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Studies on Leaf Spot of Chilli caused by *Cercospora capsici* and its management in Manipur

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Abstract
Chilli (*Capsicum annum* L.) also known as morok in Manipur is a spice crop consume all over the world. Infected chilli plants showing the CLS symptom were isolated. The disease samples were collected during September 2018-2019 & 2020-2021 from Imphal East district (*viz*., Keirao Bitra Lairembi Leikai, Keikhu Makha Leikai, Khurai Kongpal Chingangbam Leikai, Huikap Mayai Leikai, Wangkhei Keirungbam Leikai, Khongman Zone 5 & Langdum Lamkhai). Average Disease Incidence of the surveyed sites was found highest in Keirao Lairembi Leikai with 55.42% whereas the least disease incidence was found in Langdum Lamkhai (20.35%). Averaged disease severity was found highest in Keirao Bitra Lairembi Leikai with 28.50% whereas the least disease severity was observed in Khongman Zone 5 with 14.63%. The organism produced white- grey- olivaceous brown uniformly dense colonies on PDA media after incubation at 25˚C±1 for 15 days. *In vitro* evaluation of plant extracts resulted that the highest inhibition of growth & sporulation under 10% conc. was observed in garlic extract with 100% inhibition & turmeric extract was observed lowest inhibition with 5.55%. Similarly, under 15% conc. highest inhibition was observed in garlic extract with 100% inhibition & the lowest inhibition was observed in onion extract with 10.18% inhibition. The best biocontrol agent against the test fungus was found to be *Trichoderma harzianum* with 80.66% inhibition and the least effect against the test fungus was found in *Trichoderma viride* with 75.52% inhibition. *In vitro* evaluation of fungicides resulted that 100%inhibition of mycelial growth was found in four fungicides *viz*, Carbendazim 0.1% conc., Tebuconazole at 0.2% conc., (Carbendazim 12% WP+ Mancozeb 63% WP) at 0.2% conc. and mancozeb at 0.1% conc. & the least inhibition was observed in Captan at 0.2% conc. (inhibit 85.22%).

Keywords: *Cercospora* leaf spot, chilli, *Cercospora capsici*, plant extracts, bioagent, fungicides

Introduction
Chilli (*Capsicum annum* L.) is one of the major cultivated crops in India including the state of Manipur. It belongs to Solanaceae family. It was originated from tropical America and was introduced in India from Brazil by the Portuguese in 16th C (Selvakumar, 2014) [62]. It is an annual or perineal, herbaceous, cash crop having more than 25 species, and out of which only 5 five common cultivated species are known *viz*. *Capsicum annum, Capsicum chinense*, *Capsicum frutenscens, Capsicum pubescens* and *Capsicum baccatum* which comprises more than 400 different varieties in it (Reena Pundir *et al.,* 2016) [57]. Chilli is also known as 'wonder spice' because of its diverse use as spice, condiment, culinary supplement, medicine, vegetable etc. Chilli fruits contain carotenoids, phenols, vitamin C foliates and oxidation product (dehydroascorbic acid), has many biological activities in the human body due to its antioxidant properties. Chilli is a good source of vitamin A, B, C (Ascorbic acid) and E (Tocopheral), oleoresin, carbohydrate and minerals such as calcium, phosphorus, ferrous, sodium and copper in trace amounts and the allied pungent principles *viz*., dihydrocapsaicin and nordihydrocapsaicin. The pungency of chilli is due to the presence of alkaloid called capsaicin and the red colour of chilli is due to the pigment called capsanthin. India is the largest producer, consumer and exporter of chillies in the world. Total green chillies production in 2020-2021 was 4417 MT from 418 ha of land whereas total dried chillies produced was 2092 MT from 729 ha of land (Anonymous, 2021) [2]. In Manipur the total green chilli production during the year 2016-2017 was 2560 MT. Chilli is grown in all the 16 districts of Manipur's hills and plains. The two main species of chillies grown in Manipur are *Capsicum annum* L. (Morok) & *Capsicum frutescens* L. (landrace varieties -Meitei Morok & Yelhlong Morok) (Devi datt *et al.,* 2018) [18]. In Manipur February/March has been observed to be favourable for planting chilli (Pinky Chanu *et al.,* 2020) [47].

Chilli is prone to be affected by many biotic (Pests, diseases) & abiotic (drought, high soil moisture, salinity, soil poverty, etc.) stresses that cause severe yield losses (Khan *et al.*, 2009; Segnou *et al.*, 2013; Zhani *et al*., 2013) [24, 61, 79] thus reducing the marketable quality & quantity of chilli disturbing the economic stability of the farmers and our nation. Chilli is mostly prone to fungal, bacterial, viral diseases and nematode diseases. Fungi alone causes more than 40 diseases in chilli plant (Walker, 1952; Rangaswami, 1979) [74, 55].

Cercospora mainly is seed borne, however, the pathogen is also able to survive for at least one year in plant debris and soil. Primarily spores are spread by wind, splashing water & leaf to leaf contact. The suitable environmental condition required for the luxuriant growth of *Cercospora* leaf spot is when there is less than 28ºC temperature, 92% RH & soil pH ranging from 5-6. The disease does not develop below 90% RH. The disease is more severe in wet weather than in dry weather and becomes destructive in high relative humidity (Cerkauskas, 2004) [8]. Due to *Cercospora* leaf spot disease, photosynthetic process is disturbed & leaves becomes deformed resulting weakens plant, premature defoliation which ultimately lowers the yield & market value (Franc *et al.*, 2001) [25]. Generally, *Cercospora* leaf spot (CLS) disease symptoms can be observed on either side of the leaf. The initial stage of the infection shows symptoms of small dark brownish circular spots (0.5- 1mm) with ashy light-grey centre & reddish-brown margins on the leaves. As the disease progress the spots enlarges (up to 1.5cm in diameter), dark concentric rings growing around the whitish centre giving the characteristic 'frog-eye' symptom. Gradually the spots coalesce & the ashy grey centre often dries & falls out, giving rise to shot-hole effect. The leaves turn yellow, wilt and drop out exposing the fruit to sunscald. As the disease become more severe spots can also be found to observe on fruit stalk & calyx, resulting in stem end rot (Oluwafemi M. Adedire *et al*., 2018) [45]. Leaf spots are distinct on both surfaces of the leaves. Spots are circular when young becoming oval or somewhat elongated as the spots age. With light grey centres & each spot delineated by a dark brown ring. Sometimes spots are surrounded by a diffuse yellow necrotic line. Two or three spots may coalesce as they expand. Under favourable conditions, the number of lesions increases rapidly & their coalescence causes extensive necrosis of leaf tissue & defoliation. Bhat *et al.,* (2008) [4] reported that the typical disease symptoms of frog eye leaf spot of pepper caused by *C. capsici* were observed on leaves, stem & peduncles. On leaves, the spots appeared as necrotic, circular to sub-circular with greyish white centre, surrounded by brown to greyish brown area & marginated by definite darker zone. The spots enlarged up to a mean diameter of 9.8 mm, coalesced frequently & led to defoliation with or without yellowing. The spots become raised and resembled frog eye. The lesions on stem, peduncle and petioles, however, were longer rather than round. The pathogen does not infect fruit.

Cercospora leaf spot (CLS) disease is also known as frogeye leaf spot disease and it was first reported by Saccardo in 1876. Although, the disease was not considered to be severe in the past but due to indiscriminate use of chemical fungicides and introduction of newer varieties CLS has become one of the potentially destructive disease in the state of Manipur and can be observed in any stages of the chilli plant reducing the economic value of the crop. The management of CLS of chilli caused by *Cercospora capsici* is important in order to maximize the crop yield. Hence, the present study was carried out in Manipur to evaluate different management practices against the CLS of chilli through *in vitro* condition.

Materials and Methods

The present investigations were undertaken in the Department of Plant Pathology, School of Agriculture, Pandit Deen Dayal Upadhyay institute of Agricultural Sciences, Utlou Bishnupur during September 2018-2019 & 2020-2021. The experimental approaches and procedure adopted during the course of investigation are as follow:

Survey for natural incidence of *Cercospora* leaf spot of chilli in Imphal East district of Manipur

A survey for the natural incidence of CLS of chilli was carried out. Disease samples showing typical symptoms of CLS were collected during September 2018-2019 & 2020-2021 from chilli growing area of Imphal East district of Keirao Bitra Lairembi Leikai, Keikhu Makha Leikai, Khurai Kongpal chingangbam Leikai, Huikap Mayai Leikai & Wangkhei Keirungbam Leikai, Khongman Zone 5 & Langdum Lamkhai. In the field 25 plants were randomly selected and in each plant five leaves (two each from bottom and middle and one from top) were observed to determine the incidence and severity of CLS. Disease samples were collected & brought to the laboratory for further studies.

