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Antifungal effect of aqueous and cow urine extracts of native plants against *Alternaria brassicicola* causing leaf spot of cauliflower

SHALINI SHARMA, AMIT SHARMA[1] AND MONICA SHARMA*

Department of Plant Pathology, [1]Department of Basic Sciences,
College of Horticulture and Forestry,
Dr Y S Parmar University of Horticulture and Forestry (Solan), Neri, Hamirpur-177 001
**E-mail: dmonicasharma@gmail.com*

ABSTRACT

Cauliflower (*Brassica oleracea* L. var. *botrytis*) is one of the most widely grown, delicious winter vegetables of the Cruciferae family. Alternaria leaf spot is the major constraint in cauliflower production. In the present study, the effect of six aqueous-based and six cow urine-based plant extracts of native plants namely *Justicia adhatoda, Ocimum tenuiflorum*, *Tagetes erecta*, *Phyllanthus emblica*, *Vitex negundo,* and *Aegle marmelos* at various concentrations was evaluated against *Alternaria brassicicola* causing leaf spot of cauliflower. Cow urine as alone was also tested to check its effectiveness against the fungal pathogen. Among aqueous extracts, maximum mycelial inhibition of 65.47% was observed by using the extract of *P. emblica* at 50% concentration. Cow urine alone resulted in complete inhibition in mycelial growth of pathogen at different tested concentrations i.e. 10, 15, and 20%. While, in cow urine-based extracts, maximum mycelial inhibition of 53.33 and 51.67% was observed by using the extract of *O. tenuiflorum* and *P. emblica* at 20% concentration. Thus, aqueous and cow urine-based plant extracts could be exploited for the management of Alternaria leaf spot disease of cauliflower.

Key words: Bio-pesticides, natural farming, eco-friendly disease management, *Alternaria*, cole crops

Cauliflower (*Brassica oleracea* L. var. *botrytis*) is one of the most widely grown winter vegetables in the world for its edible curd. It plays an important role in the human diet due to its attractive appearance, good taste, and its nutritional value. It belongs to the cole group of vegetables which originated from a single wild ancestor *Brassica oleracea* L. var. *sylvestris* commonly known as wild cabbage. Major cauliflower-growing countries are China, India, Spain, Mexico, Italy, France, the United States of America, Poland, Pakistan and Egypt. Cauliflower follows cabbage in importance with regard to area and production in the world. However, in India, cauliflower is more widely grown than cabbage. The total cultivated area of cauliflower in India is 459 thousand hectares having a total production of 8844 thousand MT/ha with the productivity of 19.27 MT/ha (National Horticulture Board, 2019). The agro-climatic conditions in Himachal Pradesh provide an opportunity for the production of off-season cauliflower for sustainable income to the farmer. Cauliflower can be produced in the hills during the period when it cannot be grown in the adjoining plains. The annual temperate types or early summer cauliflowers have been more suited for off-season cultivation in the hills as they give curds during the early summer season when production of quality cauliflowers is limited in plains due to higher temperatures (Kumar *et al.,* 2014). As cauliflower is a very sensitive crop, it is prone to various biotic and abiotic stresses. Plant diseases have been identified as a limiting factor in cauliflower production. Among various fungal diseases, Alternaria leaf spot or blight is a major constraint in cole crop production. It causes major production losses in many parts of the world (Dillard *et al*., 1998; Pattanamahakul and Strange, 1999), including India (Meena *et al*., 2012; Sharma *et al.,* 2013). The disease is caused by two species of *Alternaria.* The vegetable *Brassicas* i.e. cauliflower, cabbage and broccoli are mainly affected by *Alternaria brassicicola* (Schwein) Wiltshire and *Alternaria brassicae* (Berkeley) Saccardo whereas,

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the oleiferous *Brassica* seed crops are mainly affected by *A. brassicae* (Michereff *et al.,* 2012; Kumar *et al.,* 2014). A maximum 64 % disease incidence and 30.4 % disease severity have been recorded from different parts of the country (Sailaja *et al.,* 2017; Ekabote *et al.,* 2017; Ansar and Ghatak**,** 2018; MadhuKiran *et al.,* 2018*;* Valvi *et al.,* 2019 a).

