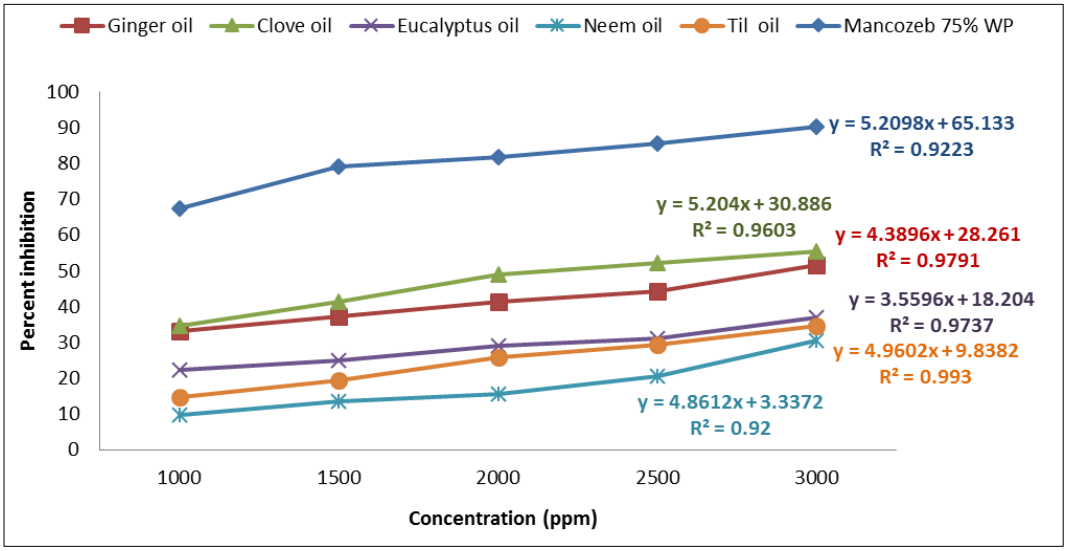
*DAI = Days after inoculation; Figure in Values in parenthesis arc sine transformed values

4. Discussion

In view of the ever-evolving fugal population and their significant impact on the yield of wheat, the focus to develop environmentally viable, socially acceptable and economically feasible alternative is the need of the hour. Since plant extracts are easy to prepare and are cheaper as compared to commercially available fungicides, they serve as a desirable alternative. In the present study, five botanical extracts, i.e., Ginger oil, Eucalyptus oil, Clove oil, Til oil and Neem oil were evaluated along with Mancozeb 75% WP against *Bipolaris sorokiniana*, the causal agent of spot blotch of wheat. Among the botanicals evaluated, clove extract exhibited highest average percent inhibition (46.49%), while the lowest percent inhibition was observed in neem extract (17.92%). Singh *et al*., (2018) [27] observed a 73.57% inhibition percent of mycelia of *Alternaria triticina* by neem leaf extract, while Gurjar *et al*., (2012) [8] reported neem extract to be a potent fungistat against a wide range of fungal pathogens. However, our study indicated neem extract to be weakly fungistatic against *B. sorokiniana*, in comparison to the other botanicals studied. Yasmin (2016) [37] reported that ginger extract inhibited the mycelial growth of *B. sorokiniana* up to 38.35% at 15% concentration, which suggested the antifungal activity of the extract. Hasan *et al*., (2005) [12] also reported similar findings when working with seed borne fungi of wheat. In the present study ginger extract was able to inhibit percent mycelial growth of the pathogen by 41.43% (average), which was the second highest inhibition among the botanicals. Our study revealed that clove oil extract brought about an average 46.49% mycelial growth inhibition of the pathogen, which was in keeping with the findings by Verma *et al*., (2017) [33], who reported clove essential oil to be highly potent against different fungal infections Araujo *et al*., (2018) [2] and Raveau *et al*., (2020) [24] reported similar findings.

It was observed that the fungistatic efficacy against *B. sorokiniana* increased with an increase in concentration of the botanical extracts. The highest inhibition was observed at 3000 ppm in the fungicide as well as the botanicals considered. Similar results have been reported by several researchers working with different botanicals (Yadav, 2015; Yasmin, 2016; Magar, 2020) [36, 37, 16]. At 3000 ppm concentration, clove extract (55.27%) significantly checked the growth of the pathogen followed by ginger (51.45%) and eucalyptus (37.05%). Shafique *et al*., (2007) [25] had

previously reported the presence of antifungal compounds in Eucalyptus extract against various fungal pathogens of wheat, which was also observed in the present study. Further, neem leaf extract exhibited least inhibitory action among the tested botanicals, to inhibit the growth of *B. sorokiniana* while clove, ginger and eucalyptus extracts exhibited more than 22% inhibitory effect at all concentrations on PDA. The percent inhibition was observed to increase gradually till 8 DAI at all concentrations in the extracts considered.



