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Original Research

Inhibitory Effect of Some Plants of Western Ghats of Karnataka against *Colletotrichum capsici*

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Abstract

Anthracnose is a serious disease of chilli which results in major crop loss. Species of *Colletotrichum* are the causative agents of chilli anthracnose. In the present study, we investigated the inhibitory effect of a total of 50 extracts from 35 plants (belonging to 23 botanical families) of Western Ghats of Shivamogga district, Karnataka, India. The powdered plant materials were extracted using methanol. The methanol extracts were screened for antifungal activity by Poisoned food technique against *Colletotrichum capsici* isolated from anthracnose of chilli. All extracts were effective in inhibiting the growth of *C. capsici* but to a varied extent (16 to 74% inhibition). The mycelial growth of fungus was found to be reduced on poisoned plates when compared to control plate. Marked inhibitory efficacy was observed in case of leaf extract of *Maesa indica* (74.19%) followed by leaf extract of *Pimenta dioica* (70.96%). Least inhibition of the fungus was shown by leaf extract of *Persea macrantha* (16.13%). The extent of inhibition of the fungus by other extracts ranged between 20 to 70%. In conclusion, the plants selected in this study appear promising as natural antifungal agents. Further field studies are to be conducted to determine the possible application of these plants in the control of chilli anthracnose.

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INTRODUCTION

Chilli (*Capsicum annuum*L.) is one of the most important economic food crops grown in various countries for domestic usage and export. It is used as a vegetable (fresh) as well as a spice (dried). India is one of the largest producers of chilli. The chilli suffers from various diseases and chilli anthracnose is one of the most important among them. It is the most important disease of chilli in tropics and subtropics worldwide. The disease drastically reduces the yield, deteriorates the fruit quality, and hence results in low returns to farmers. In severe cases, the crop loss may exceed 50%. Species of the genus *Colletotrichum* such as *C. capsici*, *C. gloeosporioides*, *C. acutatum* etc have been identified as pathogens causing chili anthracnose. Out of these, *C. capsici* is the major pathogen causing anthracnose disease (Gomathi and Kannabiran, 2000; Kaur *et al*., 2006; Montri *et al*., 2009; Susheela, 2012; Chaisemsaeng *et al*., 2013).

Various fungicides such as mancozeb, captan, bavistin, thiram, copper oxychloride, cosan, benlate and ziram are employed in order to control anthracnose disease. The resistance to these fungicides has been noticed in most fungal pathogens including *C. capsici*. Moreover, the residues of these fungicides remain in the harvested produce. Hence, search for alternative disease control strategies are of immense interest. Natural products are promising in terms of their low cost, potential efficacy as well as no or negligible side effects. Plants and their derivatives have been extensively studied for the control of phytopathogenic fungi. Several studies have been carried out on inhibitory potential of many botanical extracts against phytopathogenic fungi including species of *Colletotrichum* (Gomathi and Kannabiran, 2000; Kumaran *et al*., 2003; Nduagu *et al*., 2008; Rahman *et al*., 2011; Mukherjee *et al*., 2011; Johnny *et al*., 2011; Bajpai and Kang, 2012; Ajith *et al*., 2012; Dileep *et al*., 2013; Jagtap *et al*., 2013; Sundaramoorthy *et al*., 2014).

India has a rich floristic diversity which represents about 11% of total flora of the world. Western Ghats of India is one among the global biodiversity hotspots. The mountain ranges of Western Ghats harbors a large number of plant species with high degree of endemism. It is a mountainous range extending from the mouth of the river Tapti in Gujarat to Kanyakumari in Tamil Nadu. The Western Ghats encompass various vegetation types such as wet evergreen forests, moist and dry deciduous forests, montane forests, sholas, scrubs and savannas (Richard and Muthukumar, 2012; Sivu *et al*., 2013; Nampoothiri *et al*., 2013). The central Western Ghats of Karnataka, known as 'Sahyadri', represents a long mountain chain along the west coast of India and encompass districts namely Chikmagalur, Shivamogga, Udupi, Dakshina Kannada, Uttara Kannada, Hassan and Coorg. The present study was carried out to investigate

the antifungal efficacy of 35 plants (belonging to 23 Hulikal of Hosanagara Taluk and Maragalale of Thirthahalli Taluk of Western Ghats of Shivamogga district, Karnataka against *C. capsici* isolated from anthracnose of chilli.

