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Efficacy of Microalgae and Lemon Grass Oil in the Management of Anthracnose of Chilli (*Capsicum annuum* L.)

N. Nandin* and Abhilasha A. Lal

Department of Plant Pathology, Naini Agricultural Institute, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, (UP) India

**Corresponding author*

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A B S T R A C T

Chilli (*Capsicum annuum* L.) or red pepper, a member of solanaceae family is mainly cultivated as annual herbaceous vegetable and commercial spice crop. Anthracnose caused by *Colletotrichum capsici* is a serious disease in chilli growing areas and occur as a pre-harvest and post harvest fruit rot, causing extensive losses in chilli. In this study, citronella essential oil was tested *in vitro* at different concentrations viz., 0.25%, 0.5%, 0.75%, 1%, 1.25% and carbendazim 0.01% against radial growth of *Colletotrichum capsici*. The minimum radial growth and maximum percent inhibition was recorded at a concentration of citronella @ 1.25 % (2.50 mm and 97.22%) followed by citronella @ 1% (5.95 mm and 93.38%) as compared to treated carbendazim- (98.41%) and untreated check (00.00%). During *Rabi* 2019-2020 the effect of microalgae and citronella essential oil were tested against the disease severity, growth & yield parameters under field conditions in prayagraj, Uttar Pradesh. Among the treatments minimum disease severity (%) was recorded in T_3– citronella oil @ 1% + microalgae@ 4kg/acre (24.92%)as compared to treated T_7- carbendazim (21.68%) and untreated control T_0 - Control (35.92%). The maximum plant height (cm), number of branches, fruit length and yield was recorded maximum in T_3 – citronella oil @ 1%+microalgae @ 4kg/acre (60.18 cm, 28.00, 9.60 cm and 4.40 t/h) when compared to control. The minimum per cent fruits infected (%) was recorded in T_3 – citronella oil @ 1%+microalgae @ 4kg/acre (23.08) followed by, T_2 – citronella oil @ 1% + microalgae@ 3kg/acre (24.08), as compared to treated T_7- Carbendazim (21.02) and untreated control T_0 - control (34.60). Results showed that microalgae and citronella essential oil can be used as ecofriendly compounds for the management of anthracnose disease in chili.

Introduction

Chilli (*Capsicum annuum* L.) or red pepper, a member of solanaceae family is mainly cultivated as annual herbaceous vegetables (Mishra *et al.,* 2017) and commercial spice crop in many countries including India (Poulos, 1992). Chilli commonly refers to hot types of capsicum which is originated in the American tropics. There are five major species of cultivated capsicum, *Capsicum annuum, Capsicum chinense, Capsicum baccatum, Capsicum frutescens, and Capsicum pubescens*. It is an important spice

crop as well as major cash crop which are grown for its pungency in India and our country is rated to be the second largest exporter in the world. It is used as a principal ingredient of various dishes. Chillies are famous for its aromatic flavor, nutritional values, texture, color and pungency.

Chilies are widely used both as condiment or culinary supplement and also found to have medicinal properties. Color of the fruit is due to capsanthin pigment and pungency is due to the presence of active alkaloid compound capsicin. Chillies are known to contain 3% carbohydrates, 2% protein, 0.6% fat and being a good source of vitamin A, B and C and minerals.

Among diseases the chilli crop suffers from damping off, anthracnose (fruit rot), wilt, murda complex and root knot nematode. Anthracnose caused by *Colletotrichum capsici* is serious disease in chilli growing areas (Butler and Bibsy, 1913). Sydow (1913) reported the disease for the first time in India. Anthracnose occur as a pre-harvest as well as post harvest fruit rot, causing extensive losses in chilli grown during the warm wet season in tropical and subtropical climates (Pearson *et al.,* 1984). The disease occurs in three phases, they are seedling blight or damping off stage, leaf spot or die back and anthracnose or fruit rot (Rajeshwari *et al.,* 2004). However, fruit lesions are the most economically important aspect of this disease. Their productivity is limited by fruit losses due to anthracnose (Ali *et al.,* 2016). The disease seriously appears in every part of state in the month of November and December due to availability of favorable climatic condition. The overall disease intensity was higher during 2017- 18 (31.29%) as compared to 2018-19 (36.11%) and the disease was found to be predominant.

