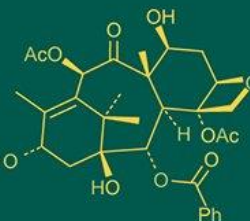
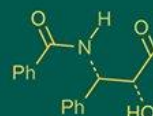
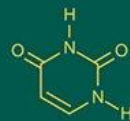


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Ecofriendly management of bacterial leaf rot of onion caused by *Enterobacter cloacae* under *in vitro*

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Abstract

An *in-vitro* evaluation of botanicals and bio-agents was conducted against bacterial leaf rot disease of onion caused by *Enterobacter cloacae*, in the Dept. of Plant Pathology, College of Horticulture, Bagalkot. Among the 15 botanicals evaluated, *Prosopis juliflora* showed highest average zone of inhibition (17.06 and 24.56 mm), followed by *Garcinia indica* (19.22 and 21.39mm) in both aqueous and alcoholic extraction methods, respectively at 1:1, 1:5, 1:10 concentration. The least zone of inhibition was shown by citronella (10.89 mm) in aqueous extraction method and amrithpani in alcoholic extraction method (10.67 mm). Among the ten bio agents PM-2A (*Brevibacillus sp.*) has shown the highest average zone of inhibition 19.33mm and the least inhibition was shown by 11.50 mm.

Key words: *In-vitro*, *Enterobacter cloacae*, *Prosopis juliflora*, *Garcinia indica*, aqueous extraction method, alcoholic extraction method, average zone of inhibition

Introduction

Onion (*Allium cepa* L.) belongs to the family *Alliaceae* and having chromosome number $2n=16$. It is the most widely cultivated species of the genus *Allium*. It is commonly known as “ullagaddi or irulli” in Kannada and “pyaaz” in Hindi. The onion is called as “queen of kitchen” (Priya *et al.*, 2015) [4]. The south-west Asia is considered as the primary centre of domestication. Onion is one of the most important commercial vegetable crops in India and throughout the world. Onion has manifold use such as spice, vegetable, salad-dressing etc.. India (288.77 lakh tons) is the largest producer of onion. In India Maharashtra is the leading producing state. In Karnataka the area under onion is 2.32 lakh hectares, with production of 38.91 lakh tons, (Anon., 2024) [1]. Onion has high value both in domestic use and export purpose, but growing of onion has some constraints such as insect pests and diseases. The pests are thrips, whiteflies, beetles etc. Now a day's bacterial leaf rot is becoming an important disease in northern part of Karnataka from past 8-10 years. There is a need to develop ecofriendly management practice for mitigating the plant diseases. Hence, commercially available bio-agents and botanicals were evaluated against the disease under *in vitro*.

Materials and method

Evaluation of different bio agents

About 10 isolates of bacterial bio agents which were isolated and maintained in laboratory in Department of Plant Pathology of College of Horticulture, Bagalkot were used to evaluate their efficacy against *Enterobacter cloacae* by inhibition zone assay method.

The botanicals and bio agents were evaluated under *in vitro* against the growth of bacteria causing leaf rot of onion at different concentrations by zone of inhibition method. A loop full of 24h old *E. cloacae* culture was inoculated into nutrient broth and incubated for 48h to allow bacterial growth. This broth was seeded into nutrient agar media and then poured into Petri plates and allowed for solidification. Media was bored with cork borer to get 9 mm well, into which different concentrations of botanicals were poured, as mentioned in Table 1. The bio agents were multiplied by following the procedure given by Vijayan *et al.*, 2013 [9]. The bio agents were poured into the well in three replications @ 10^8 cfu/ml concentration. The list of botanicals and bio agents evaluated were given in table 1.

The inoculated plates were incubated at 28 ± 2 °C for 24 h. Each concentration was repeated thrice and observations were recorded by measuring the diameter of the inhibition zone for each concentration and analyzed statistically.

