International Journal of Bio-resource and Stress Management https://ojs.pphouse.org/index.php/IJBSM

Crossref

IJBSM February 2024, 15(2): 01-09 **Article** AR5026a

Research Article *Stress Management*

DOI: HTTPS://DOI.ORG/10.23910/1.2024.5026a

Incidence, Epidemiology and Management of *Cephaleuros virescens* Causing Red Rust of Litchi under Sub Tropical Zone of Himachal Pradesh

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ABSTRACT

The present investigation was carried out during year 2020–2021 at the laboratory of Department of Plant Pathology, College of Horticulture and Forestry, Neri (Hamirpur) Himachal Pradesh, India to study the disease incidence, severity and management of red rust of litchi incited by *Cephaleuros virescens* under *in vitro* conditions. The survey was conducted in major litchi growing areas which revealed that the disease was prevalent in mild to severe form at various locations in five districts (Hamirpur, Una, Kangra, Chamba and Mandi) surveyed in Himachal Pradesh. Disease incidence was found to be ranging from 0 to 35.47% with maximum at Nagrota Bagwan in Kangra district and disease severity ranged from 0 to 47.79%. Epidemiological studies reveal that *C. virescens* can be incubated at all the temperatures tested (20, 25, 28, 30, 32 and 35°C) and at all the tested RH levels (80.5, 85.7, 89.9, 95.6 and 100%). However, temperature 32°C and RH 100% was found to be optimum for symptom development of disease under artificial inoculation conditions.. Among the fungicides tested (copper oxychloride, captan, mancozeb, potassium phosphite, potassium phosphate, Cabrio top®, azoxystrobin, carbendazim, hexaconazole and propiconazole) at different concentrations, all treatments were significantly superior over control in managing *C. virescens*. However, captan, mancozeb, Cabriotop, hexaconazole and propiconazole resulted in cent per cent inhibition of *C. virescens*.

KEYWORDS: *Cephaleuros virescens*, incubation, temperature, relative humidity, management

***Citation* (VANCOUVER):** Garima et al., Incidence, Epidemiology and Management of *Cephaleuros virescens* Causing Red Rust of Litchi under Sub Tropical Zone of Himachal Pradesh. *International Journal of Bio-resource and Stress Management,* 2024; 15(2), 01-09. HTTPS://DOI.ORG/10.23910/1.2024.5026a.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 23th November 2023 RECEIVED in revised form on 29th January 2024 ACCEPTED in final form on 12th February 2024 PUBLISHED on 26th February 2024

1. INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is the most important sub-tropical evergreen tree belonging to the soapberry family *Sapindaceae* and sub family Nephelaceae (Mitra, 2002). The genus, *Litchi*, has two species, *Litchi philippinensis* and *Litchi chinensis*, usually known as Pearl of India and 'Queen of fruits' (Mehta, 2017). The litchi is a non-climacteric fruit of Southeast Asia (Koul and Singh, 2017) and isbelieved to be originated in China and introduced to Burma and India by the end of the seventeenth century (Anal et al., 2017; Lal et al., 2022).

Litchi fruit contains 15.9% seed, 9.6% pericarp and 74.5% edible portion (Ghaffoor et al., 1999). Litchi pulp contains proteins, lipids, carbohydrates, vitamins and phytonutrient flavonoids. It also contains minerals like manganese, magnesium, copper and iron (Sarkar et al., 2018). India is the second largest producer of litchi in the world after China with an annual production of 727.0 thousand MT from 95.0 thousand ha area with a productivity of 7.7 MT per ha during 2018–19 (Anonymous, 2019). Bihar has the largest area and highest production in litchi cultivation in India (Anonymous, 2018). Bihar occupies 40% of the area with 45% of litchi production in India (Gupta et al., 1997). In Himachal Pradesh, litchi covers about 6.179 thousand ha area with a production of 4.605 thousand mt (Anonymous, 2020).