Chemical method for managing anthracnose disease is not sustainable and is associated with health hazards, residue problems and environmental pollution, accumulating potential resistance in pathogen to fungicides (Ali *et al.,* 2016). To overcome this problem use of bio- resources and essential oil is an economical and eco-friendly option to manage anthracnose disease. Bio resource like microalgae acts as bio stimulant and bio fertilizer contain high levels of macronutrients and micronutrients essential for optimal crop growth and development. Even though microalgae are applied in low doses to seed, soil or crop, are able to regulate and enhance the crop physiological processes. This improves crop growth, yield, quality, nutrient uptake, tolerance to abiotic stress and the shelf life of harvested products. Essential oils are the compound produced from plants parts which are found to have antimicrobial properties against many plant pathogens (Dikbas *et al.,* 2008) among the essential oils the lemon grass oil and citronella are most promising and which are derived from *Cymbopogon* spp which inhibits the growth and germination of pathogen and it is non-phytotoxic.

Since, chilli has an important place in spice and vegetable, the area under chilli cultivation is increasing every year and fruit rot is one of the devastating disease of chilli. Therefore, keeping these points in view, the present investigation has been taken up in detail about anthracnose or fruit rot disease, the pathogen its behavior and especially on management practices.

Materials and Methods

Isolation and identification of the pathogen

The leaves of chilli showing typical symptoms were collected from central research field SHAUTS Prayagraj and the standard tissue isolation procedure was followed to isolate the pathogen. Diseased

portion of the leaves were washed with tap water and cut under aseptic conditions into small bits and surface sterilized in sodium hypochlorite for 2 minutes. The diseased leaf bits were placed on petridish containing potato dextrose agar media. The potato dextrose agar media was prepared by peeling 200 grms of potato and boil it in 500 ml of distilled water for 15 minutes and stain through a muslin cloth. Add 20 grms of dextrose and 20 grms of agar agar into another 500 ml of distilled water and mix-up the volume. Then transfer the media into conical flask. The inoculated plates were incubated at room temperature (27±2 ◌C) for 3-4 days until visible growths are seen on the plates. The fungal colonies growing in the incubated plates were sub-cultured into fresh medium until pure cultures were obtained.

Evaluation of citronella essential oil and fungicide *in-vitro*

Essential oils were used at different concentrations (0.25%, 0.5%, 0.75%, 1% and 1.25%) using PDA as base media. Measured amount of essential oil and tween 80 at 0.1% (for oil dispersion) was added to sterile perti plates containing PDA in each treatment, measured concentration of fungicide was added to PDA then poured to sterile Petri plates and allowed them to mix homogeneously and to get solidified. Fungal disc of 5mm diameter from 7 days old pure culture was placed in centre of each Petri dish containing medium under aseptic condition incubated at 27± 2 ºC for seven days. Colony diameter was recorded every 24 hours. Per cent inhibition of mycelial growth was calculated by using the formula given by Vincent (1947) comparison with the control plate. The data obtained was averaged and analyzed statistically.

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

Where,

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

Evaluation of microalgae and citronella essential oil on different parameters in field conditions

The *in vivo* experiment was conducted in the research plot in the Department of Plant Pathology, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj. The experiment was laid out in a randomized block design with eight treatments viz., T_1 Microalgae + Citronella oil @ 2kg/ac + 1.0%, T_2 Microalgae + Citronella oil @3kg/ac + 1.0%, T_3 Microalgae + Citronella oil @4kg/ac +1.0%, T_4 Microalgae + Citronella oil @5kg/ac + 1.0%, T_5 Microalgae + Citronella oil @6kg/ac + 1.0%, T_6 Microalgae +Citronella oil @7kg/ac + 1.0%, T_7 Carbendazim @ 0.1% and T_0 Control were evaluated against disease severity of anthracnose in chili. The application of microalgae, citronella essential oil and Carbendazim was done after the appearance of disease at 25 DAT followed by sprayings at 25 days interval. The observations were recorded before each spray and the disease severity was calculated using the disease scoring scale given by (Mayee and Datar, 1986); Where,