Table 1: Disease rating score given by Galanihe *et al*., 2015.

Crop injury description	Rating
No disease symptom	0
10% of colony showing diseased symptom	1
10.1 - 25% of colony showing diseased symptom	3
25.1 - 50% of colony showing diseased symptom	5
50.1-75% of colony showing diseased symptom	7
> 75% of colony showing diseased symptom	9

The disease severity was classified into 0-9 rating given by Galanihe *et al*., 2015.

The Percent Disease Index (PDI) was calculated by the following formula:

$$PDI\% = \left(\frac{Sum\ of\ all\ disease\ rating}{Total\ no.of\ rating\ x\ Maximum\ disease\ grade}\right) x100 \quad (1)$$

The percentage of disease incidence was calculated by the following formula:

$$Percent\ Disease\ Incidence = \left(\frac{No..of\ in\ diseased\ plants}{Total\ no.of\ plants}\right) x100 \quad (2)$$

Isolation and identification of causal Pathogen

Isolation of the pathogen were carried out by cutting the infected leaves 3 mm size and surface sterilized with 0.1% sodium hypochlorite for two mins and further cut leaves were serial washed in sterile distilled water for three times to clear residue toxicity. Excess water was removed by placing them on the sterile blotting paper. Then the cut leaves were inoculated on to Petri dishes containing PDA and incubated at 25˚C±1 for 7 days in BOD chamber. Daily observations were done on the development of the fungus. The culture was purified & sub-cultured on PDA for further studies. The fungal culture was purified by adopting hyphal tip cut method from the actively growing hyphal tips by using cork-borer of 3mm diameter. The conidiophores and conidia which were observed from the fresh leaves were recorded and compared with the earlier description of the fungus. The isolated fungi

from diseased samples were identified in the laboratory by comparing with the previous culture and monographs available. Cultures were maintained on freshly prepared PDA slants inside the refrigerator at around 4-5˚C and periodically sub cultured to fresh medium. Such culture was used throughout the study period.

Pathogenicity tests: The pathogenicity test was conducted on the seedlings raised from the healthy seeds. The test was conducted under pot culture conditions in the department of plant pathology, Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences, Utlou. In this test, thirty days old healthy seedlings were given mechanical injury by using pin prick method and sprayed with the mycelial suspension of the fungus prepared in sterilized distilled water. Control plants were sprayed only with sterilized distilled water. Inoculated plants were covered with perforated polythene bags to maintain high relative humidity condition for 24 hrs. Bags were removed 7 days after inoculation and kin observation were taken on the appearance and development of the disease symptoms. After appearance of disease symptoms of CLS the pathogen fungus was re-isolated on the PDA media and observed under 40x microscope to confirm the pathogen.

In-vitro* evaluation of locally available plant extracts against growth and sporulation of *Cercospora capsici

Efficacy of plants extracts against the fungal mycelial growth were evaluated by performing poison food technique. In this studies, two different concentrations of each of the eight botanical extracts were prepared at 10% and 15%. Each plant extracts were prepared in 1:1 ratio w/v. Each treatment was replicated three times. Each of the sterilized plate were poured with 20 ml of poisoned media & such solidified medium were inoculated with 3 mm disc of actively growing fungal mycelium of *C. capsici*. Inoculated plates were incubated in BOD chamber maintaining 25˚C±1 to proliferate. Inspections were carried out every day. Data & information of the fungal colony were collected when maximum radial growth was observed in control plate. The radial growth of the fungus was taken in two direction & the average was recorded. Percent inhibition was calculated by using the formula given by Vincent (1927) [72].

$$I = \left(\frac{Radial\ growth\ of\ fungus\ in\ control - Radial\ growth\ of\ funcus\ in\ treatment}{Radial\ growth\ of\ fungus\ in\ control} \right) \mathbf{x\ 100} \quad (3)$$

Where:
I = Per cent inhibition
C = Radial growth of fungus in control,
T = Radial growth of fungus in treatment.

Table 2: List of botanicals/ plants used *in vitro* evaluation against *C. capsici*

Sl. No.	Common name	Scientific name	Parts use
1	Onion	*Allium cepa*	Bulb
2	Garlic	*Allium sativum*	Rhizome
3	Ginger	*Zingiber officinale*	Rhizome
4	Turmeric	*Curcuma longa*	Rhizome
5	Tulsi	*Ocimum sanctum*	Leaves
6	Neem	*Azadirachta indica*	Leaves
7	Darrek	*Melia azedarach*	Leaves
8	Sweet flag	*Acorus calamus*	Rhizome

In vitro* evaluation of biocontrol agent against the growth and sporulation of *Cercospora capsici

Efficacy of two biocontrol agents *viz*., *Trichoderma harzianum* & *Trichoderma viride* were evaluated under *in vitro* condition using dual culture technique (Bell *et al*., 1982) [3] against the fungus *C. capsici*.

In this test, actively growing 3 days old bioagents *viz*., *Trichoderma harzianum* and *Trichoderma viride* were cut from the periphery of the fungal colony separately with the help of sterile cork-borer (3mm diameter). The bio-agents and test fungus were inoculated side by side 3cm apart on a single Petri dish containing solidified PDA medium. 3 three replications were maintained for each treatment. The plates received only mycelial disc of the test pathogen served as control. The inoculated plates were incubated for two weeks in BOD at 25˚C±1 until the control plate is fully grown with the fungal mycelium. Therefore, inhibition percentages were calculated based on the point of contact between the test pathogen and antagonists on PDA plates following Bell's scale (Bell's *et al*., 1982) [3] (Class I-V) developed as:

a. Class I = the antagonist completely overgrew the pathogen (100% growth)
b. Class ii = the antagonist overgrew at least 2/3 of pathogen surface (75% overgrowth)
c. Class iii = the antagonist colonized on half of the growth of the pathogen (50%)
d. Class iv = the pathogen and the antagonist locked at the point of contact and
e. Class v = the pathogen overgrew the pathogen.

In vitro* evaluation of fungicides against the growth and sporulation of *Cercospora capsici

Efficacy of six fungicides having different mode of action *viz*. two systemic fungicides [Carbendazim & Tebuconazole, two systemic as well as contact fungicides [Copper Oxychloride & Carbendazim 12%+Mancozeb 63% WP] and contact fungicides [Captan & Mancozeb] were evaluated at different concentrations against the growth and sporulation of *Cercospora capsici* using poisoned food technique given by Sharvelle, 1961 [64].

In this test, accurate quantity of each fungicide was added to 50ml PDA medium to give desired concentrations after autoclaving and shaken well to mix thoroughly. The medium without any fungicides served as control. Fungal mycelium of 3mm disc from the actively growing fungal colony (3days old) were cut and inoculated at the centre of the fungicide treated and untreated plates and incubated in BOD at 25˚C±1 and the linear growth of mycelium fungus in each fungicide was recorded at 24 hours interval till the control plates were fully covered with fungal mycelium. Inhibition % were calculated by measuring the mycelial growth in two directions and averaged were recorded. Percent inhibition of the mycelial growth was calculated over control by using the formula given by vincent (1927).