High usage of fungicides over the crops to manage the disease leads to residue issues in the food chain. Though the spray of fungicides has been widely accepted and considered as important, it is imposing a high danger for pathogens to attain resistance against it. As a result, new strains of pathogens are evolving. As an alternative to fungicides, plant extracts and cow urine have anti-fungal properties, and are being used against plant pathogens. Plant extracts from many different species of plants were reported for antifungal activity against the fungal pathogens of important crops (Gurjar *et al*., 2012; Hernández-Ceja *et al*., 2021; Lee *et al*., 2022). However, very limited reports on the management of Alternaria leaf spot of cole crops, particularly on cauliflower, through plant extracts are available in literature. Keeping these points in mind, the present study was conducted to test the effect of six aqueous-based and six cow urine-based plant extracts of *Justiciaa dhatoda,Ocimum tenuiflorum*, *Tagetes erecta*, *Phyllanthus emblica*, V*itex negundo,* and *Aegle marmelos* at various concentrations against *Alternaria brassicicola* causing leaf spot of cauliflower.

MATERIALS AND METHODS

Isolation and purification of the pathogen

The cauliflower leaves showing typical symptoms of Alternaria leaf spot were collected from nearby areas and were brought to the laboratory for isolation of the pathogen. Isolation was done by following the standard isolation procedure in Petri plates containing sterilized potato dextrose agar (PDA) medium (Dhingra and Sinclair, 1985). These plates were incubated at 28°C temperature for growth of the pathogen. The culture was purified by subculturing mycelium onto the PDA slants.

Preparation of plant extracts

Aqueous and cow urine-based extracts of six native plants viz., *J. adhatoda, O. tenuiflorum, T. erecta, P. emblica, V. negundo* and *A. marmelos* were prepared from freshly harvested leaves. Leaves were thoroughly washed in running tap water and were dried overnight. 200 g of each sample leaf was ground in a mixer and blender by adding a small amount of sterilized distilled water. After grinding, the final volume of the each sample extract was adjusted to 200 ml by addition of sterilized distilled water. The plant extracts were sieved using sterilized muslin cloth and 200 ml of plant extract was collected. In the case of preparation of cow urine-based botanical extracts, the same procedure was followed as mentioned above except that distilled water was replaced with cow urine for grinding the leaves and for adjusting the final volume to 200 ml.

Evaluation of plant extracts against the pathogen

Aqueous and cow-urine based extracts of native plants were evaluated *in vitro* for their efficacy in inhibiting the mycelial growth of *A. brassicola* by Poisoned Food Technique at different concentrations (Table 1). The experiment was conducted in a completely randomized design. Each treatment was replicated thrice. The desired concentrations of the aqueous and cow urine-based botanical extracts were prepared in sterilized distilled water and by mixing them with the required quantity of sterilized double strength potato dextrose agar (PDA) medium. Sterilization of the botanical extracts was done through tyndallisation in an autoclave at a pressure 5 lb psi for 30 minutes for three consecutive days. The extracts were poured aseptically in sterilized Petri plates. The double-strength PDA medium was mixed with an equal quantity of sterilized distilled water to maintain the control treatment. After solidification of the medium amended with the desired concentration of aqueous and cow urine-based plant extracts, the Petri plates were then inoculated with mycelial discs of 5 mm diameter, taken from an actively growing culture of *A. brassicicola*, in the center and were incubated at 28°C. The Petri plates were observed periodically for the growth of the pathogen and colony diameter was measured when control Petri plates were completely filled with mycelial growth of the pathogen.

Statistical analysis was performed by two-way ANOVA using OPSTAT software (Sheoran *et al.,* 1998).

Table 1. Aqueous and cow urine extracts of native plants screened against *Alternaria brassicicola*

Plant	Common name of the plant	Concentration of aqueous extracts (%)	Concentration of cow urine extracts (%)
Justicia adhatoda	Malabar nut	10, 20, 30, 40, 50	10, 15, 20
Ocimum tenuiflorum	Holy basil	10, 20, 30, 40, 50	10, 15, 20
Tagetes erecta	African marigold	10, 20, 30, 40, 50	10, 15, 20
Phyllanthus emblica	Indian gooseberry	10, 20, 30, 40, 50	10, 15, 20
Vitex negundo	Chaste tree	10, 20, 30, 40, 50	10, 15, 20
Aegle marmelos	Japanese bitter orange	10, 20, 30, 40, 50	10, 15, 20

Per cent inhibition in mycelial growth was calculated by using following formula given by Vincent (1947):

Mycelial growth inhibition (%) = {(C-T)/C} x 100

where, C = Growth of test pathogen in absence of plant extract (mm)