0 = no symptoms, 1 = 1 to10% disease infection, 3 = 11 to 25% disease infection, 5 = 26 to 50% disease infection, 7 = 51 to 75% disease infection, 9 = >75% disease infection. The percent disease over control was calculated to know the disease reduction percentage

$$PDI = \frac{\text{Sum of all disease ratings}}{\text{No.of observations assessed} \times \text{maximum disease rate}} \times 100$$

$$\text{PDI increase or decrease over control (\%)} = \frac{\text{Disease in control plot} - \text{Disease in treatment plot}}{\text{Disease in control plot}} \times 100$$

Effect of microalgae and citronella essential oil on different parameters of chili

Pre harvest and post-harvest observations were recorded

Pre harvest observations

Plant height

Plant height was recorded at 45, 75 and 105 days after transplanting.

Number of branches

Numbers of branches were recorded at 45, 75 and 105 days after transplanting.

Post-harvest observations:

Yield (t/ha)
Per cent fruit infected (%)
Fruit length (cm)

Results and Discussion

Evaluation of citronella essential oil and fungicide *in-vitro* against *Colletotrichum capsici*

The antifungal effect of citronella essential oil at different concentrations i.e., 0.25%, 0.5%, 0.75%, 1%, 1.25%, were tested against radial growth and percent inhibition of *Colletotrichum capsici* and recorded every 24 hrs intervals. The results revealed that minimum mycelia growth was recorded in T_5 – citronella oil @ 1.25 % (2.50 mm and 97.22%) followed by T_4-citronella oil @ 1.0 % (5.95 mm and 93.38%), T_3 - citronella oil @ 0.75 % (11.58 mm and 87.13%), T_2 - citronella oil @ 0.5 % (15.44 mm and 82.84%), as compared to treated T_6– Carbendazim (1.43mm and 98.41%) and untreated control T_0 - (89.04 mm). The results obtained were significantly differed from each other (Table 1).

Effect of microalgae and citronella essential oil on per cent disease severity of chill anthracnose (before spray, 25 days after 1st spray and25 days after 2nd spray)

Results showed that all the treatments were significantly reduced the disease compared to control. The minimum disease severity was recorded in T_3–citronella oil @1% + microalgae@ 4kg/acre (24.92%), followed by, T_2 – citronella oil @1% + microalgae@ 3kg/acre (27.34%), T_4- citronella oil @ 1% + microalgae @ 5kg/acre (26.27%), T_5- citronella oil @ 1% + microalgae @ 6kg/acre (26.78%), T_1- citronella oil @1% + microalgae @ 2kg/acre (27.34%), T_6- citronella oil @ 1% + microalgae @ 7kg/acre (28.05%), as compared to treated T_7- Carbendazim (21.68%) and untreated control T_0 - Control (35.92%) (Table 2).

Results observed that the per cent fruits infected were significantly reduced compared to control. The minimum disease was observed in T_3 – citronella oil @ 1% + microalgae @ 4kg/acre (23.08), followed by, T_2 – citronella oil @1% + microalgae @ 3kg/acre (24.08), T_4- citronella oil @1% + microalgae @ 5kg/acre (25.57), T_5- citronella oil @ 1% + microalgae @ 6kg/acre (25.92), T_1-citronella oil @1% + microalgae @ 2kg/acre (25.48), T_6-citronella oil @1% + microalgae @ 7kg/acre (26.15), as compared to treated T_7- Carbendazim (21.02) and untreated control T_0 - Control (34.60).

Effect of microalgae and citronella essential oil on plant height of chilli

Results revealed that the treatments were significantly differed from each other. The maximum plant height (cm) was recorded in T_3 – citronella oil @ 1% + microalgae @

4kg/acre (60.18cm), followed by, T_2 – citronella oil @1% + microalgae 3kg/acre (58.12cm), T_4- citronella oil @ 1% + microalgae @ 5kg/acre (56.70cm), T_5- citronella oil @1% + microalgae @ 6kg/acre (56.10cm), T_1- citronella oil @ 1% + microalgae@ 2kg/acre (56.40cm), T_6- citronella oil @ 1% + microalgae @ 7kg/acre (55.10cm), as compared to treated T_7- Carbendazim (58.04cm) and untreated control T_0 - Control (45.71cm) (Table 3).