Results

In vitro evaluation of botanicals against the growth of *Enterobacter cloacae*

Aqueous extraction method

The aqueous extracts of 15 botanicals were evaluated against *Enterobacter cloacae* under *in vitro*. Among the different botanicals tested, *Prosopis juliflora* has shown the highest inhibition of 23.33, 19.16 and 15.16 mm, this was followed by *Garcinia indica* has given 22.83, 15.50 and 12.83 mm at different contrations viz., 1:1, 1:5 and 1:10 w/v respectively. The least inhibition was expressed in citronella (12.00, 10.67 and 0.00 mm). While *Pongamia pinnata* and Amrithpani were unable to inhibit the growth of pathogen. The standard check streptocycline (K-cyclin) 500 ppm + copper oxychloride 3000 ppm recorded maximum inhibition of 38.33 mm which found superior over all the botanicals tested, whereas no inhibition zone was recorded in water control (Table 2 and Plate 1).

Alcoholic extraction method

The results on effect of botanicals with alcoholic extraction are presented in Table 15. Among the tested botanicals *Prosopis juliflora* has shown the highest inhibition of 32.16, 23.33 and 18.17 mm and this was followed by *Garcinia indica*, recorded inhibition zone of 26.50, 20.17 and 17.50 mm at 1:1, 1:5 and 1:10 w/v concentrations, respectively. The least inhibition was seen in case of amrithpani 11.33, 10.66 and 0.00 mm. *Pongamia pinnata* (0.00 mm) failed to show inhibition of bacterial growth at all three concentrations. The standard check (streptocycline 500 ppm + copper oxychloride 3000 ppm) was recorded maximum inhibition of 38.33 mm, which found superior to all the botanicals tested. While the water control was shown no inhibition zone (Table 3 and Plate 1).

In vitro evaluation of antagonistic bacterial bio agents on the growth of *Enterobacter cloacae*

Ten antagonistic bio agents collected from the Dept. Plant Pathology were screened against growth of bacteria causing leaf rot of onion. The outcome of the *in vitro* evaluation showed that some bio agents did not able to inhibit the growth of the pathogenic bacterium viz., VK-6B (*Lysinibacillus xylanilyticus*), BK-6 (*Brevibacillus borstelensis*), KK-9A (*Bacillus subtilis*), KK-3A and BK-8, which showed 0.00 mm zone of inhibition. The highest mean zone of inhibition was observed in PM-2A (*Brevibacillus sp.*) (19.33 mm) which was followed by BK-5 (15.33 mm) and BK-3 (13.50 mm). The least mean zone of inhibition (11.50 mm) was observed in CK-13A. The results were depicted in table 4 and Plate 3.

Discussion

In vitro evaluation of botanicals against the growth of *Enterobacter cloacae* of onion

Fifteen botanicals were screened against the growth of *Enterobacter cloacae*. Among the different tested botanicals *Prosopis juliflora* with concentration 1:1, 1:5 and 1:10 w/v was found to be most effective with the highest mean zone of inhibition of 21.88 mm, which is followed by *Garcinia*

indica (19.22 mm) and *Tamarindus indica* (19.02mm) at different concentrations. The lowest inhibition was shown by Amrithpani (10.33 mm).

Among the tested botanicals *Prosopis juliflora* has shown the highest inhibition of 24.56 mm and this was followed by *Garcinia indica*, which recorded inhibition zone of 21.39 mm at 1:1, 1:5 and 1:10 w/v concentrations respectively. The least inhibition was seen in case of amrithpani (10.67 mm). *Pongamia pinnata* (0.00 mm) failed to show inhibition of bacterial growth at all the three concentrations. The standard check (Streptocycline 500ppm + copper oxychloride 3000ppm) recorded maximum inhibition of 28.44 mm and was found superior to all the botanicals tested.

Prosopis juliflora found to contain different group of alkaloids isolated from this plant are reported to possess significant antibacterial and antifungal activity which is comparable to the standard drugs (Ragavendra *et al.*, 2010). Kokum rind possesses an important phenolic compound called as garcinol which possess antibacterial properties (Sutar *et al.*, 2012)^[8].