In this test, 0.1% conc. of the fungicides (Carbendazim and Mancozeb) were prepared by adding 1gm of fungicide in 50ml sterilized PDA and mixed thoroughly. Similarly, 0.2% conc. of the fungicides (Copper oxychloride, Captan, Carbendazim 12%+Mancozeb 63% WP and Tebuconazole) were prepared by adding 0.2gm of fungicide in 50ml sterilized PDA and mixed thoroughly. The concentrations were adjusted to 100% active ingredient. Three replications were maintained for each and every treatment. Suitable check was maintained without addition of any fungicides. 20ml of the PDA were poured in each plate and solidify. Fungal mycelium of 3mm disc from the actively growing fungal colony (3 days old) were cut and inoculated in the plate and incubated in BOD at 25˚C±1 to proliferate. Inspection were done in every alternate day. Inhibition% were calculated by measuring the mycelial growth in two directions and average were recorded. The radial growth of colony was recorded when maximum growth was observed in control plate and per cent inhibition was calculated by using the formula given by Vincent (1927) [72]:

$$I = \left(\frac{Radial\ growth\ of\ fungus\ in\ control - Radial\ growth\ of\ funcus\ in\ treatment}{Radial\ growth\ of\ fungus\ in\ control} \right) \times 100 \quad (4)$$

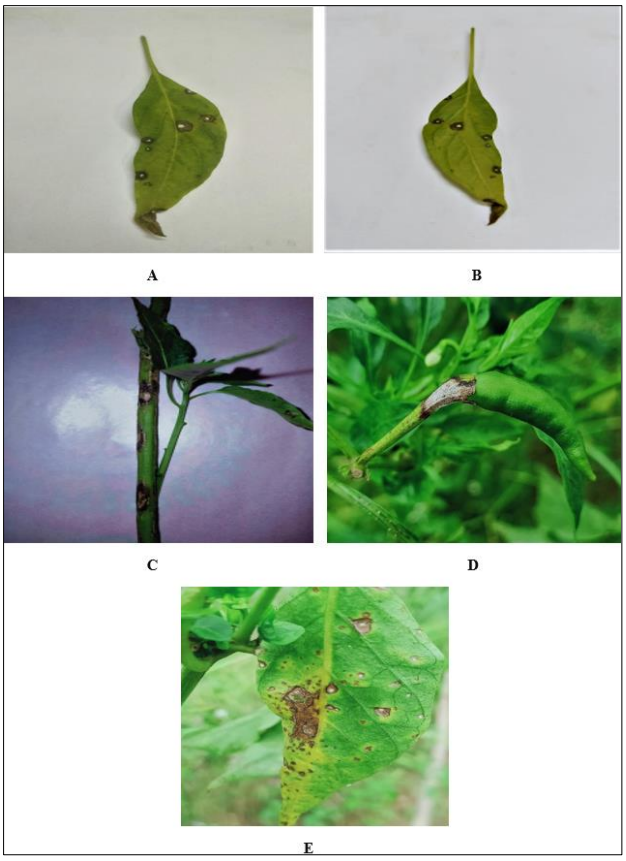
Where:
I = Per cent inhibition,
C = Radial growth of fungus in control,
T = Radial growth of fungus in treatment.

Results

Survey and collection of diseased samples.

The disease specimens showing typical symptoms of *Cercospora capsici* were collected during September 2018-2019 & 2020-2021 from chilli growing area of Imphal East district of Keirao Bitra Lairembi Leikai, Keikhu Makha Leikai, Khurai Chingangbam Leikai, Huikap Mayai Leikai, Wangkhei Keirungbam Leikai, Khongman Zone- 5 & Langdum Lamkhai. Average % disease incidence was found to be highest in Keirao Bitra Lairembi Leikai 55.42%, followed by Huikap Mayai Leikai (46.31%), Keikhu Makha Leikai (37.33%), Khurai Kongpal Chingangbam Leikai (33.43%), Wangkhei Keirungbam Leikai (26.52%) and Khongman Zone 5 (26.21%). The least inhibition was found in Langdum Lamkhai with 20.35%. The highest average % disease severity was found in Keirao Bitra Lairembi Leikai 28.50% followed by Huikap Mayai Leikai 23.69%, Khurai Kongpal Chingangbam Leikai 23.45%, Keikhu Makha Leikai 20.13%, Wangkhei Keirubgbam Leikai 17.90% & Langdum Lamkhai 14.89%. The least average % disease severity was found in Khongman Zone 5 with 14.63%.

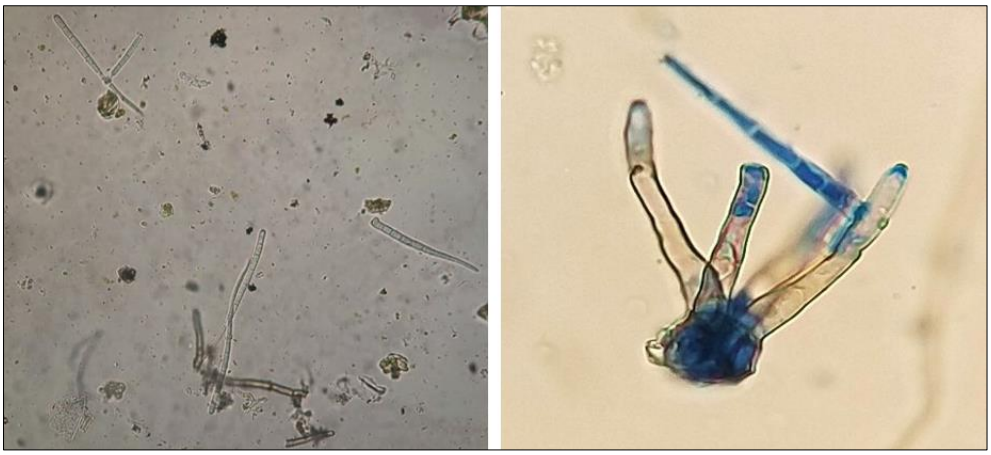
Photograph 1: Disease sample collection



Photograph 2: CLS symptoms on chilli plant. A). Upper leaf surface. B). Lower leaf surface. C). Stalk, D). Calyx. E). Spot coalesces & shot hole effect.

Isolation and identification of the causal pathogen

Diseased samples were collected from the chilli growing areas of Imphal East district of Manipur during the survey. Isolation of causal pathogen were carried out in the laboratory using PDA medium. With repeated isolations, *Cercospora capsici* was consistently found to be associated with infected chilli plants. *Cercospora capsici* isolated from leaf spot of chilli were identified microscopically by their morphological characteristics such as pale brown to olivaceous brown colour culture on PDA medium and septate, smooth, straight or slightly curved conidia as compared with the old culture available in the laboratory Dept. of Plant Pathology, PDDUIAS.



Photograph 3: A) *C. capsici* conidia/asexual spore. B) *C. capsici* Conidiophore bearing conidia

Table 3: Occurrence of *Cercospora* leaf spot disease in Imphal East district

Location	Geographical co – ordinates	DI% 2018-2020	DI% 2019-2020	Avg. DI% (2018-2019 & 2020-2021)	DS% 2018-2019	DS% 2020-2021	Avg. DS% (2018-2019 & 2020-2021
Keirao Bitra Lairembi Leikai	24˚44’42’’ N 93˚59’9.6’’ E	54.62	56.23	55.42	30.27	26.73	28.50
Keikhu Makha Leikai	24˚47’ 13.91 N 93˚58’24.75’’ E	34.51	40.12	37.33	16.12	24.11	20.13
Khurai kongpal Chingangbam Leikai	24˚49’10.24’’ N 93˚58’15.17’’E	34.65	32.21	33.43	22.80	24.10	23.45
Huikap Mayai Leikai	24˚43’35” N 94˚1’56’’ E	44.32	48.30	46.31	22.53	24.86	23.69
Wangkhei Keirungbam Leikai	24˚47’24’’ N 93˚57’8’’ E	28.43	24.61	26.52	20.91	14.89	17.90
Khongman Zone 5	24˚45’59.3338’’ N 93˚57’0.2376’’ E	24.20	28.22	26.21	12.82	16.43	14.63
Langdum Lamkhai	24˚44’22.0596’’ N 93˚59’29.436’’ E	24.15	16.56	20.35	20.93	8.86	14.89

Pathogenicity

It was observed that the fungus isolated from the diseased samples was artificially inoculated on the healthy seedlings of chilli plants could induce disease symptoms after isolation & identification of the causal pathogen. After re-isolation the same pathogen was found and thus proved pathogenicity of *C. capsici.*

Photograph 4: A) Healthy plant. B) Chilli plant showing leaf spot after inoculation of mycelial suspension

***In vitro* evaluation of locally available plant extracts against growth and sporulation of *Cercospora capsici*.**

Among the eight plant extracts evaluated against mycelial growth of *C. capsici* revealed that, under 10% concentration the most effective inhibition was found in garlic with 100% inhibition. The subsequent best fungal inhibition was followed by Ginger (inhibit 69.63%), Darrek (inhibit 68.41%), Tulsi (inhibit 54.07%), Sweet flag (inhibit 53.71%), Neem (inhibit 23.52%), Onion (inhibit 9.63%) and the least inhibition was found in Turmeric (inhibit 5.55%).