Effect of microalgae and citronella essential oil on number of branches of chilli

Results revealed that the number of branches was significantly recorded maximum in T_3 – citronella oil @ 1% +microalgae @ 4kg/acre (28.00), followed by, T_2–citronella oil @1% + microalgae @ 3kg/acre (26.40), T_4- citronella oil @ 1% + microalgae @ 5kg/acre(24.53), T_5- citronella oil @ 1% + microalgae @ 6kg/acre (24.0), T_1- citronella oil @ 1% + microalgae @ 2kg/acre (23.47), T_6-citronella oil @ 1% + microalgae @ 7kg/acre (23.13), as compared to treated T_7- Carbendazim (26.06) and untreated control T_0 - Control (16.80) (Table 3).

Effect of microalgae and citronella essential oil on fruit length of chilli

The fruit length (cm) was significantly found maximum in T_3 – citronella oil @ 1% + microalgae @ 4kg/acre (9.60cm), followed by, T_2 – citronella oil @1%+microalgae @ 3kg/acre (8.18cm), T_4- citronella oil @1% + microalgae @ 5kg/acre (7.30cm), T_5- citronella oil @ 1% + microalgae@ 6kg/acre (6.44cm), T_1- citronella oil @1% + microalgae @ 2kg/acre (6.56cm), T_6- citronella oil @ 1% + microalgae @ 7kg/acre (6.05cm), as compared to treated T_7- Carbendazim (7.33cm) and untreated control T_0 - Control (4.23cm) (Table 3).

Table.3.1 Effect of citronella essential oil on radial mycelial growth (mm) and percentage inhibition of *Colletotrichum capsici* at different time intervals

Sr. No	Treatments	Radial growth (mm)				Percentage inhibition at 120 Hrs (%)
		48 Hrs	72 Hrs	96 Hrs	120 Hrs	
T_0	Control(Untreated check)	40.62	57.19	72.95	89.04	0
T_1	Citronella oil @ 0.25%	10.63	16.76	22.19	29.02	67.75
T_2	Citronella oil @ 0.5%	7.03	8.25	11.25	15.44	82.84
T_3	Citronella oil @ 0.75%	5.72	6.15	9.12	11.58	87.13
T_4	Citronella oil @ 1. 0 %	3.25	3.51	4.33	5.95	93.38
T_5	Citronella oil @ 1.25%	1.33	2.39	2.44	2.50	97.22
T_6	Carbendazim (Treated check)	1.11	1.35	1.41	1.43	98.41
F test		S	S	S	S	-
SEd ±		0.47	0.70	0.84	0.88	-
C.D.(P=0.05)		1.02	1.53	1.83	1.91	-

Table.3.2 Effect of microalgae and citronella essential oil on per cent disease severity of chilli anthracnose (before spray, 25 days after 1st spray and 25 days after 2nd spray) and on fruit infected (%)

Sr.No	Treatments	Average disease severity (%) 25 DAT (before spray)	Average disease severity (%) (25 days after 1st spray)	Average disease severity (%) (25 days after 2nd spray)	Fruit infected (%)	
T_0	Control +NPK (Untreated check)	21.36	26.93	35.92	34.60	
T_1	Microalgae +Citronella oil	19.89	24.66	27.34	25.48	
T_2	Microalgae +Citronella oil	19.11	23.53	25.82	24.08	
T_3	Microalgae +Citronella oil	18.70	21.44	24.92	23.08	
T_4	Microalgae +Citronella oil	19.42	23.49	26.27	25.57	
T_5	Microalgae +Citronella oil	19.46	23.90	26.78	25.92	
T_6	Microalgae +Citronella oil	19.97	25.59	28.05	26.15	
T_7	Carbendazim (Treated check)	19.55	21.23	21.68	21.02	
F Test		S	S	S	S	
SEd ±		0.43	0.44	0.57	0.45	
C.D.(P=0.05)		0.93	0.96	1.24	0.98	

Table.3.3 Effect of microalgae and citronella essential oil on plant height, Number of branches, Fruit length and yield of chilli