In vitro evaluation of antagonistic bacterial bio agents on the growth of *Enterobacter cloacae*

Ten antagonistic bio agents collected from the Dept. Plant Pathology were screened against growth of bacteria causing leaf rot of onion. The outcome of the *in vitro* evaluation showed that some bio agents did not able to inhibit the growth of the pathogenic bacterium viz., VK-6B (*Lysinibacillus xylanilyticus*), BK-6 (*Brevibacillus borstelensis*), KK-9A (*Bacillus subtilis*), KK-3A and BK-8, which showed 0.00 mm zone of inhibition. The highest mean zone of inhibition was observed in PM-2A (*Brevibacillus sp.*) (19.33 mm) which was followed by BK-5 (15.33 mm) and BK-3 (13.50 mm). The least mean zone of inhibition (11.50 mm) was observed in CK-13A. While the water control was shown no inhibition zone (Table 4 and Plate 3).

Under biological control of plants disease, various antagonistic organism has been identified, which fight against the pathogen by different mechanisms viz., competition, lysis, antibiosis, siderophore production and hyper parasitism.

The result obtained in the study showed similarity with the earlier research works, by Xianling *et al.* (2008)^[11] against bacterial wilt (*Ralstonia solanacearum*) by biocontrol management of mulberry by an endophytic *Bacillus subtilis*. *In vitro*, *Ralstonia solanacearum* by 4 isolates of bacillus in these 4 isolates L4144 was effectively reduced the disease incidence. This L4144 strain was identified as *Bacillus subtilis*.

Khosro *et al.* (2012)^[2] were evaluated antibacterial activity of different *Bacillus* spp. against *X. campestris* pv. *campestris* and *Pectobacterium carotovorum* subsp. *carotovorum*. They were used 8 epiphytic *Bacilli* viz., *Bacillus pumilus* (3 strains), *Bacillus thuringiensis* (2 strains), *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus cereus*, which were isolated from plants such as cabbage, carrot, radish and kale. They used the paper disc method for screening of these bacteria against pathogen. Among the different species of *Bacillus* spp., *B. thuringiensis*, *B. cereus*, *B. subtilis*, *B. megaterium* and *Bacillus pumilus* found effective against the pathogens (*X. c. pv. campestris* and *P. c. subsp. carotovorum*).

Table 1: List of botanicals and bio agents used for *in-vitro* evaluation against *E. cloacae* of onion

Sl. No.	Botanicals	Scientific Name	Plant part used	Dosage (1:1, 1:5, 1:10)	Sl. No.	Bio agents
1	Garlic	<i>Allium sativum</i>	Clove		1	PM-2A (<i>Brevibacillus sp.</i>)
2	Kokum	<i>Garcinia indica</i>	Fruit		2	VK-6B (<i>Lysinibacillus xylanilyticus</i>)
3	Neem	<i>Azadiracta indica</i>	Leaves		3	BK-6 (<i>Brevibacillus borstelensis</i>)
4	Pongamia	<i>Pongamia pinnata</i>	Pod		4	KK-9A (<i>Bacillus subtilis</i>)
5	Prosopis	<i>Prosopis juliflora</i>	Leaves		5	VK-10C (Unidentified)
6	Citronella oil	<i>Cymbogon winteranius</i>	Oil		6	KK-3A (Unidentified)
7	Clove bud oil	<i>Eugenia caryophyllata</i>	Oil		7	BK-5 (Unidentified)
8	Eucalyptus oil	<i>Eucalyptus citriodora</i>	Oil		8	BK-3 (Unidentified)
9	Gingr	<i>Gingiber officinale</i>	Rhizome		9	CK-13A (Unidentified)
10	Tamarind	<i>Tamarindus indica</i>	Fruit		10	BK-8 (Unidentified)
11	Basil	<i>Basilla alba</i>	Leaves			
12	Marigold	<i>Tagetus erecta</i>	Leaves			
13	Pomegranate	<i>Punica granatum</i>	Rind			
14	Aloevera	<i>Aloe barbadensis</i>	Leaves			
15	Amrithpani		Cow dung, Honey, Ghee. Distilled water			
17	Streptocycline @ 500 ppm + COC @ 3000 ppm (Positive control)					
Control	Distilled Water (Negative control)					