Diseases are one of the constraints in the fruit production of litchi as they directly or indirectly reduce the yield and quality of fruit (Anal et al., 2017). Red rust disease is the one which is caused by alga *Cephaleuros virescens* (Fang et al., 2021). *C. virescens* is the incitant of leaf, stem and fruit diseases on economically important tropical plants such as litchi nut (Vasconcelos et al., 2019). Three species of *Cephaleuros* have been reported in India *viz.*, *C. parasiticus* Karsten on *Camellia sinensis* (L.) O. Kuntze in tea plantations (Ramya et al., 2013), *C. solutus* Karsten on *Pyrus* sp. in Varanasi and *C. virescens* Kunze on various host plants from various districts (Gokhale and Shaikh 2012; Suto et al., 2014). *C. virescens* is the most widespread and common species among the genus (Brooks et al., 2015; Sanahuja et al., 2018). The infections caused by *Cephaleuros* species are often referred to as red rust or algal rust and are often confused with the rust disease caused by fungi (Muthukumar et al., 2014; Scott, 2017). Algal leaf spot is a threat during nursery crop production, as it can cause primary (direct) and also lead to secondary (indirect) infection (Browne et al., 2019). *Cephaleuros* species are filamentous green algae widely known as a parasite of higher plants (Sangeetha et al., 2022; Syeful et al., 2022). The genus *Cephaleuros* belongs to the division of aquatic green algae *Chlorophyta*, class *Ulvophyceae*, order *Trentepohliales* and family *Trentepohliaceae* (Patrice et al., 2020). They need free water to germinate (Sunpapao et al., 2016). Recently, algae in the genus *Cephaleuros* were considered novel as plant pathogens (Bunjongsiri and Sunpapao, 2018). In India, *C. virescens* commonly infect mango and litchi leaves (Kumar et al., 2019). The first incidence of red rust of litchi from Sulah and adjoining areas of Kangra was recorded in 1981 and it has been identified as a major constraint at 750–950 metres elevation (Gupta et al., 1997). The harmful effects of the algal presence were attributed to (a) depletion of water, nitrogen, amino acids, sugar and other essential biochemical constituents from the host tissues (Wolf, 1930; Chanthapatchot and Satjarak, 2019), (b) secretion of harmful algal metabolites (Joubert and Rijkenberg, 1971), and (c) loss of photosynthetic area (Safeeulla and Govindu, 1948; Pereira et al., 2020). Keeping these in mind, the present investigation was undertaken to evaluate the disease incidence, severity and management of red rust of litchi incited by *Cephaleuros virescens*.

2. MATERIALS AND METHODS

2.1. Incidence of red rust of litchi

Important litchi growing areas in Kangra, Hamirpur, Una, Mandi and Chamba districts of Himachal Pradesh were surveyed during 2020-2021 for recording the prevalence of red rust disease of litchi in terms of disease incidence and severity.

The % disease incidence was calculated by using the following formula given by Mayee and Datar (1985):

Disease Incidence (%)=(Number of infected leaves/Total number of leaves sampled)×100

Disease severity was recorded on the basis of 0–4 scale as follows:

Grade	Description
0	No symptoms
1	Up to 25% of leaf area infected
2	Up to 50% of leaf area infected
3	Up to 75% of leaf area infected
4	>75% of leaf area infected

Disease severity/ index was further calculated by following the formula given by McKinney (1923).

Disease severity index (%)=(Sum of all disease ratings/Total number of ratings×Maximum disease grade)×100

The samples were collected from the litchi growing areas during survey on the basis of visual symptoms of red rust disease. Culture of *C. virescens* was isolated from the infected samples. Based on cultural and morphological

characteristics, the isolate was identified as *C. virescens*. The pure culture was transferred on PDA slants and maintained for further studies.

2.2. Epidemiological studies- temperature and relative humidity regimes on disease development

The experiment on epidemiology of the disease was conducted under *in vitro* conditions. To check the best temperature and relative humidity (RH) levels for disease development, a twig consisting of 5 to 6 healthy litchi leaves was taken and five mm diametric area was scrapped on leaves. This scrapped area was inoculated with five mm disc of algal spot from infected leaves while, 1 or 2 leaves were left uninoculated as a control and incubated in desiccators at different temperature regimes *viz.,* 20, 25, 28, 30, 32 and 35°C by maintaining different relative humidity levels *viz.,* 80.5, 85.7, 89.9, 95.6 and 100% adjusted with the help of method given by Stevens (1916). Each treatment was replicated three times and data were recorded in terms of incubation period (days) and ratings for severity were given to the leaves which exhibited symptoms as per the scale (Table 1) given below:

Table 1: Scale used for rating disease severity

Scale	Description
0	Very light brown scrapped area on adaxial side and light beige colour spot on abaxial side with no peculiar symptom
1	Light brown scrapped area exhibiting mild algal growth on adaxial side while, watery spot on the abaxial side
2	Light brown colour scrapped area with mild algal growth on adaxial side while, light brown watery spot on abaxial side
3	Rust coloured spots with algal growth on adaxial side and reddish spot on abaxial side
4	Orange to dark brown spots on the adaxial surface exhibiting algal growth while pale brown spots on abaxial surface of leaves.

