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Evaluation of the Antifungal Efficacy of Plant Extracts, Essential Oils and Cow Urine on *Curvularia lunata*, the causal Organism of Maize Leaf Spot

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ABSTRACT: Studies were carried out to assess the efficacy of botanicals, essential oils and cow urine at different concentrations against *Curvularia lunata,* causal pathogen of maize leaf spot. Maize being the third most important crop in India after rice and wheat, is cultivated throughout the year in different parts of the country for diverse purposes thereby making it a crop of economic significance. The crop is being affected by an array of pathogens majorly being managed by the chemicals. Adding on to the burden of the environment, the chemicals used also act as pollutant and can also be subsided by the pathogen by developing resistant races. Botanicals on the other hand provide an ecofriendly method of the plant disease management. Amongst the botanicals, *Zingiber officinale* was found most successful at 20% for inhibiting the growth (71.85%) of the fungus which was at par with *Zingiber officinale* at 15% (66.67%) followed by *Curcuma longa* at 20% (60.01 %). Among the essential oils, highest inhibition (87.41%) was found in *Eucalyptus globulus* oil at 500 ppm which was at par with same oil at 250 ppm, respectively. Cow urine showed maximum per cent inhibition in 10% (100 %) onwards followed by 5% (29.44%).

Keywords: Botanicals; *Curvularia lunata*; essential oils; leaf spot; maize; cow urine; screening; *in-vitro*.

INTRODUCTION

Maize (*Zea mays* L.) contributes to food security in majority of the developing countries including India after rice and wheat. The growing interest of the consumers in nutritionally enriched products and increasing demand for maize seeds are the nucleus driving forces behind emerging importance of maize crop in India. Losses encountered by biotic factors are substantial and the leaf spot caused by *Curvularia lunata* causes severe losses in different crops as it has a vast host range. Curvularia leaf spot caused by *Curvularia lunata* has apparent visible symptoms exhibiting small chlorotic spots that expand into a round or oval-shaped lesion surrounded by a spacious translucent straw yellow coloured halo (Rao and William, 1978). Bisht *et al.* (2013) reported that amongst the evaluated essential oils *viz;* Peppermint oil, Geranium oil, Mentha oil, Palmroza oil and Citronella oil at three different concentrations; 2µL, 4µL and 8 µL against *Curvularia lunata*, complete inhibition was found in Citronella oil at all three concentrations while Peppermint oil showed complete inhibition at 4µL and 8 µL concentrations, respectively. Mourao *et al*. (2017) reported that among the tested concentrations of essential oil *Cymbopogon citratus* showed promising results under *in vitro* conditions for the control of *Curvularia lunata* and suggested it as a great alternative for the management of Curvularia leaf spot disease on maize. The concentrations of 5.0 and 7.5 µL mL^{-1} of the *C. citratus* oil also totally inhibited the conidia germination. Yet the concentrations 0.625; 1.25 and 2.5 µL Ml^{-1} had a low inhibition percentage of 3.3%, 2.3%, and 25%, respectively, indicating oil activity upon the conidia, even in the lower concentrations. Kishore and Pande (2007) reported that evaluation of cinnamon oil, clove oil, and five different essential oil compounds (Geraniol, Eugenol, Citral, Limonene and Linalool) for growth inhibition by paper disc agar diffusion method of 14 phytopathogenic fungi found that citral gave complete inhibition on growth of Curvularia *lunata, Alternaria alternata, Aspergillus flavus, Fusarium moniliforme* and *Phoma sorghina.* Varma and Dubey (1999) reported that a better alternative to synthetic pesticides appears to be plant metabolites and plant based products as they are known to have minimal environmental hazards and danger to consumers. Kuri *et al*. (2010); Rahman (1992) reported that extracts of many higher plants exhibit antifungal properties under *in vitro* conditions. Similarly, Rahman *et al*. (1999) found that the plant extracts show antifungal activity against a wide range of fungi. Rahman (1992) reported that bishkatali, garlic, ginger and neem extract were effective against seed-borne *Curvularia lunata*. Similarly, Rahman (1999); Mansur (2013) reported that extract of garlic was found to be superior followed by ginger and neem in terms of reducing seed-borne infections by *Alternaria* spp., *Bipolaris sorokiniana,*