Table 2: Effect of Aqueous extracts of botanicals against *Enterobacter cloacae* under *in-vitro* condition

Sl No.	Treatments	Mean zone of inhibition (mm)			Mean (Botanicals)
		Dilutions			
		1:1	1:5	1:10	
1	Aloevera (<i>Aloe barbadensis</i>)	12.67 (3.69)	10.83 (3.44)	10.00 (3.32)	11.17 (3.48)
2	Amrithpani	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
3	Basil (<i>Osimum basillum</i>)	13.83 (3.85)	12.33 (3.65)	10.83 (3.44)	12.33 (3.65)
4	Citronella (<i>Cymbopogan citratus</i>)	12.00 (3.60)	10.67 (3.41)	0.00 (1.00)	10.89 (3.44)
5	Clove oil (<i>Syzygium aromaticum</i>)	19.17 (4.49)	15.67 (4.08)	13.00 (3.74)	15.94 (4.10)
6	Eucalyptus (<i>Eucalyptus citriodora</i>)	12.50 (3.67)	11.00 (3.46)	10.50 (3.39)	11.33 (3.51)
7	Garlic (<i>Allium sativum</i>)	15.17 (4.02)	14.17 (3.89)	11.50 (3.53)	13.61 (3.81)
8	Ginger (<i>Gingiber officinalis</i>)	14.83 (3.98)	14.17 (3.89)	10.83 (3.44)	13.28 (3.77)
9	Kokum (<i>Garcinia indica</i>)	22.83 (4.88)	15.50 (4.06)	12.83 (3.71)	17.06 (4.22)
10	Marigold (<i>Tagetus erecta</i>)	15.17 (4.019)	12.67 (3.69)	0.00 (1.00)	12.61 (3.68)
11	Neem (<i>Azadiracta indica</i>)	15.17 (4.02)	12.67 (3.69)	9.83 (3.29)	12.56 (3.67)
12	Pomegranate (<i>Punica granatum</i>)	16.37 (4.54)	17.20 (4.53)	16.50 (4.42)	16.67 (4.49)
13	Pongamia (<i>Pongamia pinnata</i>)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
14	Prosopis (<i>Prosopis juliflora</i>)	23.33 (4.93)	19.17 (4.49)	15.16 (4.09)	19.22 (4.48)
15	Tamarind (<i>Tamarindus indica</i>)	22.50 (4.85)	13.00 (3.73)	10.50 (3.39)	15.33 (3.99)
16	Streptocyclin @ 500ppm + copper oxy chloride - 3g/lit	38.33 (6.27)			
17	Water control	0.00 (1.00)			
	Mean (Dilutions)	16.89 (4.16)	14.65 (3.89)	13.04 (3.68)	
	Source			SEm±	CD @ 1%
	Botanicals			0.055	0.16
	Dilutions			0.027	0.08
	B x D			0.04	0.28

*Figures in the parenthesis are square root transformed

Table 3: Effect of alcohol extracts of botanicals against *Enterobacter cloacae* under *in-vitro* condition