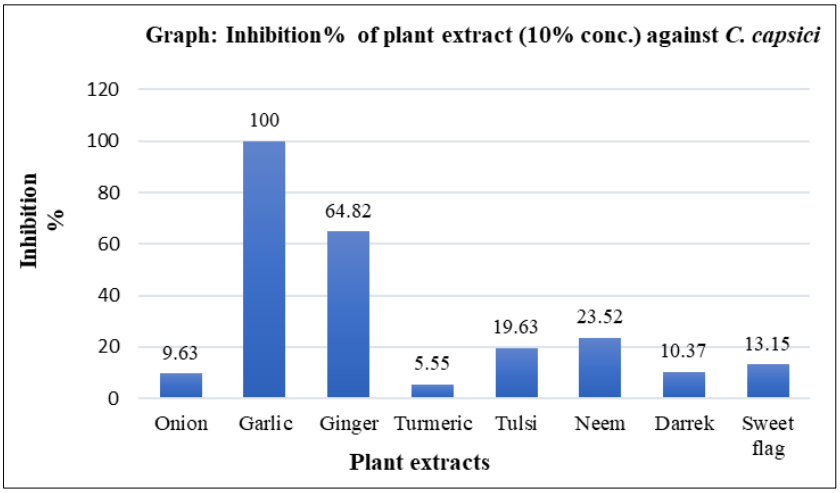
Under 15% concentration the most effective inhibition was also found in Garlic extract with 99.22% followed by the Neem (inhibit 88.71%), Ginger (inhibit 64.82%), Turmeric (inhibit 50.37%), Tulsi (inhibit 19.63%), Sweet flag (inhibit 13.15%), Darrek (inhibit 10.37%) and the least inhibition was observed in Onion (inhibit 10.18%).

Table 4: *In vitro* evaluation of plant extract against *Cercospora capsici* at 10% concentration

Treatment	Mean colony diameter (cm)	Inhibition (%)
Onion	8.13(2.93)	9.63
Garlic	0(0.7)	100
Ginger	3.16(1.92)	64.82
Turmeric	8.5(3.00)	5.55
Tulsi	7.23(2.78)	19.63
Neem	6.88(2.72)	23.52
Darrek	8.06(2.93)	10.37
Sweet flag	7.82(2.16)	13.15
Control	9.00(3.08)	0.00
SEd ±	0.11	
CD (5%)	0.32	

Mean of 3 replications

Figure in () parentheses are square root transformed value $\sqrt{x+0.5}$



Graph 1: Inhibition% of plant extract (10% conc.) against *C. capsici*

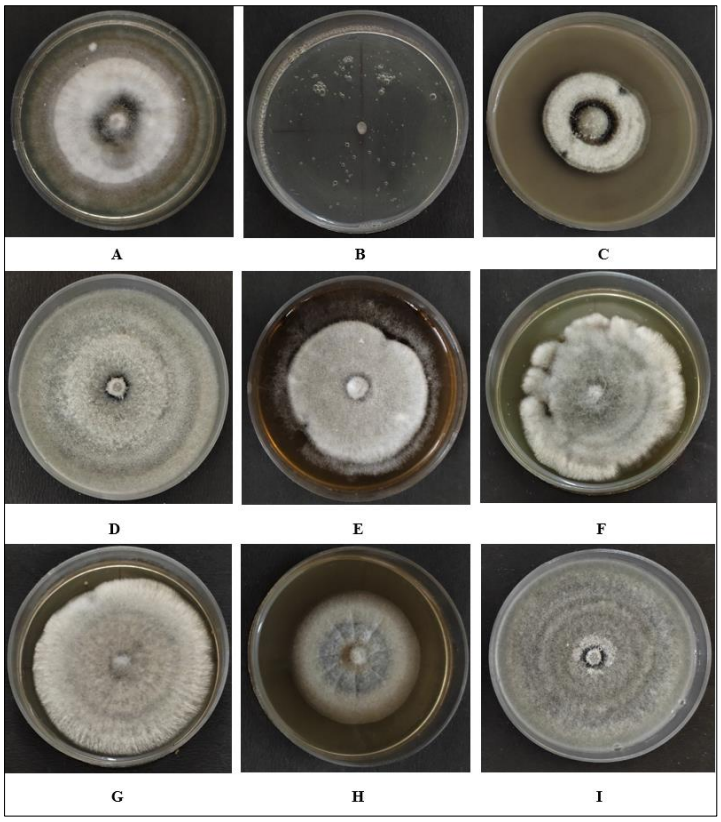


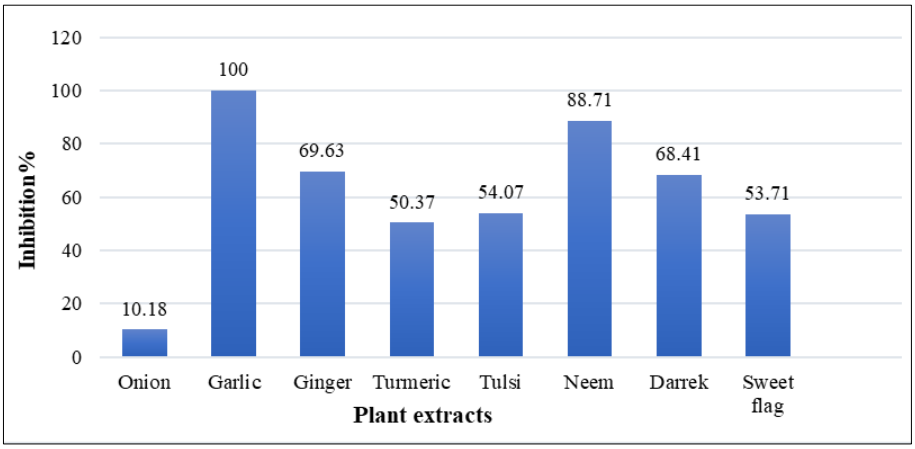
Plate 1: Efficacy of Plant Extract against the growth of *Cercospora* leaf spot of chilli at 10% conc. A) onion. B) Garlic. C) Ginger. D) Turmeric. E) Tulsi. F) Neem. G) Darrek. H) Sweet flag and I) Control.

Table 5: *In vitro* evaluation of plant extract against *Cercospora capsici* at 15% concentration

Treatment	Mean colony diameter (cm)	Inhibition (%)
Onion	8.08(2.93)	10.18
Garlic	0(0.7)	100
Gin	2.73(0.79)	69.63
Turmeric	4.46(2.22)	50.37
Tulsi	4.13(2.15)	54.07
Neem	1.02(1.23)	88.71
Darrek	2.84(1.83)	68.41
Sweet flag	4.16(2.16)	53.71
Control	9.00(3.08)	0.00
SEd ±	0.08	
CD (5%)	0.24	