Curvularia lunata, *Fusarium* spp. of wheat. Choi *et al.* (2004); Bandara *et al*. (1988) reported that *Acorus calamus*, *Zingiber zerumbet* and *Curcuma longa* possessed numerous significant biological including antifungal activities. Similarly, Sanit (2012) reported that the crude extracts of *Curcuma longa*, *Zingiber officinale*, *Allium sativum*, evaluated *in vitro* showed significant antifungal activity against *Curvularis s*pecies. Bisht *et al.* (2013) reported that amongst the plant extract evaluated *Lantana* showed maximum mycelial growth inhibition. Behura *et al*. (2000) reported that leaf and rhizome oil of *Curcuma longa* suppressed the growth of common rice pathogen including *Curvularia lunata*. Ankita and Kanika (2011) reported that there was inhibition of *Dreschlera graminae*, *Curvularia lunata*, *Aspergillus fumigatus and Candida albicans* by leaf of *Lawsonia inermis.* Pawar *et al*. (2012) reported that Garlic (*Allium sativum*) completely inhibited the mycelial growth of *Curvularia lunata* and *Curvularia pallescens* followed by A*zadirachta indica.* Garhwal (2013) reported that among Neem and Aloe vera extract evaluated against *Curvularia lunata* of maize and found that Neem (2000 ppm) was most effective followed by Aloe vera at 5000 ppm. Bhadauria (2002) reported that cow urine contains 95% water, 2.5% urea, and the remaining 2.5% a mixture of salts, hormones, enzymes, and minerals. Krishnamurthi *et al*. (2004) reported several beneficial properties of cow urine such as antioxidant, antidiabetic, antitumor, antiprotozoal, and molluscicidal. Similarly, Dharma *et al.* (2005) reported that cow urine is vital for agricultural operations in the form of biofertilizer and biopesticide. It can kill number of pesticide and herbicide resistant bacteria, viruses, and fungi. Mandavgane *et al.* (2005) also reported the use of cow urine in combination with plant extracts to prepare disinfectant that is biodegradable and ecofriendly with good antibacterial action. Majority of people in India use cow urine to get rid of various diseases due to its therapeutic values. Vahanka *et al.* (2010) reported that, in crops especially cotton, groundnut, maize, castor, chilli, regular use of cow urine by the farmers led to an increase the population of soil microorganism along with increased crop production and enhanced soil texture and structure. Sharma *et al.* (2010) reported that cow urine when evaluated at different concentrations, the percentage germination was minimum at 5% of the four fungal species *Alternaria alternata*, *Fusarium oxysporium*, *Colletotrichum capsici* and *Curvularia lunata* that were used for their germination attributes. Keeping in view of public health, environmental safety and the economic importance of disease, the present studies were carried out by *in vitro* evaluation of botanicals, essential oils and cow urine against *Curvularia lunata* .

MATERIALS AND METHODS

A. Screening of Botanicals against Curvularia lunata

The rhizome extracts of *Zingiber officinale* and *Curcuma longa* whereas leaf extracts *Melia azedarach* , *Cassia fistula*, *Cinnamomum tamale*, *Syzygium cumini*, *Aloe vera*, *Nerium oleander*, *Cinnamomum camphoraa*, *Murraya koenigii* and *Phyllanthus emblica* were prepared by method described by Ansari (1995) with a slight modification. The plant parts of certain selected plants supposed to have active antifungal properties were collected from different locations at Pantnagar. New leaves from different plants were thoroughly washed with tap water, then surface sterilized with 2 per cent Sodium hypochlorite and successively washed with distilled water. Leaves were then semi dried under shade so that it could be crushed easily. Each sample was then homogenized using (1:1 w/v) sterilized distilled water using a pestle-mortar and squeezed through fine muslin cloth and lastly through Whatmann No. 1 fiter paper. The filter pure extracts were mentioned in the study as 100% extract solution. The correct amount of plant extract was mixed in sterilized distilled water to make the preferred concentration (v/v) for experiments. For bioassay, double strength concentrations of botanicals were prepared by dissolving 10, 20, 30 and 40 ml of plant extract in 90,80,70 and 60 ml of sterilized distilled water, correspondingly to get the final concentrations of 5,10,15 and 20%.