MATERIALS AND METHODS

Collection and Identification of Plants

A total of 35 plant species belonging to 23 botanical families were used in this study. The plants were collected at different regions of Western Ghats *viz*., Haniya and Hulikal of Hosanagara Taluk and Maragalale of ThirthahalliTaluk of Shivamogga district, Karnataka. The plants used in this study are mentioned in Table 1. The plants were authenticated by Dr. Vinayaka K.S, Department of Botany, KFGC, Shikaripura, Karnataka.

Extraction

25g powder of each of the selected plants was transferred into separate conical flasks containing 100ml of methanol (HiMedia, Mumbai) and mixed well. The flasks were kept at room temperature for two days with occasional stirring. The extracts were filtered through Whatman No. 1 filter paper, concentrated in vacuum under reduced pressure and dried in the desiccator (Manasa *et al*., 2013).

Antifungal Activity of Extracts of Selected Plants

Poisoned food technique was carried out to determine antifungal effect of extracts of various plants against *Colletotrichum capsici* isolated in our previous study from anthracnose of chilli (Kambar *et al*., 2013). Potato dextrose agar (HiMedia, Mumbai) was prepared, poisoned with extracts (1mg/ml of medium), autoclaved, dispensed into sterile petri dishes and allowed to solidify. The test fungus was inoculated aseptically at the centre of poisoned plates and the plates were incubated for 5 days at 28°C. The colony diameter in mutual perpendicular directions was recorded using a ruler. Antifungal activity, in terms of inhibition of mycelial growth (%), was calculated using the formula:

Mycelial growth inhibition (%) = (A–B/A) × 100,

where 'A' is average colony diameter in control plate and 'B' is average colony diameter in poisoned plates (Kambar *et al*., 2013).

Statistical Analysis

The experiments were done in triplicates and the results were mentioned as Mean±Standard deviation.

RESULTS AND DISCUSSION

In the present study, we investigated the efficacy of 50 extracts from 35 plants to inhibit *C. capsici* isolated previously from chilli anthracnose by Poisoned food technique. The result of inhibitory potential in terms of mycelial growth inhibition is shown in Table 2 and Figure 1 and 2. Poisoning of medium with extracts resulted in reduction of mycelial diameter when compared to control. All extracts were able to inhibit the fungus but to a varied extent. The extent of inhibition of *C. capsici* ranged between 16.13 and 74.19% by extracts of selected plants. Highest and least inhibition of the fungus was observed in case of leaf extract of *M. indica* (74.19%) and leaf extract of *P. macrantha* (16.13%) respectively. Next to *M. indica*, leaf extract of *P. dioica* caused high inhibition of fungus (70.96%). An inhibition of 60-70% was observed in case of leaf extract of *R. tetraphylla*, *F. montana*, *P. scandens*, *L. roxburghii* and *C. odorata* and bark extract of *F. zeylanica*. Inhibition of fungus ranged 50-60% in case of leaf extract of *J. arborescens*, *F. zeylanica*, *O. dioica*, *A. lakoocha*, *A. indica* and *C. roxburghii*, bark extract of *D.montana* and *P. macrantha*, root extract of *A. curassavica* and whole plant extract of *H. indicus*. All other extracts (except leaf extract of *P. macrantha*) inhibited the fungus to an extent which ranged between >20 and <50%.

Bark extract of *D. montana* inhibited the fungus to high extent than leaf extract. Leaf extract of *D. buxifolia* was more effective than that of leaf extract of *D. montana*. Leaf extract of *T. heyneana* inhibited fungus to high extent when compared to flower extract. Leaf extract of *C. odorata* was more inhibitory to fungus than inflorescence extract. In case of *F. zeylanica*, bark extract was more effective in inhibiting the fungus when compared to leaf extract. The extract from roots of *A. curassavica* inhibited the growth of fungus to high extent when compared to leaf and flower extracts which showed similar inhibition. Extracts from all parts of *L. speciosa* exhibited similar inhibition of the fungus. The bark extracts of *P. macrantha* and *A. occidentale* exhibited stronger inhibitory activity when compared to leaf extracts. In case of leaf and bark extract of *P. dioica*, leaf extract caused higher suppression of fungal growth. Rhizome extract of *A. galanga* was effective in inhibiting fungus to high extent than leaf extract. Leaf and flower extracts of *P. ferrugineum*, *D. regia* and *C. pulcherrima* exhibited more or less similar inhibition of *C. capsici*.