Graph 1: Functional relation between the concentration and mycelia growth inhibition of different botanicals against *B. sorokiniana*

5. Conclusion

Based on the evaluation of the five botanical extracts, it can be concluded that the best fungistatic activity is exhibited by clove oil followed by ginger oil, eucalyptus oil, til oil and need oil, against *Bipolaris sorokiniana*. The mycelial growth inhibition of *Bipolaris sorokiniana* increased gradually with increased concentration of the extracts. The highest inhibition was observed by the application of clove oil, followed by garlic oil at 55.27% and 51.45% respectively, at a concentration of 3000 ppm. Neem oil was found to be the least effective among the tested botanicals at all the concentrations. Further investigation may be conducted at different concentrations of the same botanicals in order to test their efficacy. The scope of the research can be further extended to test the botanicals against other important fungal pathogens of wheat.

6. Reference

1. Annual progress Report. ICAR - Indian Institute of Wheat and Barley Research, Karnal-132001, Haryana, India 2018-19.
2. Araujo EF, Mauri AL, Araujo RF, Amaro HT, da Silva DJ, dos Santos Dias DC. Physiological and sanitary quality of organic tomato seeds treated with clove basil extracts. Comunicata Scientiae 2018;9(1):26-33.
3. Burt S. Essential oils: their antibacterial properties and potential applications in foods-a review. Int. J Food Microbiol 2004;94:223-253.
4. Dasgupta B, Barman S, Sarkar S, Das S. Efficacy of some essential oils against stem rots of Groundnut (A*rachis hypogaea* l.) Caused by *Sclerotium rolfsii* (sacc.). The Bioscan 2015;10(4):1829-1832.
5. Devi HM, Mahapatra S, Dutta S, Das S. Influence of phenological growth stages and meteorological parameters on leaf blight infestation of wheat in Gangetic plains of West Bengal. J Wheat Res 2017;9(2):101-107.
6. Devi HM, Mahapatra S, Das S. Assessment of yield loss of wheat caused by spot blotch using regression model. Indian Phytopathol 2018;71(2):291-294.
7. Forrest W, Nuttar JR. Detection and Management of plant diseases gradient in peanut with a Multispectral Radiometer. Phytopathology 1989;79(9):959-963.
8. Gurjar G, Ali MSS, Akhtar M, Singh KS. Efficacy of plant extracts in plant disease management 2012.
9. Gomez KA, Gomez AA. Statistical procedures for agricultural research. 2nd ed. John Wiley & Sons, New York, NY 1984.
10. Hasan MM, Ahmad F, Islam MR, Murad KFI. *In-vitro* effect of botanical extracts and fungicides against *Bipolaris sorokiniana*, causal agent of leaf blotch of barley. Journal of Agroforestry and environment 2012;6:82-87.
11. Hasan MM. Biological control of wheat diseases caused by *Bipolaris sorokiniana*, *Fusarium graminearum* and *Aspergillus flavus* with antagonists of *Trichoderma* spp. Persian Gulf Crop Protection 2013;2:1-9.
12. Hasan MM, Chowdhury SP, Alam S, Hossain B, Alam MS. Antifungal effects of plant extracts of seed-borne fungi of wheat seed regarding seed germination, seedling health and vigour index. Pakistan Journal of Biological Sciences 2005;8(9):1284-1289.
13. Kumar S, Singhora G, Bharadwaj SC, Bala R, Saharan MS, Gupta V *et al*. Multi environmental evaluation of wheat (*Triticum aestivum* L.) germplasm identifies donors with multiple fungal disease resistance. Genet Resour and Crop Evt 2019. DOI: 10.1007/s10722-019-00751-3.
14. Kumar S, Singroha G, Bhardwaj SC, Saharan MS, Gangwar OP, Mishra CN *et al*. Characterization of exotic germplasm lines for resistance to wheat rusts and spot blotch. Indian Phytopath 2020;73:237-243 https://doi.org/10.1007/s42360-020-00232-z
15. Kumar U, Joshi AK, Kumar S, Chand R, Roger MS. Quantitative trait loci for resistance to spot blotch caused by *Bipolaris sorokiniana* in wheat (*T. astivum*) lines Ning 8201 and Chirya 3. Melecular breeding 2010;26(3):477-491.