Disease severity / index was further calculated as mentioned above

2.3. Management studies

Ten chemicals *viz.,* copper oxychloride, captan, mancozeb, potassium phosphite, potassium phosphate (all tested at 500, 1000, 1500 and 2000 ppm) Cabrio top®, azoxystrobin, carbendazim, hexaconazole and propiconazole (all tested at 125, 250, 375 and 500 ppm) were evaluated *in vitro* for their efficacy against *C. virescens* by using poisoned food technique (Falck, 1907) and compared with untreated control. The Petri plates containing respective concentration of the test chemical in Host Leaf Extract were inoculated with the pathogen culture bits of 5 mm diameter and incubated at 25°C temperature until the untreated control plates were completely filled with the mycelial growth of the alga. Data were recorded in terms of diametric growth of the alga (mm) and growth inhibition (per cent) in relation to untreated control was further calculated on the basis of formula given by Vincent (1947) as follows:

Inhibition (per cent)=(C-T/T)×100

Where,

C=Diametric growth of the pathogen in control

T=Diametric growth of the pathogen in treatment

2.4. Data analysis

Laboratory experiments were conducted in completely randomized design with three to four replications in each treatment and results were statistically analysed by using online programme OPSTAT (Sheoran et al., 1998).

3. RESULTS AND DISCUSSION

3.1. Incidence of red rust of litchi

The symptoms on leaves (Figure 1) were recorded as orange, rust coloured, velvety spots which later led to curling of leaves in the presence of mites. The symptoms were not seen on the fruits. The algal attack caused reduction in photosynthetic activity and defoliation of leaves thereby lowering vitality of the host plant.

Figure 1: Symptoms of red rust of litchi in different orchards

The data presented in Table 2 clearly depict that the disease was prevalent in mild to severe form at various locations surveyed. In Kangra district, maximum disease incidence (35.47%) was recorded at Nagrota Bagwan. The incidence of disease ranged between 0 to 35.47% depending upon the climate and micro climate of the plants being minimum (0.60%) at Ghuggar in Kangra district. However, no incidence of red rust disease was found in Gummer, Baroh, Puhara and Ramnagar areas of Kangra and Una districts. As far as disease severity was concerned, it ranged between 7.37–47.79% at various locations surveyed being

Table 2: Occurrence of red rust of litchi at different locations in Himachal Pradesh

District	Area	Disease incidence (%)	Disease severity (%)
Hamirpur	Neri	6.00	16.67
	Bhota	24.28	20.83
	DeraParol	3.00	7.37
	Jahu	5.00	10.67
Una	Mehatpur	20.02	18.01
	Amb	7.20	13.30
	Ispur	11.12	20.15
	Ramnagar	0.00	0.00
Mandi	Pingla	22.00	16.00
	Dharampur	10.00	25.17
	Sarkaghat	9.00	28.57
	Gopalpur	11.00	18.12
Kangra	Gummer	0.00	0.00
	Baroh	0.00	0.00
	Pharer	21.75	11.11
	Faddu	23.75	21.15
	Nagrota Bagwan	35.47	47.79
	Puhara	0.00	0.00
	Indora	2.57	15.00
	Jachh	1.87	10.94
	Dramman	5.00	9.62
	Shahpur	2.60	12.50
	Ladwada	2.50	8.33
	Daulatpur	4.00	8.33
	Icchhi	1.40	12.50
	Gaggal	1.60	10.42
	Ghuggar	0.60	8.33
	Laddhi	5.00	15.63
Chamba	Balana	2.20	11.67
	Hatli	1.80	8.33
	Kamla	3.20	13.33
	Malara	2.50	16.67
	Sihunta	3.00	12.50

maximum at Nagrota Bagwan (47.79%) in district Kangra and minimum (7.37%) at Dera Parol in Hamirpur district. The visualised symptoms were similar to those explained by Pitaloka et al. (2015) and Kumar et al. (2019). Further the explanation of symptoms by McMillan (1994) also supports our observations. The results of disease incidence in Himachal Pradesh were supported by Gupta et al. (1997) who conducted survey of litchi orchards in Kangra Valley of Himachal Pradesh and revealed that red rust problem was more prevalent at 750–950 m altitude where infestation ranged from 50–90%.