Mean of 3 replications

Figure in () parentheses are square root transformed value $\sqrt{x + 0.5}$



Graph 2: Inhibition% of plant extract (15% conc.) against *C. capsici*

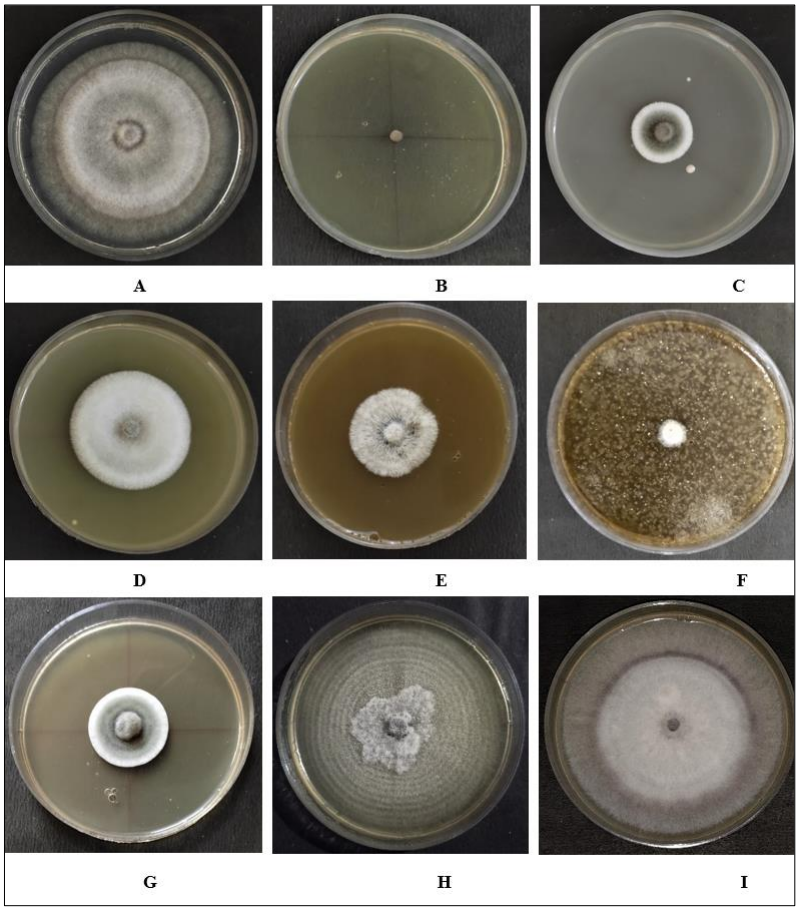


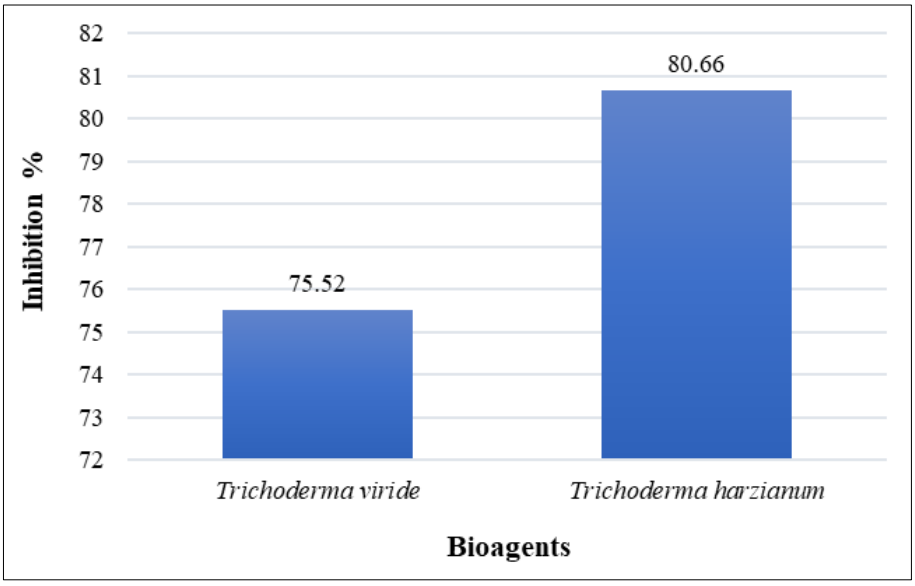
Plate 2: Efficacy of Plant Extract against growth of *Cercospora* leaf spot of chilli at 15% conc.. A) onion. B) Garlic. C) Ginger. D) Turmeric. E) Tulsi. F) Neem. G) Darrek. H) Sweet flag and I) Control.

In vitro* evaluation of biocontrol agent against the growth and sporulation of *Cercospora capsici.

Dual culture technique: The best biocontrol gent against the test fungus was found to be *Trichoderma harzianum* with 80.66% inhibition & *T. viride* was found less inhibition with 75.52% inhibition.

Table 6: *In vitro* evaluation of bioagent against *Cercospora capsici*.

Biocontrol agent	Duration for point of contact (Days)	% Inhibition of mycelial growth	Bell's scale
Trichoderma viride	3	75.52	Class ll
Trichoderma harzianum	3	80.66	Class ll



Graph 3: Inhibition% of bioagents against *C. capsici*

Plate 3: Efficacy of Bioagent against the growth of *C. capsici.* A) *Trichoderma viride.* B) Control plate. C) *Trichoderma harzianum.*

In vitro* evaluation of fungicides against the growth and sporulation of *Cercospora capsici.

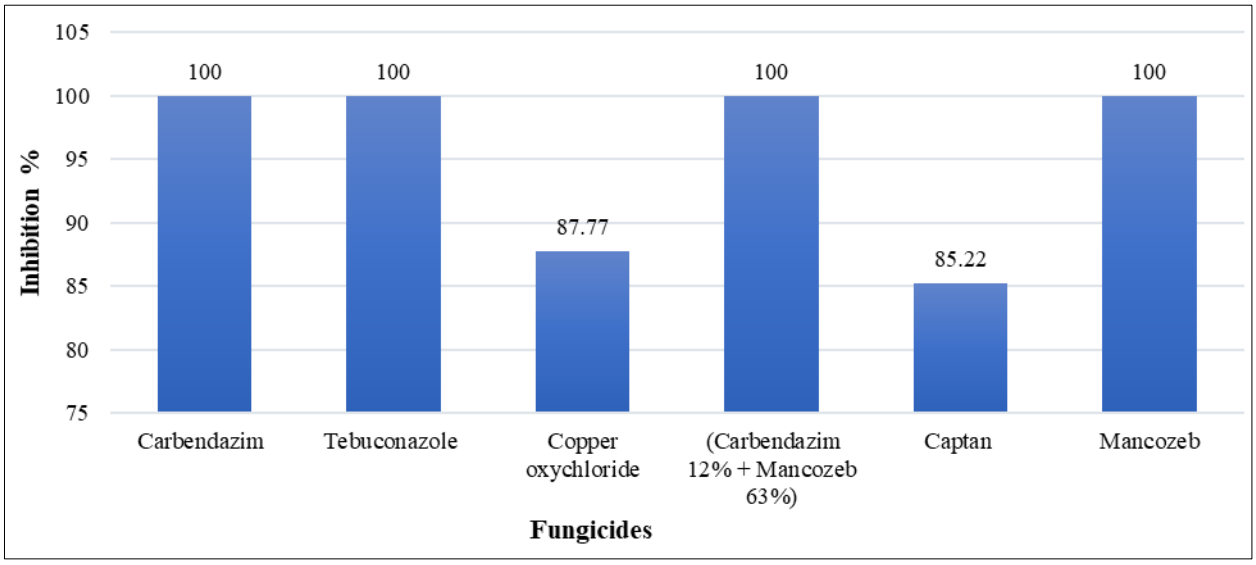
It was observed that 100% inhibition of mycelial growth was found in four fungicides *viz*, Carbendazim at 0.1% conc., Tebuconazole @ 0.2% conc., (Carbendazim 12%+ Mancozeb 63%) at 0.2% conc. & Mancozeb at 0.1% conc. The next best fungicides against the test fungal growth and sporulation were observed as follow, However Copper oxychloride @ 0.2% and Captan @ 0.2% showed 87.77% and 85.22% inhibition over control.

Table 7: *In vitro* evaluation of fungicides

Treatment	Conc. (%)	*Cercospora* Growth (cm)	Inhibition (%)
Carbendazim	0.1	0.00(0.7)	100
Tebuconazole	0.2	0.00(0.7)	100
Copper Oxychloride	0.2	1.1(1.26)	87.77
(Carbendazim 12%+ mancozeb 63%)	0.2%	0.00(0.7)	100
Captan	0.2%	1.33(1.35)	85.22
Mancozeb	0.1	0.00(0.7)	100
Control		9(3.08)	0.00
SEm ±		0.05	
CD 5%		0.16	