T = Growth of test pathogen in presence of plant extract (mm)

RESULTS AND DISCUSSION

Evaluation of aqueous plant extracts against *A. brassicicola*

Aqueous extracts of *P. emblica, T. erecta*, *O. tenuiflorum,* and *A. marmelos* had antifungal effects against the mycelial growth of *A. brassicicola* at different concentrations. The overall mean indicated that the minimum average radial growth of *A. brassicicola* was observed by using the extract of *P. emblica* (37.07 mm radial growth) with a maximum inhibition of 47.05% (Table 2). It was followed by significantly different 12.72% inhibition by an extract of *T. erecta* (61.10 mm radial growth) (Fig. 1). In contrast, the maximum average radial growth of 70.00 mm, which was equal to control, was observed in the aqueous extract of *V. negundo* and *J. adhatoda*.

At 50 % concentration, the radial growth of *A. brassicicola* was minimum in aqueous extract of *P. emblica* (24.17 mm) with maximum mycelial inhibition of 65.47 % followed by an extract of *T. erecta (*44.83 mm) with inhibition of 35.95%. No inhibition in the mycelial growth of the pathogen was observed with aqueous extract of *V. negundo* and *J. adhatoda* at all the tested concentrations i.e., 10, 20, 30, 40, and 50 %. In addition, no inhibition in mycelial growth of pathogen was observed with aqueous extracts of *A. marmelos* at 10% concentration, *T. erecta* at 10 and 20 % concentration, and *O.*

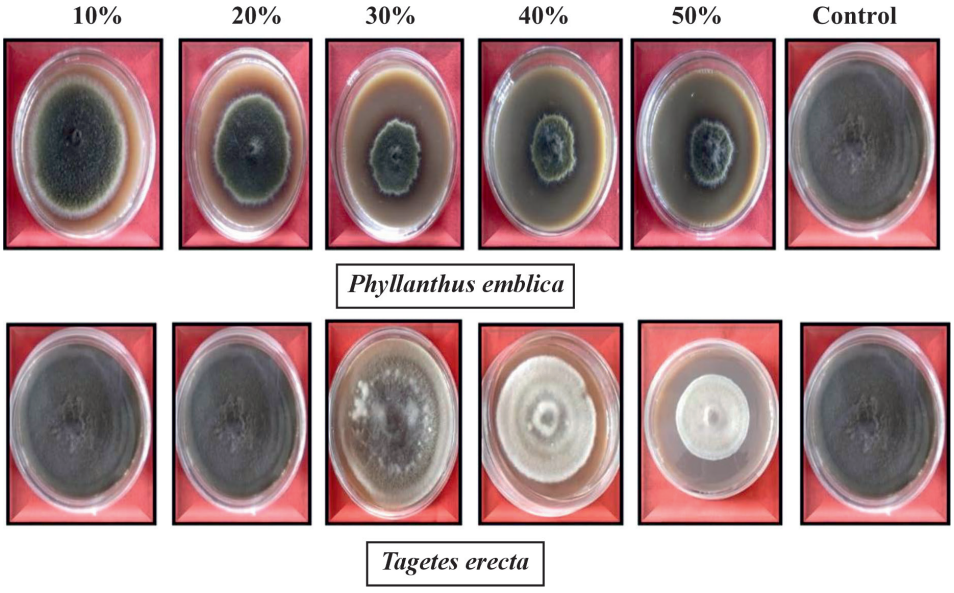

Fig. 1. Effect of aqueous extract of *Phyllanthus emblica* and *Tagetes erecta* on mycelial growth of *Alternaria brassicicola*

Table 2. Effect of aqueous plant extracts on mycelia growth of *Alternaria brassicicola*