B. Bioassay procedure

The plant extract of desired concentrations (10%, 15 % and 20 %) were prepared and then mixed in double strength PDA medium in separate conical flask and sterilized in autoclave (15 lb p.s.i. for 20 min). The prepared media (poisoned with plant extract) was poured into sterilized Petri plates. In control plates, sterilized water was added as an alternative of plant extracts. Each treatment was replicated three times along with control. After solidification of the medium, the amended and non-amended agar plates were inoculated in the centre with 5 mm discs cut from 7 days old culture by sterilized cork borer. Inoculated Petri plates were kept in incubation at 25± 2ºC and observations of colony diameter (mm) were recorded at every 24 hour interval until the check Petri plates were fully covered with the growth of the test fungus. Percent growth inhibition was calculated according to the formula given by Vincent (1947).

$$PI = (C-T)/C \times 100$$

Where, PI = Inhibition percentage
C = Colony diameter in check plate (mm)
T = Colony diameter in treatments (mm)

C. Screening of essential oils against Curvularia lunata

Ten essential oils *viz*; *Syzygium aromaticum*, *Curcuma longa*, *Zingiber officinale*, *Eucalyptus globulus globulus*, *Mentha arvensis*, *Mentha piprata*, *Mentha spicata*, *Pogostimon patchouli*, *Cymbopogon martini* and *Verbena officinalis* oils were evaluated at 25, 50, 100, 250, 500 ppm each using poison food technique. Different essential oils were collected from Central Institute of Medicinal and Aromatic Plants (CIMAP) Research Centre, Nagla, U.S. Nagar whereas *Curcuma longa* oil, *Zingiber officinale* oil, *Eucalyptus globulus* oil were extracted using clevenger apparatus. Solvent to dissolve the oils in PDA was prepared. To prepare 1

litre of solvent 10ml of Tween 20 (1%) and 100 ml of DMSO (20%) was mixed in 890ml of sterilised distilled water. Stock solution of 10,000 ppm was prepared subsequently desired quantity from stock solution was added to the sterilised PDA media and pouring was done in sterilised petriplates. Three replications of each concentration was taken. Petri plates inoculated were then incubated at 25 ± 2ºC and observations of the colony diameter (mm) was recorded at every 24 hour interval until the check petri plates were fully covered with the growth of the fungus mycelium. Per cent growth inhibition was calculated by the formula given by Vincent (1947).

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

Where, PI = Percent inhibition of mycelial growth
C = Radial growth of test pathogen in control plates (mm)
T = Radial growth of test pathogen in dual culture (mm)

D. In vitro screening of cow urine against Curvularia lunata

(i) Cow urine collection and preparation. Cow urine was collected from Indian cow of Instructional Dairy Farm, Nagla (Pantnagar) from. The urine was stored in a refrigerator at 4ºC prior to use. In this technique, double strength PDA medium was used as described earlier. Subsequently, 25 ml sterilized PDA was poured in sterilized conical flask of 100 ml capacity. Stock solution was prepared of 10,000 ppm in distilled water (sterilized) in separate 250 ml flask. Stock solution was made ready by mixing required volume of Cow Urine in distilled water (sterilized) and supplemented to double strength PDA medium. Treatments were planned to mix freshly collected Cow Urine in different concentration *i.e*., 1, 5, 10, 20, 30, 40 and 50% of cow urine in 20 ml of PDA medium. Poisoned medium with various concentrations of Cow Urine was poured into sterilized Petri plates. For each treatment, three replications were maintained along with check (control). In control, normal PDA was poured in sterilized Petri plates. Thereafter the Observations were recorded and percent growth inhibition was calculated by the formula given by Vincent (1947)

$$PI = (C-T)/C \times 100$$

Where,
PI = Inhibition percentage
C = Colony diameter of check plate (mm)
T = Colony diameter of treatments (mm)