Mean of 3 repliactions

Figure in () parentheses are square root transformed value $\sqrt{x + 0.5}$



Graph 4: Inhibition% of fungicides against *C. capsici*

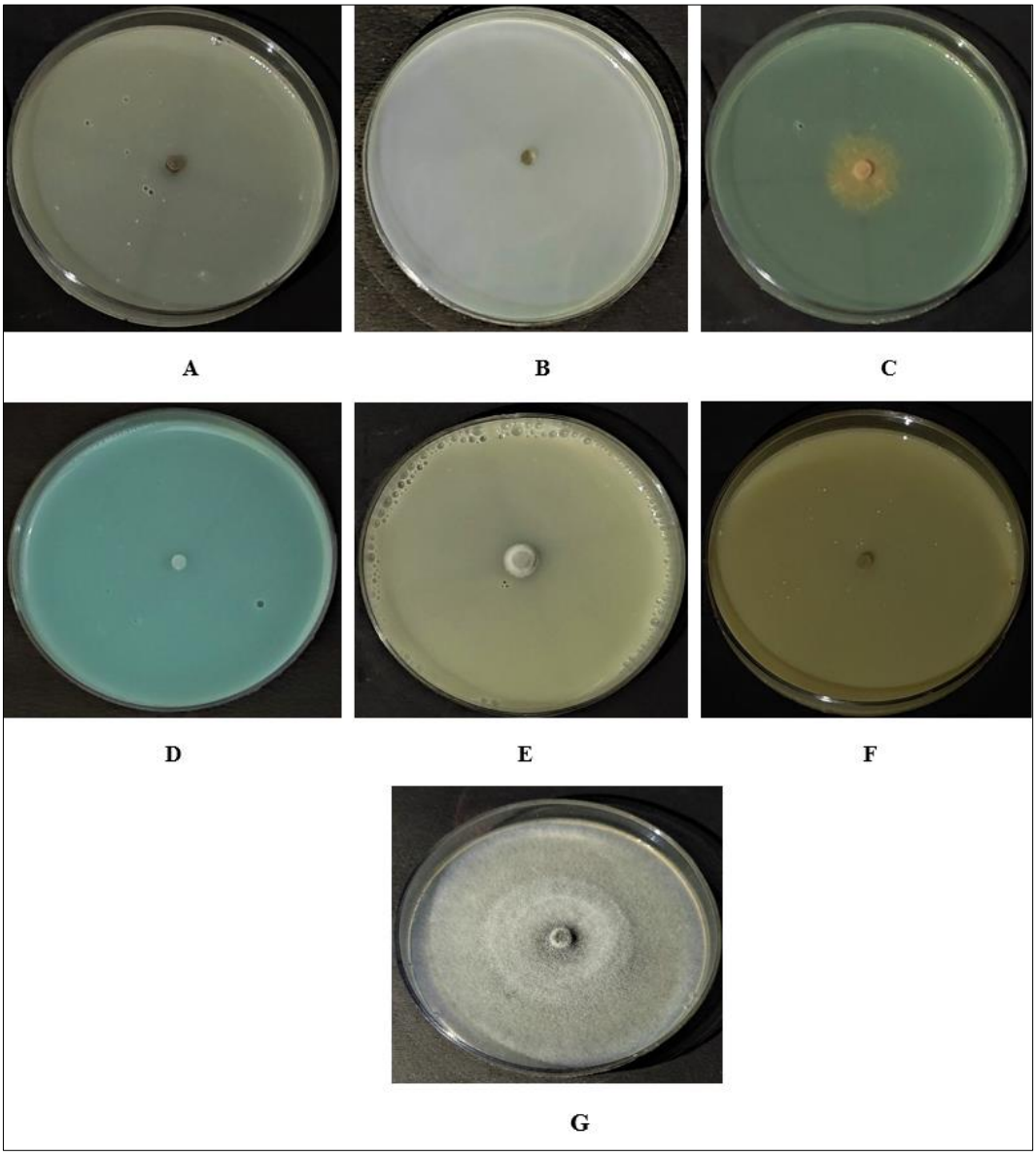


Plate 4: Efficacy of fungicides against the growth of *C. capsici.* A) Carbendazim. B) Tebuconazole. C) Copper oxychloride. D) Carbendazim 12% WP + Mancozeb 63% WP. E) Captan. F) Mancozeb and G) Control.

Discussion & conclusion

Chilli (*Capsicum annum.* L) the wonder spice is one of the cultivated vegetable crops in India. Total green chillies production in 2020-2021 was 4417 MT from 418 ha of land

whereas total dried chillies produced was 2092 MT from 729 ha of land (Anonymous, 2021) [2]. In Manipur the total green chilli production during the year 2016-2017 was 2560 MT. *Cercospora* mainly is seed borne, however, the pathogen is also able to survive for at least one year in plant debris and soil resulting in immense economic loses to the farmers. Average % D.I were observed in Keirao Bitra Lairembi Leikai 55.42% and Langdum Lamkhai 20.35% respectively. Whereas the highest and the least average % D.S were observed in Keirao Bitra Lairembi Leikai 28.50% and Khongman Zone-5 14.63% respectively. In this study, several disease management methods were evaluated against the pathogen *Cercospora capsici. In vitro* evaluation of plant extracts at different conc., (*viz*. 10% & 15%) revealed that under 10% conc. amongst the tested plant extract most effective inhibition was found in garlic with 100% inhibition followed by ginger (64.82%), neem (23.52%), tulsi (19.63%), sweet flag (13.15%), darrek (10.37%), onion (9.63%) & the least inhibition was found in turmeric (5.55%). Under 15% concentration the most effective inhibition was found in garlic with 100% followed by the plant extract of neem (88.71%), ginger (69.63%), darrek (68.41%), tulsi (54.07%), sweet flag (53.71%), turmeric (50.37%) & the lowest disease inhibition was observed in onion with 10.18%. The present findings in agreement with KI Vasava *et al.,* 2020 [35], who tested 8 botanical plant extracts against *C. malayensis* and reported that cent per cent inhibition of mycelial growth over control was recorded in garlic clove extractat 10% concentration. Similarly, Bambode RS *et al.,* 1973, conclude that *Allium sativum* clove showed maximum potential both in aqueous and ethanol extracts showed 100% or more than 90% inhibition of spore germination of all the fungal pathogens.

The result of the biocontrol agent tested in dual culture for their antagonistic activity against the pathogen fungus associated with the diseased sample found that *Trichoderma spp.* Could come in contact with the pathogen within 3 days of incubation. *T. harzianum* inhibited 80.66% (class II) of the fungal mycelium were as *T. viride* inhibited 75.52% (class II). The present findings in agreement with those of Pun M. *et al*., 2020 [49], who reported that *T. harzianum* was effective on percent mycelium growth inhibition of *C. capsici* (*in vitro*) showed maximum inhibition (72.02%) on the pathogen followed by *T. viride* (65.56%) which was found statistically at par with *P. fluorescens* (63.46%). Dipankar Sarkar *et al.,* 2017 [22], also evaluated six bioagent *in vitro* against the growth of the fungus *Alternaria alternata* and found that *Trichoderma harzianum* was most effective inhibiting 76.23%.

The result of the six different fungicides tested against the growth & sporulation of the test fungus observed that absolute inhibition (100%inhibition) of mycelial growth was on par with four fungicides *viz*, carbendazim at 0.1% concentration, tebuconazole at 0.2% conc., SAAF (carbendazim 12% + mancozeb 63% WP) at 0.2% conc. & mancozeb at 0.1% conc. The next best fungicide was Copper oxychloride at 0.2% conc. (inhibit 87.77%) & the least inhibition was found to be Captan at 0.2% conc. (inhibit 85.22%). Similar observation was also reported by Thejakumar, M.B. *et al.,* 2016 [68] who reported that maximum inhibition of mycelial growth over control was recorded in propiconazole 25% EC (100%) which was on par with difenconazole 25% EC (100%), benomyl 50% WP (100%) and mancozeb 63% + carbendazim 12% WP (100%) followed by carbendazim 50% WP (93.87%), mancozeb 75% WP (87.98%), thiophanate methyl 70% WP (78.67%), myclobutanil 10% WP (77.32%), copper oxychloride 50% WP (75.04%) and the least inhibition was observed in copper hydroxide 77% WP (72.10%). A. Ronil kumar, 2017 [1] also suggested that tebuconazole 430 SC (500 ml/ha) is more beneficial and effective in controlling CLS and powdery mildew diseases. Pun M. *et al*., 2020 [50], also reported that hexaconazole was found most effective which inhibited 100% growth of fungus at each concentration which was statistically at par with carbendazim which inhibited 100% radial growth.