Aqueous extract	Radial growth (mm)						Inhibition in mycelia growth (%)					
	Concentration (%)						Concentration (%)					
	10	20	30	40	50	Mean	10	20	30	40	50	Mean
Vitex negundo	70.00	70.00	70.00	70.00	70.00	70.00^{e}	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)^{d}
Justicia adhatoda	70.00	70.00	70.00	70.00	70.00	70.00^{e}	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)^{d}
Aegle marmelos	70.00	63.83	62.83	62.50	60.67	63.97^{c}	0.00 (0.00)	8.81 (17.24)	10.24 (18.64)	10.71 (19.10)	13.34 (21.39)	8.62 (15.27)^{b}
Phyllanthus emblica	54.83	44.67	34.83	26.83	24.17	37.07^{a}	21.67 (27.72)	36.19 (36.96)	50.24 (45.11)	61.67 (51.74)	65.47 (53.99)	47.05 (43.10)^{a}
Tagetes erecta	70.00	70.00	68.00	52.67	44.83	61.10^{b}	0.00 (0.00)	0.00 (0.00)	2.86 (9.52)	24.76 (29.79)	35.95 (36.82)	12.72 (15.23)^{b}
Ocimum tenuiflorum	70.00	70.00	70.00	70.00	52.17	66.43^{d}	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	25.48 (30.29)	5.10 (6.06)^{c}
Control	70.00	70.00	70.00	70.00	70.00	70.00^{e}						
Mean	67.83^{e}	65.50^{d}	63.67^{c}	60.29^{b}	55.98^{a}		3.61 (4.62)^{e}	7.5 (9.03)^{d}	10.56 (12.21)^{c}	16.19 (16.77)^{b}	23.37 (23.75)^{a}	
$CD_{(0.05)}$ Aqueous extract = 1.02 Concentration = 0.86 Aqueous extract × Concentration = 2.29							$CD_{(0.05)}$ Aqueous extract =1.58 (1.02) Concentration = 1.44 (0.93) Aqueous extract × Concentration = 3.54 (2.28)					

Figures in parentheses are arc sine transformed values; Figures denoted by same letters in the columns are not significantly different at p=0.05.

tenuiflorum at 10, 20, 30 and 40 % concentration.

The use of various herbal extracts and natural products is being encouraged for management of plant diseases because they pose no health hazard. Plant extracts have both direct and indirect effects on plant disease management. The plant extracts have a direct antifungal effect on the growth of the pathogens and hence can directly manage the plant diseases. On the other hand, they can also alter many biochemical changes in the plants which in turn activate defence mechanisms against pathogens. The data from the present study demonstrated that the extracts of native plants *viz., P. emblica* and *T. erecta* could be used for the management of Alternaria leaf spot of cauliflower. Sasode *et al*. (2012) evaluated the fungitoxicity of crude extracts of some plants *viz*. *Azadirachta, Eucalyptus, Datura, Mentha, Ocimum* and *Lantana* against *A. brassicae* and *A. brassicicola* causing leaf spot of rapeseed- mustard and found minimum radial growth in extract of *Azadirachta indica* (20.73 mm) followed by *Eucalyptus* sp. (23.15 mm), *Ocimum* sp. (32.12 mm), *Lantana camera* (35.50 mm), *Datura stramonium* (39.22 mm) and *Mentha* sp. (45.26 mm) at 10% concentration. Khalse *et al.* (2017) reported that the maximum mycelial inhibition of 58.69 % was observed by using *Eucalyptus* leaf extract at 10 % concentration against *A. brassicae* isolated from cabbage leaves followed by *Lantana* leaf extract (54.96 %) and *Datura* leaf extract (52.17 %) at 10 % as compared to untreated control. In another study, Valvi *et al.* (2019 b) observed that maximum per cent inhibition of *A. brassicae* over control was obtained by fruit extract of soap nut (*Sapindus trifoliatus*) (80.56 %). Meena *et al.* (2022) evaluated the efficacy of various plant extracts (at two concentrations *viz*., 5 and 10 per cent) against *Alternaria brassicae*. Among five plant extracts, the garlic extract was found significantly superior in inhibiting mycelial growth of the pathogen (89.40% and 91.68%) followed by neem (83.45% and 87.50%) and extract of ginger (80.30% and 84.68%).

Table 3. Effect of cow urine based biopesticides on mycelial growth of *Alternaria brassicicola*