3.2. Epidemiological studies

3.2.1. Effect of different temperature and RH levels on incubation period of Cephaleuros virescens

Irrespective of temperature regimes under study, the shortest mean incubation period (46.94 h, Table 3) was recorded at 100% RH followed significantly by that at 95.6 (57.67 h) and 89.9 (70.44 h) % RH. However, longest mean incubation period (83.22 h) was recorded at 80.5% RH which was statistically at par with that recorded at 85.7 (80.50 h)% RH.

Irrespective of different RH levels under study, the shortest mean incubation period (41.20 h) was recorded at 32°C followed significantly by that at 35°C (48.53 h) and 30°C (55.13 h). Whereas, the longest incubation period (104.60 h) was recorded at 20°C followed significantly by that at 25 (85.93 h) and 28°C (71.13 h).

Table 3: Effect of different temperature and RH levels on incubation period of *Cephaleuros virescens*

RH	Mean incubation period (hours) at temperature (°C)						OM
	20	25	28	30	32	35	
80.5	113.33	100.67	95.67	65.67	52.00	72.00	83.22
85.7	110.67	100.67	92.00	65.33	48.67	65.67	80.50
89.9	104.33	95.67	71.67	55.00	47.67	48.33	70.44
95.6	104.33	82.00	49.33	48.00	30.33	32.00	57.67
100	90.33	50.67	47.00	41.67	27.33	24.67	46.94
OM	104.60	85.93	71.13	55.13	41.20	48.53	
Factors		CD $p \geq 0.05$			SE(d)		
RH		2.88			1.44		
Temperature		3.16			1.58		
Interaction		7.06			3.52		

RH: Relative humidity (%); OM: Overall mean

Interaction of different temperature and relative humidity regimes revealed that incubation period was significantly minimum (24.67 h) when incubated at 35°C temperature and 100 RH which was statistically at par with that at 32°C and 100 RH (27.33 h). However, longest incubation period (113.33 h) was recorded when incubated at 80.5 RH and 20°C temperature which was statistically at par with that when incubated at 85.7% RH and 20°C (110.67 h). An intermediate range of incubation period was recorded at

rest of the temperature and RH combinations.

3.2.2. Effect of different temperature and RH levels on severity of red rust in litchi

As far as disease severity (%) was concerned (Table 4), irrespective of the temperature regimes under study, minimum disease severity (23.37%) was recorded at 80.5% followed significantly by 85.7 (29.12 %) and 89.9% (33.78%) RH. However, maximum disease severity (55.08%) was recorded at 100% RH followed significantly by 95.6% (37.29%) RH.

Irrespective of different RH levels, significantly minimum disease severity (24.87%) was recorded at 20°C followed significantly by that at 25 (30.87%) and 28°C (35.37%). However, significantly maximum disease severity (45.40%) was recorded at 32°C followed by that at 30 (39.98%) and 35°C (37.87%).

Interaction reveals that minimum and equal mean disease severity (16.22%) was recorded at 80.5 and 85.7% RH at 20°C temperature which was statistically at par with disease severity (16.55%) recorded at 80.5% RH and 25°C temperature. Maximum disease severity (67.22%) was recorded at 100% RH and 32°C which was statistically at par with that recorded at 30°C temperature (66.22%) at same RH level.