Therefore, under this research work it is concluded that the growth and sporulation of *Cercospora capsici* could be absolutely inhibited (100%) with chemical fungicides *viz*., Carbendazim at 0.1% conc., Tebuconazole at 0.2% conc., Carbendazim 12% WP + Mancozeb 63% WP at 0.2% conc. & Mancozeb at 0.1% conc., likewise under botanical extract Garlic can inhibit 100% mycelial growth at both 10% & 15% conc. whereas the bioagent *T. harzianum* can control the pathogen *C. capsici* significantly. Even though the chemical fungicides were very effective, reducing or substituting such harmful fungicides with effective biocontrol agents or plant extract could be used as a component of IDM in the advancing Organic farming in the state. Hence further research work may be taken up in this condition.

References

1. Ronil Kumar, Singh BK, Singh AK, Moharana DP, Singh RP. Bioefficacy studies of new fungicidal formulation against *Cercospora* leaf spot and powdery mild ew of chilli (*Capsicum annum* L.) cv. Punjab Guchhedar. Res. On Crops. 2017;18(4):758-761. Doi: 10.5958 / 2348-7542.2017.00124.3.
2. Anonymous. Agriculture Statistics at a glance www.agricoop.nic.in 2021
3. Bambode RS, Shukla VN. (1973). Antifungal properties of certain plant extracts against some fungi. P.K.V. Res.J 2(1):1-8.
4. Bell D, Well H, Markham C. *In vitro* antagonism of *Trichoderma* species against six fungal pathogens. Phytopathology. 1982;73:379-382. Doi; 10.1094-72-379.
5. Bhat FA, Dar DM, Teli MA, Ahmad MF. Frogeye leaf spot of bell pepper in Kashmir: prevalence and cause. Karnataka Journal Agricultural Science. 2008;21(3):460-461
6. Bosland PW, Votava EJ. Peppers: Vegetables and Spices Capsicums. CABI, New York, 2000, 14.
7. Braun, 1996. Taxonomic notes on some species of the *Cercospora* complex (iv). Sydowia. 1996;48:205-217.
8. Brhan Khiar Saleh, Abdella Omer, Belay Teweldemedhin. Medicinal uses and health benefits of chilli pepper (*Capsicum spp*.): a review. MOJ FPT. 2018;6(4).
9. Cerkauskas R. Pepper Diseases: *Cercospora* Leaf Spot Published by AVRDC- The World Vegetable Center, Shanhau, Taiwan, 2004, 741.
10. Chandrashekhran S, Rangaswami G. studies on *Cercospora cruenta* occurring on Vigna catjang. Indian Phytopath. 1960;13:96-99.
11. Chuup C. A monograph of fungus Genus Cercospora, Ithaca. New York, 1953, 667.
12. Crous, Braun. Mycosphaerella and its anamorphs: 1. Names published in *Cercospora* and *Passalora.* CBS Biodiversity Series. 2003;1:1-569
13. Crous PW, Braun U, Groenewald JZ. Mycosphaerella is

polyphytic. Studies in Mycology. 2007;58:1-32.
14. Culbreath AK, Stevenson KL, Brenneman TB. Management of leaf spot diseases of peanut with Prothioconazole applied alone or in combination with Tebuconazole or Trifloxystrobin. Peanut Sci. 2008;35(2):149-158.
15. Dale WI. The Rainfall of Malaya, Part I. In Readings on the Climate of West Malaysia and Singapora, ed. Ooi jin Bee and Chia Lin Sien. Singapore: OUP, 1974.
16. Daub ME. Cercosporin. a photosensitizing toxin from *Cercospora species*, Phytopathology. 1982;72:370-374
17. Daub ME, Ehrenshaft M. The photoactivated *Cercospora* toxin cercosporin: contributions to plant disease and fundamental biology. Annu. Rev. Phytopathol. 2000;38:461-490.
18. Dayal R, Ram A. Phytopathological studies of *Cercospora jasminicola* Muller & Chupp. II. Nitrogen requirements. Proc. Nat. Acad. Sci., India. Sect. B. 1968;37:293-298.
19. Devappa V, Thejakumar MB. Integraed management of chilli leaf spot caused by *Alternaria alternata* and *Cercospora capsici* under field conditions. Internat. J. Adv. Res. 2016;4(4):1468-1474.
20. Devi Datt Joshi, Sapu Changkija, Wangkheirakpam Sujata and Bharat Gopalrao Somkuwar. Characterization of Pungent Red Chillis (*Capsicum spp*.); Cultivar of Manipur and Nagaland, part of Indo Burma Mega Biodiversity Hotspot. BEPLS. October 2018;7(11):116-126.
21. Dharendra Reang Khalko S, Roy A. Formulation of effective chemical management Strategy against Cercospora leaf Spot Disease of Chilli. IJCMAS. 2018;7(10):1240-1245. ISSN: 2319-7706 (online).
22. Dinesha LG. Studies on grey leaf spot of sorghum (*Sorghum bicolour* L.) caused by *Cercospora sorghi* Ell. and Ev. M. Sc. (Agri) Thesis, Univ. Agric. Sci., Bangalore (India), 1984.
23. Taoe DNA, Nyarko HD, Akpaka R. A Comparison of antifungal Properties of Onion (*Allium cepa*), Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*) against *Aspergillus flavus*, *Aspergillus niger* and *Cladosporium herbarum*. Res. J. Med. Plant. 2011;5:281-287.
24. Dipankar Sarkar, Barhate BG, Joshi VR. Studies on leaf spot of chilli. Internat. J. Plant Protec. oct 2017;10(2):369-374.
25. Faheem A, Razdan VK, Mohiddin FA, Bhat KA, Sheikh PA. Effect of volatile metabolites of *Trichoderma species* against seven fungal plant pathogens *in vitro*. J Phytopathology. 2010;2:34-37.
26. Faroog Ahmad Bhat, Mushtaq Ahmad Tell, Qazi Nissar Ahmad, Shahzad Ahmed. Host range and epidemiology of *Cercospora capsici*. IJPS, January to June 2009;4(1):44-48.
27. Franc GD, Harveson RM, Kerr ED, Jacobsen BJ. Disease management in sugarbeet production. M.Sc. Thesis University Nebraska Coop, 2001, 131-160.
28. Fresenius JB. *Cercospora* Fresenius Beitr. Mykol. 1863;3:91-93.
29. Galanihe S, Jorge S, Michl D, Ridif RK. Evaluation of Benomyl and Hexaconazole against leaf blight of chilli. Agron, Sustainable Dev. 2015;35:317-324.
30. Groenewald JZ, Nakashima C, Nishikawa J, Shin HD, Park JH, Jama AN, *et al*. Species concepts in *Cercospora:* Spotting the weeds among the roses. Stud. Mycol., 2013;75:115–170.
31. Heald FD, Wolf FA. New species of texas: Fungi. Mycologia. 1911;3:5-22.
32. Izge AU, Mohammed ZH, Goni A. Levels of variability in groundnut (*Arachis hypogaea* L.) to *Cercospora* leaf spot disease- implication for selection. Afr. J. Agric. Res. 2007;2(4):182-186.
33. Jackson LF. Distribution and severity of peanut leafspot in Florida. Phytopathology. 1981;71:324-328.
34. Kamal. Cercosporoid fungi of India. Bishen Singh Mahendra Pal Singh, Dehradun India, 2010, 351.
35. Khan AA, Khan RU, Singh R. Management of *Cercospora* Leaf spot and anthracnose diseases of mungbean by fungicides, Ann. Plant Sci. 2005;13(2):514-515.
36. Kathrine Xin Yee tan, Azlinda Binti Ibrahim and Hideyuki Nagao., 2020, effect of culture medium and lighting condition on induction of conidiation in Cercospora citrullina. Songklanakarin J. Sci. Technol. 42(4), 935-940, 2020.
37. Khan ZH, Qadir I, Yaqoob S, Khan RA, Khan MA. Response of range grasses to salinity levels at germination and seedling stage. J. Agric. Res. (Lahore). 2009;47(2):179-184.
38. Vasava KI, Patel PR. *In vitro* evaluation of various botanicals against *Cercospora malayensis* causing leaf spot of okra. JPP. 2020;9(5):2645-2648.
39. Landers KE. Growth of *Cercospora arachidicola* in glucose phosphate asparagine's thiamine agar medium. Phytopath. 1964;54:1236-1239
40. Kumar ND, Karegowda C, Murali R, Sayiprathap BR, Mahesh M, Nagaraj H, *et al*. Frogeye Leaf Spot disease of FCV Tobacco caused by Cercospora nicotianae in Southern Districts of Karnataka. J Pure Appl Microbiol. 2016;10(1):401-406.
41. Lily VG, Barnett HL. Physiology of the fungi. Mc GrewHill Book Company Inc., New York, 1951.
42. Lim YS, Kim BS. Sporulation of *Cercospora Capsici* causing *Cercospora* leaf spot of pepper. Res. Plant Dis. 2003;9(3):162-165.
43. Meah MB, Khan AA. Check List of fruit and vegetable disease in Bangladesh. 1st ed. Department of Plant Pathology, Bangladesh Agricultural University, Mymen Singh, Bangladesh, 1987, 13.
44. Islam MS, Fatema K, Alam KMB, Meah MB. Diagnosis and Prescription for *Cercospora* leaf spot of chilli. J Bangladesh Agril. Univ. 2015;13(2):191-196.
45. Mahamuda Begum M, Teresita U. Dalisay and Christian Joseph R. Cumagun. Taxonomic Review of and Development of Lucid Key for Philippine Cercosporoids and related fungi. Research Gate, 2012. DOI 10.5772/30214.
46. Megha Pun, Vijay Kumar, Anand Shubham Pandey. Growth Study of *Cercospora capsici* Causing Leaf Spot Disease of Chilli on Different Media, pH and Temperature. IJSR, 2018. ISSN: 2319-7064.
47. Nagel CM. Conidial production in species of *Cercospora* in Pure Culture. Phytopathology. 1934;24:1101-1109.
48. Naresh Kumar, Sunil Kumar, Satyadev Prajapati, Shivam Maurya. *Cercospora* leaf spot disease of greengram and its management: a review. J Pharmacogn Phytochem. 2020;9(1):1574-1576. E-ISSN: 2278-4136.
49. Oluwafemi Adedire M, Ayotunde Pitan, Adekunle Farinu O, Wuraola Ogundipe F. The Biocontrol of Soil