Sl. No.	Treatments	Mean zone of inhibition (mm)			Mean (Botanicals)
		Dilutions			
		1:1	1:5	1:10	
1	Aloevera (<i>Aloe barbadensis</i>)	15.00 (3.99)	12.83 (3.72)	10.67 (3.41)	12.83 (3.71)
2	Amrithpani	11.33 (3.51)	10.67 (3.41)	0.00 (3.317)	10.67 (3.41)
3	Basil (<i>Ocimum basillum</i>)	16.50 (4.17)	10.67 (3.41)	11.17 (3.49)	12.78 (3.69)
4	Citronella (<i>Cymbopogan citratus</i>)	15.17 (4.02)	12.83 (3.72)	11.67 (3.56)	13.22 (3.76)
5	Clove oil (<i>Syzygium aromaticum</i>)	21.83 (4.77)	17.17 (4.26)	14.83 (3.98)	17.94 (4.34)
6	Eucalyptus (<i>Eucalyptus citriodora</i>)	19.17 (4.48)	17.17 (4.26)	13.17 (3.76)	16.50 (4.17)
7	Garlic (<i>Allium sativum</i>)	16.33 (4.16)	13.83 (3.85)	11.17 (3.49)	13.78 (3.83)
8	Ginger (<i>Gingiber officinalis</i>)	16.67 (4.20)	14.17 (3.89)	12.83 (3.72)	14.56 (3.94)
9	Kokum (<i>Garcinia indica</i>)	26.50 (5.24)	20.17 (4.59)	17.50 (4.30)	21.39 (4.71)
10	Marigold (<i>Tagetus erecta</i>)	17.50 (4.30)	14.67 (3.95)	11.50 (3.54)	14.56 (3.93)
11	Neem (<i>Azadiracta indica</i>)	23.17 (4.91)	17.17 (4.26)	10.00 (3.32)	16.78 (4.16)
12	Pomegranate (<i>Punica granatum</i>)	24.67 (5.06)	18.83 (4.45)	16.17 (4.14)	19.89 (4.55)
13	Pongamia (<i>Pongamia pinnata</i>)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
14	Prosopis (<i>Prosopis juliflora</i>)	32.16 (5.76)	23.33 (4.93)	18.17 (4.38)	24.56 (5.02)
15	Tamarind (<i>Tamarindus indica</i>)	26.00 (5.19)	19.83 (4.56)	16.17 (4.14)	20.67 (4.63)
16	Streptocycline @ 500ppm + copper oxy chloride - 3g/lit	38.33 (6.271)			
17	Water control	0.00 (1.00)			
	Mean (Dilutions)	20.02 (4.51)	16.57 (4.13)	14.19 (3.83)	
	Source	SEm±			CD @ 1%
	Botanicals	0.055			0.16
	Dilutions	0.027			0.08
	B x D	0.04			0.28

*Figures in the parenthesis are square root transformed

Table 4: *In vitro* evaluation of antagonistic bacterial bio agents against *Enterobacter cloacae* of onion

Sl. No.	Code of the bio agents (10 ⁻⁸ CFU)	Mean zone of inhibition (mm)
1	PM-2A (<i>Brevibacillus sp.</i>)	19.33 (4.51)
2	VK-6B (<i>Lysinibacillus xylanilyticus</i>)	0.00 (1.00)
3	BK-6 (<i>Brevibacillus borstelensis</i>)	0.00 (1.00)
4	KK-9A (<i>Bacillus subtilis</i>)	0.00 (1.00)
5	VK-10C(Un identified)	12.67 (3.69)
6	KK-3A(Un identified)	0.00 (1.00)
7	BK-5(Un identified)	15.33 (4.04)
8	BK-3(Un identified)	13.50 (3.81)
9	CK-13A(Un identified)	11.50 (3.53)
10	BK-8(Un identified)	0.00 (1.00)
11	Control	0.00 (1.00)
	SEm±	10.32 (0.04)
	CD @ 1%	0.90 (0.12)