In an earlier study, Johnny *et al*. (2011) showed dose dependent inhibitory activity of leaves of *A. galanga* and *A. muricata* against *C. capsici*. Extract of *A. galanga* exhibited stronger inhibition of fungus than extract of *A. muricata*. However, in our study, leaf extract of *A. muricata* inhibited *C. capsici* to higher extent than leaf extract of *A. galanga*. In an earlier study, Nduagu *et al*. (2008) found that extract of *C.odorata* failed to cause reduction in the colony diameter of *C. capsici*. However, in our study, the leaf and inflorescence extract of *C. odorata* inhibited mycelial growth of the fungus. Leaf extract was found to be more effective. Kumaran *et al*. (2003) found low inhibitory potential of *L. aspera* when compared to *R. tetraphylla* against *C. capsici*. In our study also, similar result was observed. The study of Sarathambal *et al*. (2011) revealed the efficacy of solvent extracts of *L. aspera* against a panel of fungi which included *C. capsici*. In a previous study, we reported inhibitory effect of leaf and bark extracts of *P. dioica* and *A. occidentale* against *Fusarium oxysporum* f.sp. *zingiberi* isolated from soft rot of ginger. Leaf extracts of both the plants were more effective in inhibiting mycelial growth of fungus when compared to bark extracts (Vivek *et al*., 2013). In the present study, similar result was observed only in case of *P. dioica* but not in case of *A. occidentale* as bark extract of *A. occidentale* inhibited fungus to higher extent than leaf extract.

Table 1: Plants used in this study.

No.	Name of the plant	Family	Habit	Part/s used	Place of collection
1	*Tabernaemontana heyneana* Wall.	Apocyanaceae	Tree	Leaf, flower	Haniya
2	*Rauvolfia tetraphylla* L.	Apocyanaceae	Shrub	Leaf	Haniya
3	*Psychotria nigra* (Gaert.) Alston	Rubiaceae	Shrub	Leaf	Haniya
4	*Flacourtia montana* Graham	Flacourtiaceae	Tree	Leaf	Haniya
5	*Jasminum arborescens* Roxb.	Oleaceae	Shrub	Leaf	Haniya
6	*Rubia cordifolia* Linn.	Rubiaceae	Climbing herb	Whole plant	Haniya
7	*Aglaia roxburghiana* (W.&.A) Miq. Var. Beddomei	Meliaceae	Tree	Leaf	Haniya
8	*Canthium dicoccum* (Gaertn.) Teys. & Binn.	Rubiaceae	Tree	Leaf	Haniya
9	*Pothos scandens* L.	Araceae	Climbing shrub	Leaf	Haniya
10	*Diospyros montana* Roxb.	Ebenaceae	Tree	Leaf, bark	Haniya
11	*Leucas aspera* (Willd.) Linn.	Lamiaceae	Herb	Leaf	Maragalale
12	*Chromolaena odorata*a (Linn.) R. King & H. Robinson	Asteraceae	Perennial shrub	Leaf, inflorescence	Haniya
13	*Fahrenheitia zeylanica* (Thw.) Airy	Euphorbiaceae	Tree	Leaf, bark	Hulikal
14	*Olea dioica* Roxb.	Oleaceae	Tree	Leaf	Haniya
15	*Maesa indica* (Roxb.) A.DC	Myrsinaceae	Small tree	Leaf	Haniya
16	*Asclepias curassavica* L.	Asclepidiaceae	Sub-shrub	Leaf, root, flower	Haniya
17	*Elaegnus kologa* Schlecht	Elaegnaceae	Shrub	Leaf	Haniya
18	*Artocarpus lakoocha* Roxb.	Moraceae	Tree	Leaf	Hulikal
19	*Croton roxburghii* Balak.	Euphorbiaceae	Tree	Leaf	Haniya
20	*Lagerstroemia speciosa* (L.)	Lythraceae	Medium sized tree	Leaf, seed, flower	Haniya
21	*Ligustrum roxburghii* C.B. Clarke	Oleaceae	Tree	Leaf	Haniya
22	*Annona muricata* Linn.	Annonaceae	Tree	Leaf	Maragalale
23	*Persea macrantha* (Nees) Kosterm.	Lauraceae	Tree	Leaf, bark	Haniya
24	*Pimenta dioica* (Linn.) Merill	Myrtaceae	Tree	Leaf, bark	Maragalale
25	*Anacardium occidentale* L.	Anacardiaceae	Tree	Leaf, bark	Maragalale
26	*Ziziphus mauritiana* Lam.	Rhamnaceae	Small tree	Leaf	Maragalale
27	*Alpinia galanga* Willd.	Zingiberaceae	Herb	Leaf, rhizome	Maragalale
28	*Capsicum frutescens* Linn.	Solanaceae	Sub-shrub	Leaf	Haniya
29	*Diospyros buxifolia* (Blume) Hiern	Ebenaceae	Tree	Leaf	Haniya
30	*Mucuna pruriens* Linn.	Fabaceae	Twining herb	Flower	Haniya
31	*Anisomeles indica* Linn.	Lamiaceae	Herb	Leaf	Haniya
32	*Hemedesmus indicus* R.Br	Asclepiadaceae	Semi-erect shrub	Root	Maragalale
33	*Caesalpinia pulcherrima* Linn.	Fabaceae	Shrub	Leaf and flower	Maragalale
34	*Delonix regia* (Bojer Ex. Hook.)	Fabaceae	Tree	Leaf and flower	Maragalale
35	*Peltaphorum ferrugineum*	Fabaceae	Tree	Leaf and flower	Maragalale