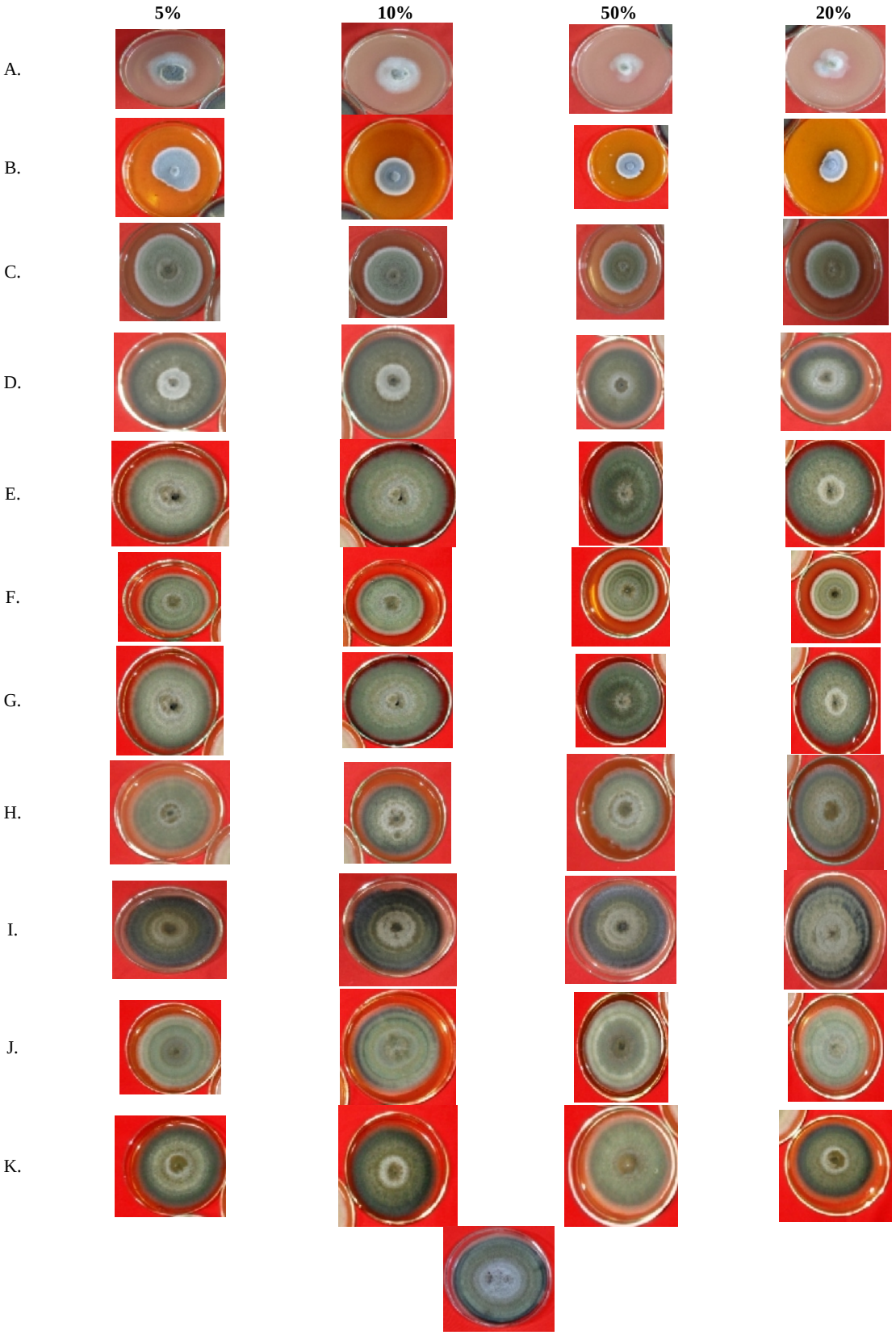
E. Statistical analysis

The data obtained were statistically analyzed using ANOVA for randomized block design with the help of STPR-1 statistical programme developed by Mathematics and Statistics Department at College of Basic Science and Humanities of G.B. Pant University of Agriculture and Technology, Pantnagar. In case of significant result of F test, critical difference at 5 % level of probability was taken for comparison between treatment means (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

A. Effect of various botanicals on radial growth of Curvularia lunata

Different botanicals *viz*; *Zingiber officinale,Curcuma longa, Phyllanthus emblica, Cinnamomum tamale, Murraya koenigii, Syzygium cumini, Nerium oleander, Cassia fistula, Aloe vera, Melia azedarach* and *Cinnamomum camphora*were evaluated at various concentrations *viz*; 5, 10, 15 and 20%. The results revealed (Table 1), at 5 per cent, maximum inhibition per cent of the fungus was reported in *Zingiber officinale* (77.41%) followed by*Curcuma longa* (48.52%) and *Phyllanthus emblica* (29.26%) while minimum inhibition was recorded in *Aloe vera* (7.04%) . At 10 per cent, maximum inhibition per cent of the fungus was reported in *Zingiber officinale* (62.59%) followed by *Curcuma longa* (50.92%) and *Phyllanthus emblica* (31.85%) while minimum inhibition was recorded in *Aloe vera* (5.56%). At 15 per cent, maximum inhibition per cent of the fungus was reported in *Zingiber officinale* (66.67%) followed by *Curcuma longa* (49.81%) and *Phyllanthus emblica* (37.04%) while minimum inhibition was recorded in *Aloe vera* (5.37%). At 20 per cent, maximum inhibition per cent of the fungus was reported in *Zingiber officinale* (71.85%) followed by *Curcuma longa* (60.00%) and *Phyllanthus emblica* (37.22%) while minimum inhibition was recorded in *Aloe vera* (5.93%). Hence, the experiment conducted concludes that *Zingiber officinale* was more effective at all the concentrations as compared to other botanicals evaluated, followed by *Curcuma longa* and *Phyllanthus emblica*. The reason to the inhibition of test fungus can be attributed to the anti fungal nature of *Zingiber officinale*, *Curcuma longa* and *Phyllanthus emblica*. The results were in accordance with the findings of Choi *et al*. (2004); Bandara *et al*. (1988) who reported that *Acorus calamus, Zingiber zerumbet* and *Curcuma longa* obsessed numerous significant biological as well as antifungal activities. Similarly Sanit (2012) reported that the crude extracts of *Curcuma longa, Zingiber officinale, Allium sativum*, evaluated *in vitro* possesed significant antifungal activity against *Curvularis sp*ecies. Behura *et al*. (2000) reported leaf and rhizome oil of *Curcuma longa* concealed the growth of common rice pathogen including *Curvularia lunata*. Guleria and Kumar (2006) reported antifungal action of *Vitex negundo*, *Zantoxylum alatum*, *Ipomea carnea*, *Thuja orientalis* and *Cinnamomum Cinnamomum camphora*against *Alternaria alternata* and *Curvularia lunata* using bioautography by using method like lipophilic (dichloromethane) leaf extract and the top results were found by lipophilic leaf extract of *T. orientalis.*