*Figures in the parenthesis are square root transformed

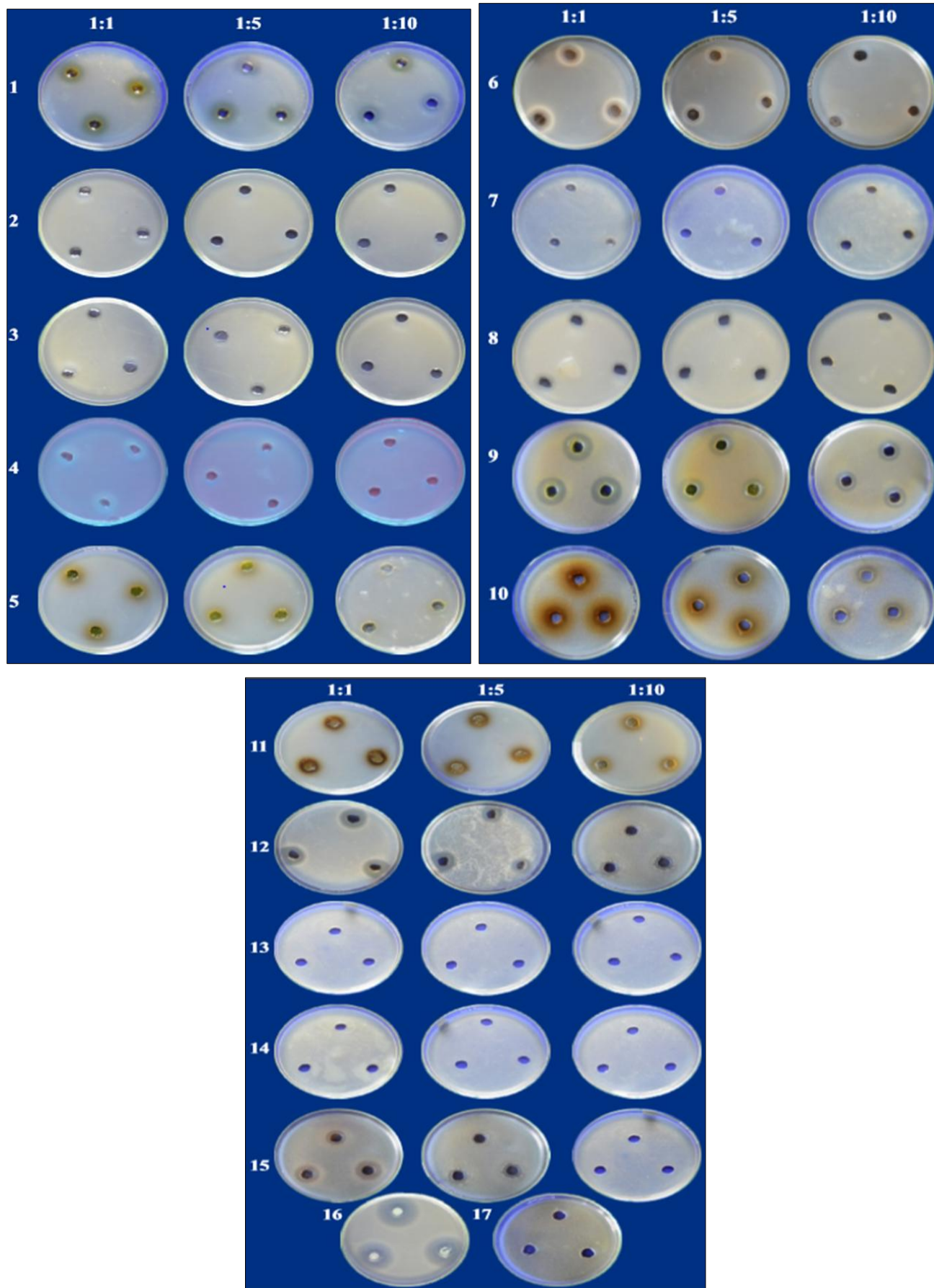


Plate 1: In vitro evaluation of aqueous extracts of botanicals against leaf rot disease causing bacteria

1) Clove oil (*Eugenia caryophyllata*), 2) Eucalyptus oil (*Eucalyptus citriodora*), 3) Basil (*Basilla alba*), 4) Pomegranate (*Punica granatum*), 5) Tamarind (*Tamarindus indica*), 6) Amrithpani (*Amrith paniosa*), 7) Prosopis (*Prosopis juliflora*), 8) Citonella (*Cymbopogan citrates*), 9) Aloevera (*Aloe barbadensis*), 10) Marigold (*Tagetus erecta*), 11) Neem (*Azardirachta indica*), 12) Kokum (*Garcinia indica*), 13) Garlic (*Allium sativum*), 14) Ginger (*Ginger officinalis*), 15) Pongemia (*Pongemia pinnata*). 16) Positive control (Streptocycline @ 500ppm + Copper oxy chloride @ 3g/l.), 17) Negative control.