Transmitted *Cercospora capsici* with Lactobacillus plantarum. Journal of Advances in Microbiology. 2019;18(3):1-8. Article no. JAMB.51763 ISSN: 2456-7116.
50. Pinky Chanu Laiphrakpam. Evaluation of antibiotics and bactericides against the bacterial wilt of chilli through *in vitro* condition. The Pharma Innovation. 2020;9(10):148-151.
51. Poornima Yashodhara RH, Prashanthi SK, Nargund VB, Venugopal CK. Antifungal effect of botanicals against *Cercospora beticola*, the incitant of leaf spot of palak. Karnataka J Agric. Sci. 2011;24 (4):575-576.
52. Pun M, Kumar V, Bhist SS, Upadhyay S. Efficacy of fungicides and biocontrol agents in the management of *Cercospora* leaf spot of chilli. J Bio.Innov. 2020;9(2):141-148. 2020 | ISSN 2277-8330 (Electronic).
53. Pun M, Kumar V, Bisht SS, Upadhyay S. Efficacy of Fungicides and Biocontrol Agents in the Management of *Cercospora* Leaf Spot of Chilli. J Bio. Innov. 2020;9(2):141-148. 2020 | ISSN 2277-8330 (Electronic).
54. Purkayastha J, Alam SI, Gogoi HK, Singh L, Veer V. Molecular characterization of 'bhut jolokia' the hottest chilli. Journal of biosciences. 2012;37:757-768.
55. Rangaswami G. Diseases of Vegetables. In: Diseases of crop Plants in India. 2nd Ed. McGraw Hill Book Company, New Delhi, 1979, 345-347
56. Rao VCS, Rao GVK. An insight into chilli cultivation and risk management procedures with special reference to Karnataka and Andhra Pradesh. Int. J Business and Admin. Res. Rev. 2014;2:144-55.
57. Reena Pundir, Ruby Rani, Shivi Tyagi, Poonam Pundir. Advance Review on Nutritional Phytochemical, Pharmacological and Antimicrobial Properties of Chilli. Int. J Ayurveda Res. 2016;4(4):ISSN: 2322-0902 (P).
58. Saccardo PA. Fungi veneti novi vel criti. Series V. *Nouvo Giorn.* Bot. Ital. 1876;8:161-211.
59. Sarkar Dipankar, Barhate BG, Joshi VR. Studies on leaf spot of chilli. Int. J Plant Protec. 2017;10(2):369-374.
60. Sariah Meon. Infection of chilli by *Cercospora capsici*. Pertanika. 1990;13(3):321-325.
61. Segnou J, Amougou A, Youmbi E, Njoya J. Effect of Chemical Treatments on Pests and Diseases of Pepper (*Capsicum annuum* L.). Greener J Agric. Sci. 2013;3(1):12-20
62. Selvakumar R. A textbook of Glaustas Olericulture. New Vishal Publications. New Delhi, 2014, 255-256.
63. Sharma SK. Efficacy of different fungi toxicant against frogeye leaf spot of bell pepper. Plant Dis. Res. 1998;13:62-63.
64. Siddaramaiah AL, Desai SA, Hegde RK. Studies on estimation of loss due to rust and tikka of groundnut, Mysore J Agric. 1983;17:365-367.
65. Singh UB. Studies on *Cercospora indica* parasitic on Cajanus indicus species. Indian J Agri. Sci. 1934;4:343-360.
66. Subash N, Meenakshi, Sundaram M, Sasikumar C. *In vitro* evaluation of different strain of *Trichoderma harzianum* as bio control agent of chilli. IJBPAS. 2013;2(2):495-500.
67. Thejakumar MB, Devappa V. Efficacy of different fungicides against *Alternaria alternata* and *Cercospora capsici* under invitro condition. IJARBS. 2016;3(5):126-129, ISSN: 2348-8069.
68. Urszula 'Swiderska- Burek, Margaret E Daub, Elizabeth Thomas, Magdalena Jaszek, Anna Pawlik, Grzegorz Janusz. Phytopathogenic Cercosporoid Fungi – From Taxonomy to Modern Biochemistry and Molecular Biology. Int. J. Mol. Sci. 2020;21:8555.
69. Vasudeva RS. Indian Cercosporae. IARI, New Delhi, 1963, 254.
70. Veer Singh, Neeraj Kumar Rajvanshi, Subhash Chandra, Susheel Kumar. To evaluate the efficacy of botanicals and chemical against *Cercospora* leaf spot of Sarpagandha (*Rauvolfia serpentine* L.) Benth Ex Kurz. *In vivo*. IJCS. 2019;SP6:732-734.
71. Vincent JM. Distortion of Fungal hyphae in presence of certain inhibitors. Nature, 1947, 159: 850.
72. Vivekananda Ravi S, Mishra RC, Bahugauna P. Evaluation of Various Management Techniques against Anthracnoses, *Colletotrichum capsici* (Sydow) in Western Himalayan Zone of Uttarakhand, Int. J. Pure App. Biosci. 2018;6(2):861-867.
73. Walker JC. Diseases of Vegetable crops. Inc. 1st Ed. McGraw Hill Book Company. New York, USA, 1952, 306-308.
74. Weiland JJ, Chung KR, Suttle JC. The role of cercosporin in the virulence of *Cercospora spp*. to plant hosts. In: *Cercospora* leaf spot of sugar beet and related species (Lartey, R. T., Weiland, J. J., Panella, L., Crous, P. W., Windels, C. E. Eds.). APS Press, Minnesota USA. 2010, 39-53.
75. William MAJ. CMI descriptions of pathogenic fungi and bacteria. *Cercospora zonata*, No. 939. CAB International. *Martinus Nijhoff /* Dr. W. Junk Publishers, Dordrecht, the Netherlands, 1987, 185-186.
76. Windels CE, Lamey HA, Hilde D, Widner J, Knudsen T. A *Cercospora* leaf spot model for sugar beet. Plant Dis. 1998;82:716-726.
77. Yu TF. *Cercospora* leaf spot of broad bean. Phytopathology. 1947;37:174-179.
78. Zhani K, Hermans N, Ahmad R, Hannachi C. Evaluation of Salt Tolerance (NaCl) in Tunisian Chili Pepper (*Capsicum frutescens* L.) on Growth, Mineral Analysis and Solutes Synthesis. J. stress physiol. Biochem. 2013;9(1):209-228.