A. *Zingiber officinale* B. *Curcuma longa* C. *Phyllanthus emblica* D. *Cinnamomum tamale* E. *Murraya koenigii* F. *Syzygium cumini* G. *Nerium oleander* H. *Cassia fistula* I. *Aloe vera* J. *Melia azedarach* K. *Cinnamomum camphora*

Plate 1. Effect of different botanicals on radial growth of *Curvularia lunata* at 28±2°C.

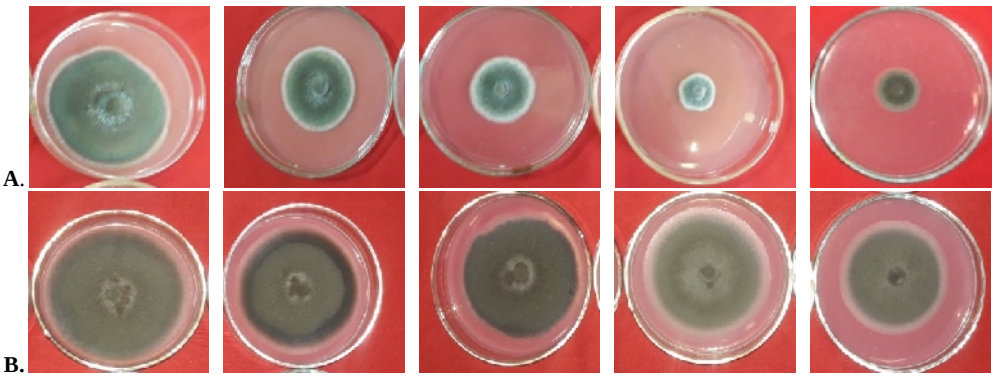
Table 1: Effect of different botanicals on radial growth of *Curvularia lunata.*