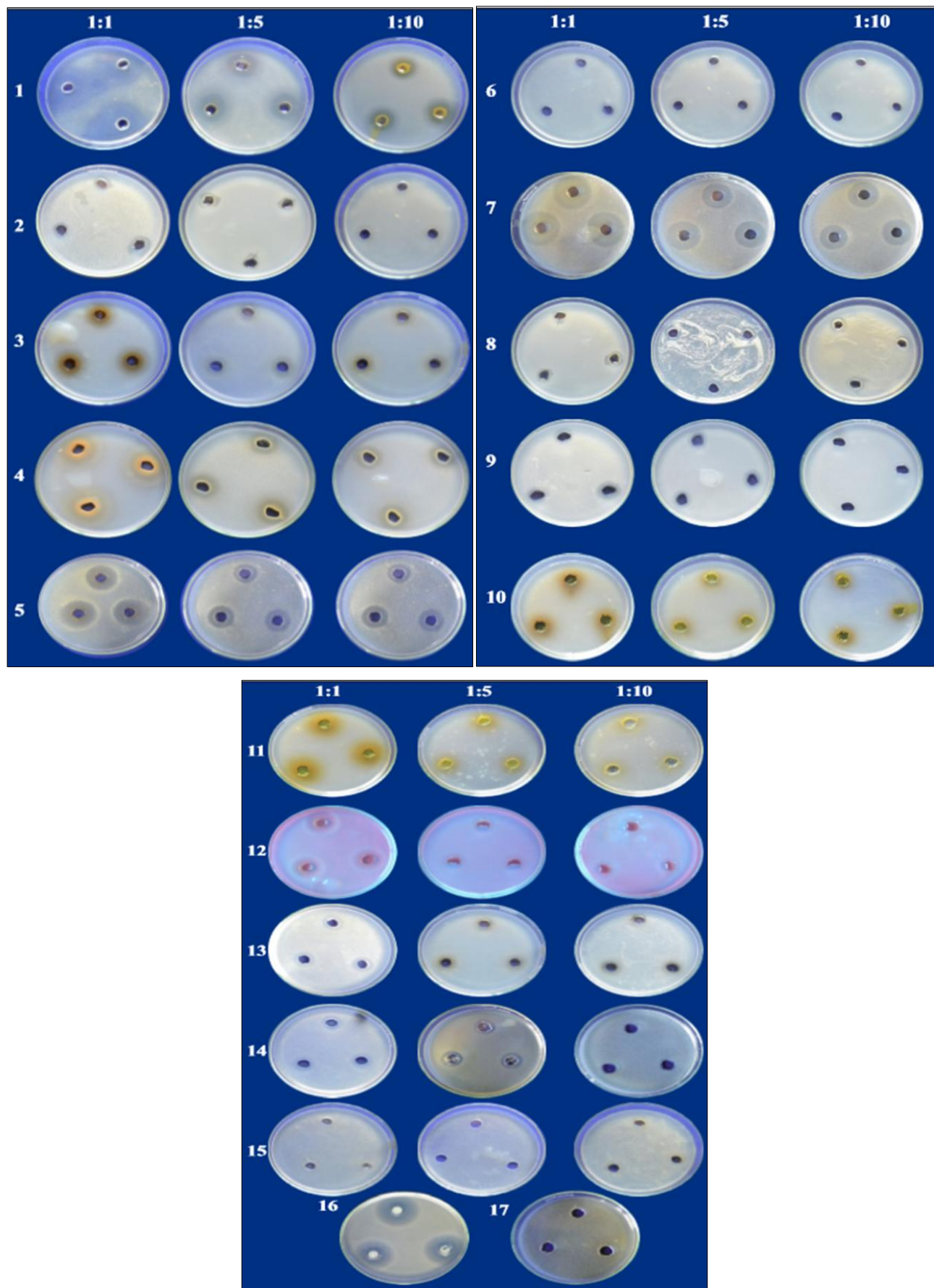


Plate 2: *In vitro* evaluation of alcoholic extracts of botanicals against leaf rot disease causing bacteria