16. Magar PB, Baidya S, Koju R, Adhikary S. *In-vitro* evaluation of botanicals and fungicides against Bipolaris sorokiniana, causing spot blotch of wheat. Journal of Agriculture and Natural Resources 2010-2020;3(2):296-305.
17. Mahapatra S, Devi HM, Kumar S, Mukherjee D, Das S. Performance and Stability of Different Zone Specific Genotypes Evaluated against Spot Blotch of Wheat. Int. J Curr. Microbiol. App. Sci 2020;9(06):2968-2981. doi.org/10.20546/ijcmas.2020.906.357
18. Mahapatra S, Das S. Efficacy of botanical oils and chemicals to control chilli anthracnose. In. J Plant Prot 2011;39(4):325-326.
19. Mahapatra S, Das S. Bioefficacy of botanicals against *alternaria* leaf blight of mustard under field condition. The Bio scan 2013;8(2):675-679.
20. Mahapatra S, Das S. Evaluation of Fungicides and Botanicals against *Alternaria* Leaf Blight of Mustard. In. J Plant Prot 2013;41(1):61-65.
21. Nema KG, Joshi LM. Flag leaf susceptibility of wheat to *H. sativum* in relation to grain weight. Indian Phytopathology 1971;24:526-532.
22. Prabhu AS, Singh A. Appraisal of yield loss in wheat due to foliage diseases caused by *Alternaria triticina* and *Helminthosporium sativum.* Indian Phytopath 1974;27(4):632-634.
23. Prashith Kekuda1 TR, Akarsh1 S, Noor Nawaz AS, Ranjitha1 MC, Darshini1 SM, Vidya1 P. *In vitro* Antifungal Activity of Some Plants against *Bipolaris sorokiniana* (*Sacc.*) Shoem. International Journal of Current Microbiology and Applied Sciences. ISSN: 2319-7706 2016;5:331-337.
24. Raveau R, Fontaine J, Lounès-Hadj Sahraoui A. Essential oils as potential alternative bio control products against plant pathogens and weeds: A review. Foods 2020;9(3):365.
25. Shafique S, Javaid A, Bajwa R, Shafique S. Effect of aqueous leaf extracts of allelopathic trees on germination and seed-borne mycoflora of wheat. Pak. J Bot 2007;39(7):2619-2624.
26. Singh D, Sinhg M, Verma VS. Prevalence of foliar blight pathogens attacking wheat in Shivalik foothills and significance of sowing dates for its management. Indian Phytopathology 2007;60(2):202-207.
27. Singh Kakraliya S, Choskit D, Pandit D, Abrol S. Effect of Bio-Agents, Neem Leaf Extract and Fungicides Against Alternaria Leaf Blight of Wheat (*Triticum aestivum* L.). International Journal of Advanced Biological and Biomedical Research 2018;6(1):23-34.
28. Singh TB, Tiwari AN. Role of weather conditions in the development of foliar diseases of wheat under Tarai conditions of North-Western India. Plant Dis. Res 2001;16(2):173-178.
29. Swaminathan. One man's quest for a hunger-free world. Education development centre, Inc 2000, 95.
30. Tamang S, Kumar S, Das Srikanta, Mahapatra S. Role of abiotic factors on disease progression of Spot blotch of Wheat 2020. Indian Phytopathology https://doi.org/10.1007/s42360-020-00280-5
31. Tzortzakis NG. Methyl jasmonate-induced suppression of anthracnose rots in tomato fruit. Crop Prot 2007;26:1507e1513.
32. Vaughn SF, Spencer G. Volatile monoterpenes inhibit potato tuber sprouting. Potato J 1991;68:821-831.
33. Verma M, Sharma S. Efficacy of Six Plant Essential Oils against Phytopathogenic Fungi Aspergillus Niger. International Journal of Basic and Applied Biology 2017;4(1):51-56.
34. Viani A, Sinha P, Sharma T, Bhar LM. A model for forecasting spot blotch disease in wheat. Australasian Plant Pathol 2017. DOI 10.1007/s13313-017-0514-z
35. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 150: 850. ICAR-IIWBR. Progress Report of AICRP on Wheat and Barley 2017-18, Social Sciences. Eds: Satyavir Singh, Anuj Kumar, Sendhil R and GP Singh. ICAR-Indian Institute of Wheat and Barley Research, Karnal, India 1947-2018, 50.
36. Yadav B, Singh R, Kumar A. Management of spot blotch of wheat using Fungicides, Bio-agents and Botanicals. African Journal of Agricultural Research 2015;10(25):2494-2500.
37. Yasmin S. *In vitro* evaluation of botanical extracts against some soil and seed borne fungi of economic importance. Journal of Agriculture and Ecology Research International 2016, 1-11.
38. Singh DP, Sharma Indu, Singh Iswar, Jindal Madhumeeta, Mann KS, Chowdhury AK *et al*. Evaluation of sources of resistance of leaf blight (*Bipolaris sorokiniana* and *Alternaria triticina*) in wheat *(Triticum aestivum*) and Triticale. Indian Phytopathology 2015;68(2):221-222.