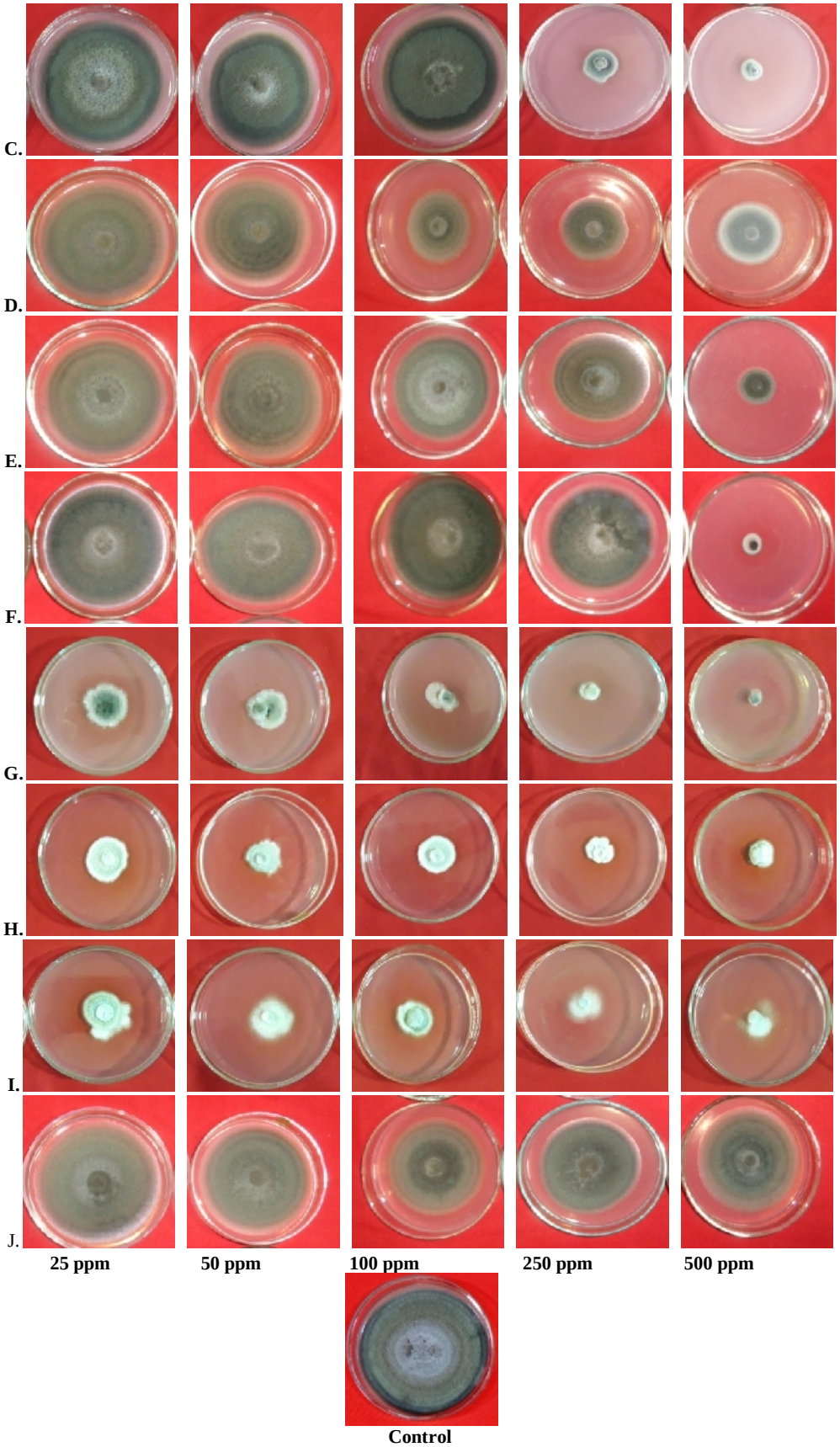
Sr. No.	Botanicals	Concentrations							
		5%		10%		15%		20%	
		Radial growth (mm)	Per cent inhibition	Radial growth (mm)	Per cent inhibition	Radial growth (mm)	Per cent inhibition	Radial growth (mm)	Per cent inhibition
1.	*Zingiber officinale*	20.33	77.41	33.67	62.59	30.00	66.67	25.33	71.85
2.	*Curcuma longa*	46.33	48.52	44.17	50.92	45.17	49.81	36.00	60.00
3.	*Phyllanthus emblica*	63.67	29.26	61.33	31.85	56.67	37.04	56.50	37.22
4.	*Cinnamomum tamale*	77.67	13.70	75.33	16.29	81.67	9.26	80.17	10.92
5.	*Murraya koenigii*	74.00	17.78	76.33	15.18	76.83	14.63	73.33	18.52
6.	*Syzygium cumini*	70.50	21.67	69.67	22.59	65.17	27.59	56.67	37.04
7.	*Nerium oleander*	79.83	11.30	79.50	11.67	77.83	13.52	78.83	12.41
8.	*Cassia fistula*	75.00	16.67	76.17	15.37	74.00	17.78	70.00	22.22
9.	*Aloe vera*	83.67	7.04	85.00	5.56	85.17	5.37	84.67	5.93
10.	*Melia azedarach*	75.67	15.92	76.33	15.18	76.83	14.63	71.33	20.74
11.	*Cinnamomum camphora*	77.17	14.25	71.17	20.92	78.50	12.78	75.00	16.67
	Check	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00
CD (A) at 5% = 2.65; CD (B) at 5% = 1. 53; CD (A*B) at 5% = 5.30; SEM (A*B) ± = 1.88; CV= 4.74 A= for oils B= for concentration A*B = for interaction									