1) Clove oil (*Eugenia caryophyllata*), 2) Eucalyptus oil (*Eucalyptus citriodora*), 3) Basil (*Basilla alba*), 4) Pomegranate (*Punicagranatum*), 5) Tamarind (*Tamarindus indica*), 6) Amrithpani (*Amrith paniosa*), 7) Prosopis (*Prosopis juliflora*), 8) Citonella (*Cymbopogan citrates*), 9) Aloe vera (*Aloe barbadensis*), 10) Marigold (*Tagetus erecta*), 11) Neem (*Azardirachta indica*), 12) Kokum (*Garcinia indica*), 13) Garlic (*Allium sativum*), 14) Ginger (*Ginger officinalis*), 15) Pongamia (*Pongamia pinnata*), 16) Positive control (Streptomycin @ 500ppm + Copper oxy chloride @ 3g/l.), 17) Negative control.

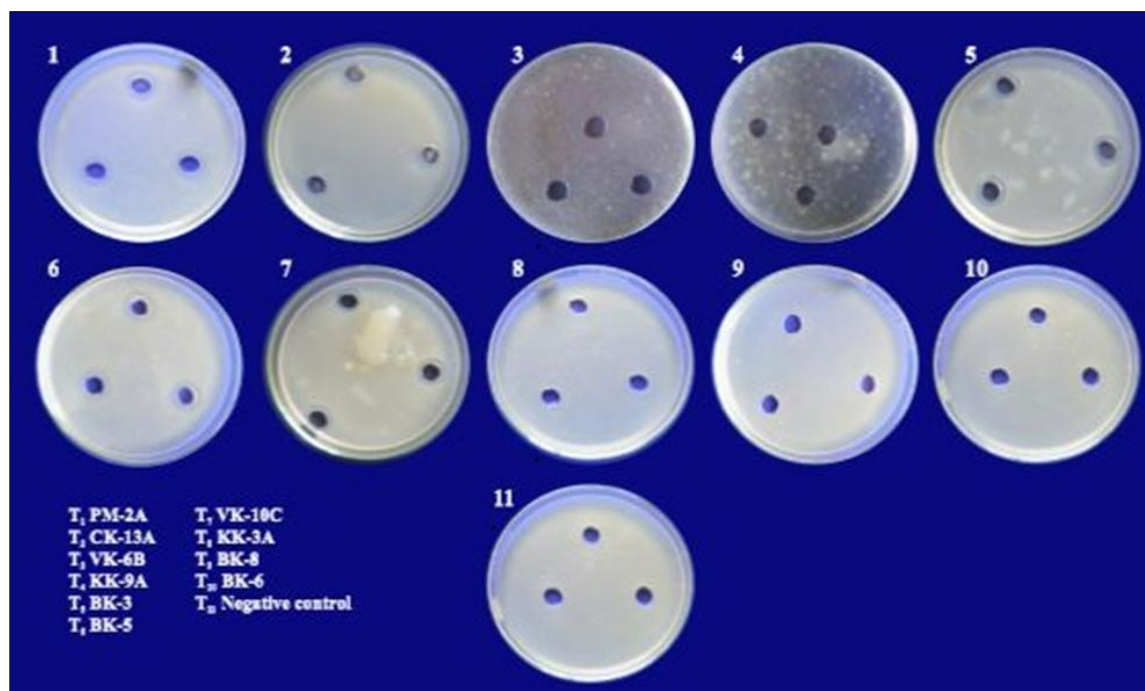


Plate 3: *In vitro* evaluation of bioagents against *Enterobacter cloacae* of onion

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