Table 4: Effect of different temperature and RH levels on severity of red rust in litchi

Relative humidity (%)	Disease severity (%) at temperature (°C)						Overall mean
	20	25	28	30	32	35	
80.5	16.22 (23.74)	16.55 (24.00)	25.00 (29.99)	26.00 (30.64)	31.53 (34.14)	24.89 (29.91)	23.37 (28.74)
85.7	16.22 (23.74)	28.56 (32.29)	31.31 (34.01)	32.78 (34.91)	34.11 (35.72)	31.75 (34.28)	29.12 (32.49)
89.9	25.00 (29.99)	30.33 (33.41)	32.89 (34.98)	33.67 (35.45)	43.27 (41.11)	37.50 (37.75)	33.78 (35.45)
95.6	25.17 (30.10)	32.33 (34.64)	32.89 (34.98)	41.22 (39.93)	50.89 (45.49)	41.22 (39.93)	37.29 (37.51)
100	41.71 (40.21)	46.56 (43.01)	54.75 (47.71)	66.22 (54.45)	67.22 (55.05)	54.00 (47.28)	55.08 (47.95)
Overall mean	24.87 (29.55)	30.87 (33.47)	35.37 (36.33)	39.98 (39.08)	45.40 (42.30)	37.87 (37.83)	

Factors	CD $p \geq 0.05$	SE(d)
Relative humidity	0.37	0.19
Temperature	0.41	0.20
Interaction	0.91	0.46

Figures in parentheses represent angular transformed value

Temperature range of 30-35°C and RH ranging between 95 to 100% was favourable for the disease initiation and development, 32°C temperature with 100% RH being the optimum (Figure 2). However, the symptoms appeared even at a temperature as low as 20°C and RH as low as 80%.

Present findings of epidemiological studies are in accordance with the findings of Sanahuja et al. (2018) who reported that symptoms caused by *C. parasiticus* appeared at an average temperature of 26°C and RH 80% on *Neoregelia* spp. However, there are no reports in the literature regarding the *in vitro* studies pertaining to effect of temperature and RH on development of red rust in any of the plant species but, Kumar et al. (2019) reported the prevalence of red rust in longan at a maximum temperature between 31.7–35.4°C and maximum RH between 82.6–93.6%, which support

Figure 2: Disease severity of red rust in litchi as influenced by different RH levels (a) 80.5% (b) 85.7% (c) 89.9% (d) 95.6% (e) 100% at 32°C temperature

our results. As per Pereira et al. (2020), rainy periods with temperature ranging between 28–32°C are ideal to break the envelope membrane of sporangia, thus facilitating the zoospore dispersal. During present studies also, high humidity might have facilitated the breaking of sporangial envelope, thus releasing the zoospores for infection to initiate, as the incubation period was shorter at higher RH levels and longer at lower RH levels.

3.3. Management studies- in vitro evaluation of chemicals

A perusal of data (Table 5) revealed that all test fungicides at all concentrations significantly inhibited the radial growth of *C. virescens* over control.

Captan, mancozeb, Cabrio top®, hexaconazole and propiconazole completely inhibited the growth of the test alga at all four concentrations tested, as the alga failed to grow in these treatments. However, among other chemicals tested, significantly mean minimum (4.45 mm) diametric growth was recorded in carbendazim followed by azoxystrobin (14.17 mm) and copper oxychloride (39.61 mm) and mean maximum (61.06 mm) diametric growth was recorded in potassium phosphite in comparison to full growth of the alga (90.00 mm) in control treatment, irrespective of the concentrations used. Whereas, irrespective of the fungicides tested, mean diametric growth of the alga was significantly maximum (25.61 mm) at lowest concentration evaluated which decreased significantly with increase in each level of concentration being significantly minimum at highest concentration tested (21.84 mm).

Captan, mancozeb, Cabriotop®, hexaconazole and

Table 5: *In vitro* evaluation of different chemicals against *Cephaleuros virescens*

Treatment	Diametric growth (mm) at concentration (ppm)				Overall mean	Inhibition (%) in diametric growth at concentration (ppm)				Overall mean
	500	1000	1500	2000		500	1000	1500	2000	
Copper oxychloride	41.67 (40.19)	41.11 (39.86)	38.89 (38.57)	36.78 (37.32)	39.61 (38.98)	53.7	54.32	56.79	59.13	55.99
Captan	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	100	100	100	100	100
Mancozeb	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	100	100	100	100	100
Cabrio top*	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	100	100	100	100	100
Azoxystrobin*	19.00 (25.83)	15.22 (22.96)	12.89 (21.03)	9.56 (18.00)	14.17 (21.95)	78.89	83.09	85.69	89.38	84.26
Carbendazim*	6.11 (14.30)	6.11 (14.30)	5.56 (13.62)	0.00 (4.05)	4.45 (11.57)	93.21	93.21	93.82	100	95.06
Hexaconazole*	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	100	100	100	100	100
Propiconazole*	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	100	100	100	100	100
Potassium phosphite	65.89 (54.24)	62.89 (52.45)	60.45 (51.01)	55.00 (47.85)	61.06 (51.39)	26.79	30.12	32.83	38.89	32.16
Potassium phosphate	59.00 (50.17)	56.33 (48.62)	54.00 (47.28)	48.89 (44.35)	54.56 (47.60)	34.44	37.41	40	45.68	39.38
Control	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)					
Overall mean	25.61	24.70	23.80	21.84	25.61					
Factors	CD $p \geq 0.05$				SE(d)					
Relative humidity	0.37				0.19					
Temperature	0.41				0.20					
Interaction	0.91				0.46					