B. Effect of various essential oils on radial growth of Curvularia lunata

Different essential oils *viz*; Peppermint, Lemon grass, Corn mint, Patchouli, Spearmint, Clove, Eucalyptus, Turmeric, Ginger and Lemon tulsi were screened on the growth of *Curvularia lunata* 28±2°C. The results (Table 2) revealed, at 25 ppm maximum inhibition per cent of the fungus was reported in *Eucalyptus globulus* oil (66.85%) which was at par with that of *Curcuma longa* oil (61.48%) followed by *Zingiber officinale* oil (60.00%) while minimum inhibition was recorded in *Syzygium aromaticum* oil (5%). At 50 ppm, maximum inhibition per cent of the fungus was reported in *Curcuma longa* oil (68.52%) which was at par with that of *Eucalyptus globulus* oil (67.41%) followed by *Zingiber officinale* oil (62.59%) while minimum inhibition was recorded in *Syzygium aromaticum* oil (5.92%). At 100 ppm, maximum inhibition per cent of the fungus was reported in *Eucalyptus globulus* oil (79.63%) followed *Curcuma longa* oil (71.85%) while minimum inhibition was recorded in *Syzygium aromaticum* oil (6.11%). At 250 ppm, maximum inhibition per cent of the fungus was reported in *Eucalyptus globulus* oil (85.86%) which was at par with that of *Curcuma longa* oil (80.74%) followed by *Mentha arvensisint* oil (72.22%) while minimum inhibition was recorded in *Syzygium aromaticum* oil (15.74%).

At 500 ppm, maximum inhibition per cent of the fungus was reported in *Eucalyptus globulus* oil (87.41%) which was at par with that of *Curcuma longa* oil (81.85%) followed by *Syzygium aromaticum* oil (72.22%) while minimum inhibition was recorded in *Cymbopogon martini* oil (15.74%). Thus, from the current study it can be concluded that *Eucalyptus globulus* oil gave better results at all the concentrations evaluated as in comparison to different other essential oils evaluated. The results were in accordance with the findings of Bisht *et al.* (2013) who reported that amongst the evaluated essential oils *viz*; Peppermint oil, Geranium oil, Mentha oil, Palmroza oil and Citronella oil at three different concentrations *viz*; 2µL, 4µL and 8 µL, complete inhibition was found in Citronella oil while Peppermint oil showed complete inhibition at 4µL and 8 µL concentrations respectively. Similar results were in case of Mourão *et al.* (2017) they reported that among the tested concentrations of essential oil, *Cymbopogon citratus* showed promising results under *in vitro* conditions for the control of *Curvularia lunata* and suggested it as a massive alternative for the management of Curvularia leaf spot disease on maize. The various concentrations of 5.0 and 7.5 µL mL^{-1} of the *C. citrates* oil also totally inhibited the conidia germination. Also, the results were similar to findings of Kishore and Pande (2007).





***A. M. piprata* B.** *C. martini* **C.** *M.arvensis* **D.** *P. patchouli* **E.** *M. spicata* **F.** *S. aromaticum* **G.** *E. globulus* **H.** *C. longa* **I.** *Z. officinale* **J.** *V. officinalis.*

Plate 2. Effect of different essential oils on radial growth of *Curvularia lunata* at 28±2°C.

Table 2: Effect of different essential oils on radial growth of *Curvularia lunata.*

Sr. No.	Essential oils	Concentrations									
		25 ppm		50 ppm		100 ppm		250 ppm		500 ppm	
		Radial growth (mm)	Per cent inhibition	Radial growth (mm)	Per cent inhibition	Radial growth (mm)	Per cent inhibition	Radial growth (mm)	Per cent inhibition	Radial growth (mm)	Per cent inhibition
1.	*M. piprata*	75.83	15.74	64.83	27.96	42.00	53.33	26.50	70.56	26.5	70.56
2.	*C. martini*	81.17	9.81	80.00	11.11	75.33	16.29	73.00	18.89	62.33	30.74
3.	*M. arvensis*	84.17	6.48	83.83	6.85	81.50	9.44	25.00	72.22	20.67	77.04
4.	*P. patchouli*	76.33	15.19	68.00	24.44	51.50	42.77	50.50	43.89	41.17	54.26
5.	*M. spicata*	80.17	10.93	81.00	10.00	76.00	15.55	78.00	13.33	28.50	68.33
6.	*S. aromaticum*	85.50	5.00	84.67	5.92	84.50	6.11	75.83	15.74	18.17	79.81
7.	*E. globulus*	29.83	66.85	29.33	67.41	18.33	79.63	13.00	85.56	11.33	87.41
8.	*C. longa*	34.67	61.48	28.33	68.52	25.33	71.85	17.33	80.74	16.33	81.85
9.	*Z. officinale*	36.00	60.00	33.67	62.59	29.00	67.78	27.33	69.63	27.33	69.62
10.	*V. officinalis*	82.50	8.33	68.33	24.07	70.33	21.85	70.5	21.67	66.67	25.92
	Check	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00
CD (A) at 5% = 2.48; CD (B) at 5% = 1.67; CD (A*B) at 5% = 5.55; SEM (A*B) ± = 1.98; CV= 6.14 A= for oils B= for concentration A*B = for interaction											