Cow urine extract	Radial growth (mm)				Inhibition in mycelia growth (%)			
	Concentration (%)				Concentration (%)			
	10	15	20	Mean	10	15	20	Mean
Vitex negundo	70.00	64.67	63.33	66.00^{e}	0.00 (0.00)	7.62 (16.00)	9.52 (17.89)	5.71 (11.30)^{e}
Justicia adhatoda	70.00	69.33	69.00	69.44^{f}	0.00 (0.00)	0.95 (4.58)	1.43 (6.86)	0.79 (3.81)^{f}
Aegle marmelos	53.33	52.00	48.33	51.22^{d}	23.81 (29.18)	25.71 (30.46)	30.95 (33.72)	26.82 (31.12)^{d}
Phyllanthus emblica	41.33	35.33	33.83	36.83^{b}	40.95 (39.77)	49.52 (44.71)	51.67 (45.94)	47.38 (43.48)^{b}
Tagetes erecta	70.00	69.67	69.33	69.67^{f}	0.00 (0.00)	0.48 (2.29)	0.95 (4.58)	0.48 (2.29)^{f}
Ocimum tenuiflorum	50.83	41.67	32.67	41.72^{c}	27.38 (31.54)	40.48 (39.48)	53.33 (46.90)	40.40 (39.30)^{c}
Cow urine	0.00	0.00	0.00	0.00^{a}	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)^{a}
Control	70.00	70.00	70.00	70.00^{f}				
Mean	53.19^{c}	50.33^{b}	48.31^{a}		27.45 (27.13)^{c}	32.11 (32.41)^{b}	35.41 (35.04)^{a}	
CD (p=0.05) Cow urine extract =1.54 Concentration = 0.95 Cow urine extract × Concentration =2.68					CD (p=0.05) Cow urine extract = 2.37 (2.04) Concentration = 1.55 (1.33) Cow urine extract × Concentration =4.10 (3.53)			

Figures in parentheses are arc sine transformed values; Figures denoted by same letters in the columns are not significantly different at p=0.05.

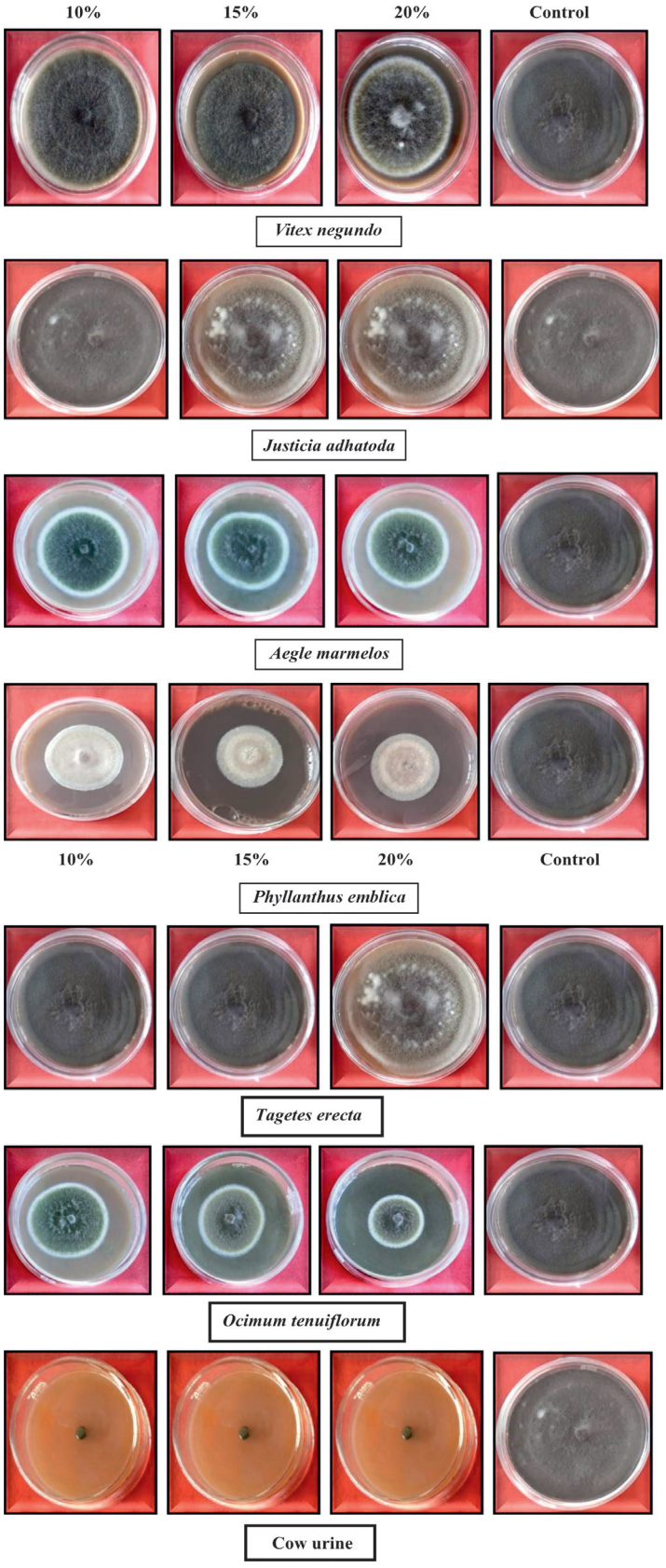

Fig. 2. Effect of cow urine plant extracts against mycelial growth of *Alternaria brassicicola* causing leaf spot of cauliflower