*Concentrations tested were 125, 250, 375 and 500 ppm; Figures in parentheses indicates Angular transformed value

propiconazole at all concentrations tested resulted in cent% inhibition of the growth followed by carbendazim which resulted in 95.06% growth inhibition (Figure 3 and 4). Minimum inhibition (38.89 mm) was recorded by using potassium phosphite. An intermediate level of growth inhibition was recorded in rest of the treatments at different concentrations tested.

The results of management studies were in accordance with Ramaya et al. (2013) who reported that carbendazim and hexaconazole provided better disease control than copper oxychloride. These results were further supported by the findings of Oliver et al. (2020) who revealed that copper products have not been found effective against red rust. Studies of Sanahuja et al. (2018) further support our results which revealed that use of copper-based fungicides is not effective in prevention of algal leaf spot.

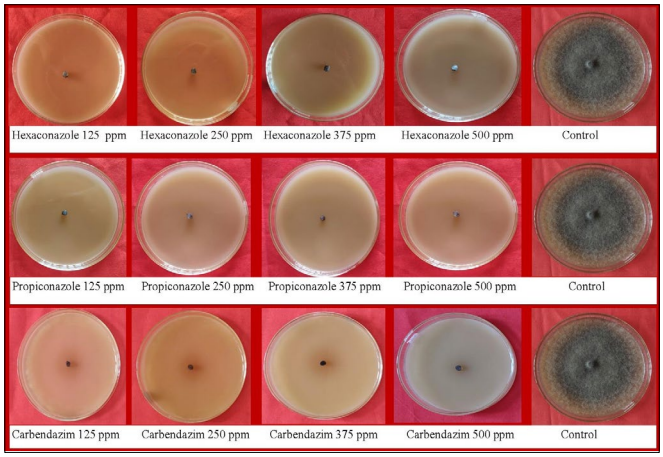

Figure 3: *In vitro* evaluation of chemicals against *Cephaleuros virescens*

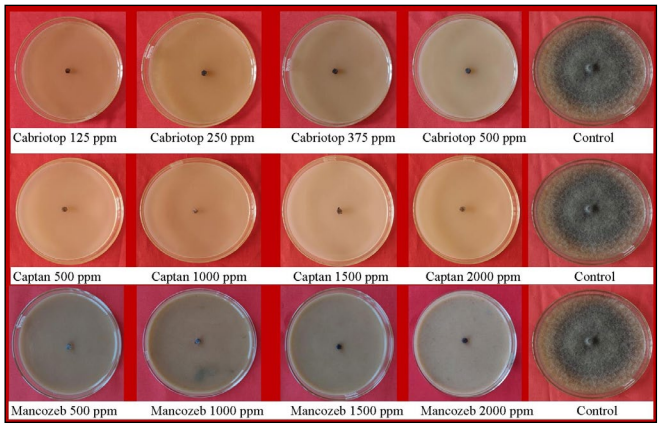

Figure 4: *In vitro* evaluation of chemicals against *Cephaleuros virescens*

4. CONCLUSION

The red rust of litchi caused by *C. virescens* was prevalent in mild to severe form in sub-tropical zone of Himachal Pradesh. Temperature 32°C and relative humidity 100% was most favourable for disease development. The disease was managed effectively *in vitro* by the use of captan, mancozeb, CabrioTop®, hexaconazole and propiconazole. So, their efficacy can be further evaluated at different development stages for the effective management of disease under field conditions.

5. ACKNOWLEDGEMENT

We are grateful to the Department of Plant Pathology, College of Horticulture and Forestry, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India for providing opportunity and necessary facilities during the research work.

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