C. Effect of different cow urine concentrations on radial growth of Curvularia lunata at 28±2 °C

The results (Table 3) revealed, maximum per cent inhibition in 10 percent onwards followed by at 5% (29.44%) and at 1 % (18.70%).

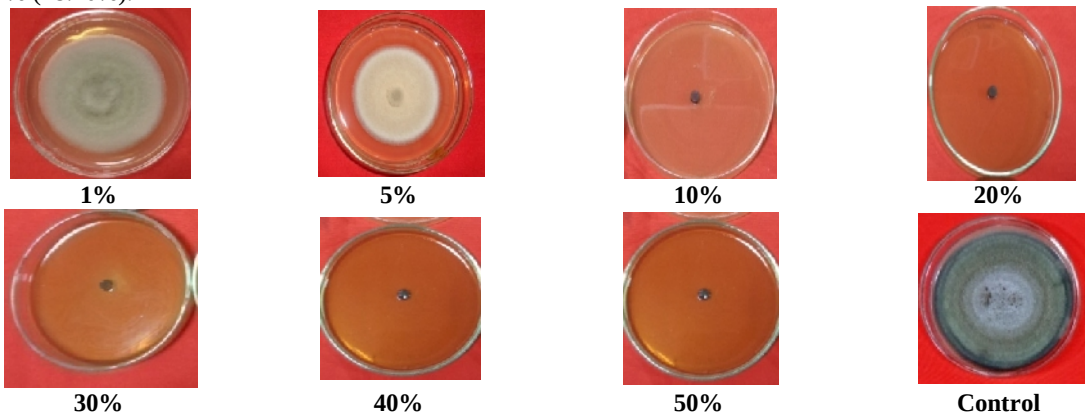


Plate 3. Effect of different cow urine concentrations on radial growth of *Curvularia lunata* at 28±2°C.

Table 3: Effect of different cow urine concentrations on radial growth of *Curvularia lunata* at 28±2°C.

Sr. No.	Per cent concentration	Radial growth (mm)	Per cent inhibition
1.	1	73.17	18.70
2.	5	63.50	29.44
3.	10	0.00	100.00
4.	20	0.00	100.00
5.	30	0.00	100.00
6.	40	0.00	100.00
7.	50	0.00	100.00
8.	Check	90.00	0.00
CD (at 5%) = 3.37; SEM ± = 1.12; CV = 6.87			

The results were in accordance with the findings of Sharma *et al.* (2010), they reported that cow urine when evaluated at different concentrations, the percentage germination was minimum (average, 5.75%) at 5% of four fungal species *Alternaria alternata*, *Fusarium oxysporium*, *Colletotrichum capsici* and *Curvularia lunata* that were used for germination characters.

CONCLUSIONS

The findings of the study revealed that evaluation of essential oils, botanicals and cow urine *in vitro* conditions, against the test pathogen was done for better understanding of the host pathogen relationship. The salient findings of the investigations were that amongst the different essential oils screened against the growth of *C. lunata* 28±1°C it was found that *E. globulus* oil gave better results at all the concentrations evaluated as compared to other essential oils as highest inhibition (87.41%) was found in *E. globulus* oil at 500 ppm which was at par with same oil at 250 ppm. Whereas in case of different botanicals evaluated at different concentrations *viz*; 5, 10, 15 and 20%, it was found that *Z. officinale* was most effective at 20% for inhibiting the growth (71.85%) of the fungus which was at par with *Z. officinale* at 15% (66.67%) followed by *C. longa* at 20% (60.01 %). Similarly, cow urine was also evaluated at different concentrations *viz;* 1, 5, 10, 20, 30, 40 and 50 per cent for growth inhibition of *C. lunata* and the results were recorded as maximum per

cent inhibition in 10 % onwards followed by at 5% (29.44%) and minimum was found at 1 % (18.70%).

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Conflict of Interest. None.

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