Evaluation of cow urine-based plant extracts against *A. brassicicola*

Minimum average radial growth of *A. brassicicola* was observed by using cow-urine based extract of *P. emblica* having 36.83 mm colony diameter with maximum mycelial inhibition of 47.38% followed by significantly different cow-urine based extracts of *O. tenuiflorum* and *A. marmelos* with 41.72 and 51.22 mm mycelial growth, respectively and inhibition of 40.40 and 26.82%, respectively (Table 3, Fig. 2). In contrast, maximum average radial growth of *A. brassicicola* was observed by using cow-urine based extract of *T. erecta* with 69.67 mm diameter and 0.48% inhibition which was statistically at par with diametric colony growth of 69.44 mm in cow-urine based extract of *J. adhatoda* with only 0.79% inhibition. Cow urine alone used as a biopesticide resulted in no radial growth with 100% mycelia inhibition at different tested concentrations i.e. 10, 15 and 20%.

Analysis of interaction between cow-urine based extracts of native plants with different concentrations revealed that minimum radial growth of the pathogen (32.67 mm) was observed by using extract of *O. tenuiflorum* at 20 % concentration with maximum 53.33% inhibition which is statistically at par with *P. emblica* at 15 and 20 % concentration with 35.33 mm and 33.83 mm colony diameter and mycelial inhibition of 49.52 % and 51.67 %, respectively. No inhibition in mycelial growth of the pathogen was observed by using extract of *V. negundo*, *J. adhatoda* and *T. erecta* at 10 % concentration (Fig. 2).

The antifungal activity of cow-urine based plant extracts against a few plant pathogens has been reported in the literature (Ashlesha and Paul, 2011; Ashlesha *et al.,* 2013; Akarsh *et al.,* 2016; Shridhar *et al*., 2019). However, such reports are lacking against the *Alternaria* spp. The efficacy of cow urine at 15 % concentration was reported against *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii* (Jandaik *et al*., 2015). Akarsh *et al.* (2016) reported inhibition of 90 % from cow urine extract of *Peristrophe bicalyculata* and *Crotalaria filipes* against *Colletotrichum capsici* causing anthracnose of chilli. Shridhar *et al*. (2019) reported that among five cow-urine based biopesticides against *Phytophthora nicotianae* var. *parasitica* causing buckeye rot of tomato*, Eucalyptus tereticornis* was found most effective with 71.89 % inhibition in mycelial growth of the pathogen followed by *Artemisia vulgaris* (68.74 %), *Lantana camara* (63.58 %) and *Murraya koenigii* (59.51 %). Cow-urine based botanical extract of *Melia azedarach* was the least effective with 55.41 % mycelial growth inhibition of *P. nicotianae* var. *parasitica*. A cow urine-based formulation known as 'Panchagavya,' which contains urine, dung, milk, curd, and ghee, has been shown to have antifungal activity against a variety of phytopathogenic fungi (Pathak and Kumar, 2003; Sugha, 2009; Ashlesha *et al.,* 2013; Rathore and Patil, 2019; Ram *et al.,* 2020). Furthermore, cow urine has the ability to kill pesticide-resistant and herbicide-resistant bacteria, viruses, and fungi, making it an excellent biofertilizer and biopesticide in agricultural operations (Dharma *et al.,* 2005). As a result, the use of cow urine may be a better alternative to synthetic chemicals, which are costly and may pose a risk to farmers, marketers, consumers, and the environment.

Alternative chemical research is currently a major concern for the food industry. The antifungal and antibacterial activity of plant extracts needs to be exploited to broaden knowledge in this area. The plant extracts have a direct antifungal effect and also have resistance mechanisms that are useful in controlling various plant diseases. Based on the results of the present study, it is possible to conclude that the aqueous extract of *Phyllanthus emblica*, cow urine extracts of *Ocimum tenuiflorum*, *Phyllanthus emblica* and cow urine alone has promising potential for the organic and environmentally friendly management of Alternaria leaf spot of cauliflower.

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