Sr.No	Treatments	Plant height	Number of branches	Fruit length (cm)	Yield (t/ha)	
T_0	Control (Untreated check)	45.71	16.80	4.23	1.73	
T_1	Microalgae +Citronella oil	55.90	23.47	6.56	2.77	
T_2	Microalgae +Citronella oil	58.12	26.40	8.18	3.82	
T_3	Microalgae +Citronella oil	60.18	28.00	9.60	4.40	
T_4	Microalgae +Citronella oil	56.70	24.53	7.30	3.45	
T_5	Microalgae +Citronella oil	56.10	24.0	6.44	3.29	
T_6	Microalgae +Citronella oil	55.40	23.13	6.05	2.60	
T_7	Carbendazim (Treated check)	58.04	26.06	7.33	4.42	
F Test		S	S	S	S	
SEd ±		0.48	0.61	0.29	0.19	
C.D.(P=0.05)		1.06	1.33	0.63	0.38	

Effect of microalgae and citronella essential oil on yield of chilli

Results revealed that the maximum yield(t/h) of chilli was recorded significantly in T_3 – citronella oil @ 1% +microalgae@ 4kg/acre (4.42 t/h), followed by, T_2–citronella oil @1% + microalgae @ 3kg/acre (3.82t/h), T_4-citronella oil @ 1% + microalgae @ 5kg/acre(3.45 t/h), T_5- citronella oil @ 1% + microalgae @ 6kg/acre (3.29 t/h), T_1-citronella oil @ 1% + microalgae @ 2kg/acre (2.77t/h), T_6-citronella oil @ 1% + microalgae @ 7kg/acre (2.60 t/h), as compared to treated T_7- Carbendazim (4.40 t/h) and untreated control T_0 - Control (1.73 t/h) (Table 3).

Chilli (*Capsicum annuum* L.) or red pepper, a member of solanaceae family, annual herbaceous vegetables and commercial spice crop. It is an important spice crop as well as major cash crop which are grown for its pungency. Chillies are famous for its aromatic flavor, nutritional values, texture, colour and pungency. Chilies are widely used both as condiment or culinary supplement and also found to have medicinal properties.

Anthracnose caused by *Colletotrichum capsici* is serious disease in chilli growing areas. Anthracnose occurs as a pre-harvest as well as posts harvest fruit rot, causing extensive losses in chilli. The disease occurs in three phases, they are seedling blight or damping off stage, leaf spot or die back and anthracnose or fruit rot. Their productivity is limited by fruit losses due to anthracnose has become major problem in chilli cultivation.

Reduction in radial growth of *Colletotrichum capsici* is due to the antifungistatic activity of citronella oil have shown direct antimicrobial and antifungal effect on fungal conidia with different concentrations of same essential oil have inhibited the mycelial growth and reduced the conidial germination in *Colletotrichum capsici*. Citronella @ 1.25% have significantly inhibited the mycelial germination, was found most promising essential oil against *Colletotrichum capsici*. Similar findings have been reported by Nikos *et al.,* (2007), Jagan (2018) The presence of antifungal activity in essential oil may have prevented the hyphal growth and sporulation of *Colletotrichum capsici*. These are also responsible for altering the fungal physiology by inducing changes in cell wall compositions, plasma membrane disruption, mitochondrial structure, disorganization and interference with respiratory enzymatic reactions of the mitochondrial membrane.

Citronella oil @ 1% +microalgae @ 4kg/acre have given the best result. Similar findings are reported by Lokhande *et al.,* (2019) and Ali *et al.,* (2017). Microalgae contain high levels of macro and micronutrients essential for an optimal crop growth and development. Even though microalgae are applied in low doses to seed, soil or crop, are able to regulate and enhance the crop physiological processes. This improves crop growth, yield, quality, nutrient uptake, tolerance to abiotic stress. In addition to this essential oils control disease and helped for good plant growth which may have lead plant to maximum height. Similar findings are reported by Arroussi, (2016) and Dineshkumar, (2017).

In conclusion use of alternative methods such as citronella essential oil (lemongrass) and microalgae i.e., bio fertilizers and bio stimulants increased yield of chilly quantitatively and qualitatively, crop growth and less attack of disease by stimulating the physiological process of plant. Essential oils are found to have antimicrobial and antifungal properties considered, these are environmentally safe, economically feasible, no residual problems and cost effective alternatives to synthetic products.

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