Table 2: Antifungal activity of selected plants

Sl. No.	Plant name	Part used	C.D in cm	% inhibition
1	Control	-	3.1±0.1	-
2	*T. heyneana*	Leaf	1.9±0.0	38.70
		Flower	2.0±0.0	35.48
3	*R. tetraphylla*	Leaf	1.0±0.0	67.74
4	*P. nigra*	Leaf	2.1±0.1	32.26
5	*F. montana*	Leaf	1.2±0.1	61.29
6	*J. arborescens*	Leaf	1.5±0.0	51.61
7	*R. cordifolia*	Leaf	2.0±0.1	35.48
8	*A. roxburghiana*	Leaf	2.0±0.0	35.48
9	*C. dicoccum*	Leaf	2.4±0.2	22.58
10	*P. scandens*	Leaf	1.1±0.0	64.52
11	*D. montana*	Leaf	2.0±0.1	35.48
		Bark	1.5±0.2	51.61
12	*L. aspera*	Leaf	2.2±0.2	29.03
13	*C. odorata*	Leaf	1.1±0.0	64.52
		Inflorescence	2.2±0.1	29.03
14	*F. zeylanica*	Leaf	1.4±0.0	54.83
		Bark	1.2±0.0	61.29
15	*O. dioica*	Leaf	1.5±0.0	51.61
16	*M. indica*	Leaf	0.8±0.1	74.19
17	*A. currasavica*	Leaf	1.7±0.1	45.16
		Root	1.5±0.0	51.61
		Flower	1.7±0.2	45.16
18	*E. kologa*	Leaf	1.6±0.1	48.39
19	*A. lakoocha*	Leaf	1.5±0.0	51.61
20	*C. roxburghii*	Leaf	1.5±0.0	51.61
21	*L. speciosa*	Leaf	2.2±0.2	29.03
		Seed	2.2±0.2	29.03
		Flower	2.2±0.1	29.03
22	*L. roxburghii*	Leaf	1.2±0.0	61.29
23	*A. muricata*	Leaf	1.6±0.1	48.39
24	*P.macarantha*	Leaf	2.6±0.1	16.13
		Bark	1.5±0.0	51.61
25	*P. dioica*	Leaf	0.9±0.1	70.96
		Bark	1.9±0.0	38.70
26	*A. occidentale*	Leaf	2.4±0.1	22.58
		Bark	1.7±0.1	45.16
27	*Z.mauritiana*	Leaf	1.9±0.1	38.70
28	*A.galanga*	Leaf	2.0±0.0	35.48
		Rhizome	1.9±0.1	38.70
29	*C. frutescens*	Leaf	2.2±0.0	29.03
30	*D. buxifolia*	Leaf	1.6±0.1	48.39
31	*M. pruriens*	Flower	2.3±0.1	25.80
32	*A. indica*	Leaf	1.5±0.0	51.61
33	*H. indicus*	Whole plant	1.4±0.0	54.83
34	*P. ferrugineum*	Leaf	2.0±0.0	35.48
		Flower	2.0±0.1	35.48
35	*D. regia*	Leaf	2.0±0.0	35.48
		Flower	2.1±0.0	32.25
36	*C. pulcherrima*	Leaf	2.0±0.0	35.48
		Flower	1.9±0.1	38.70

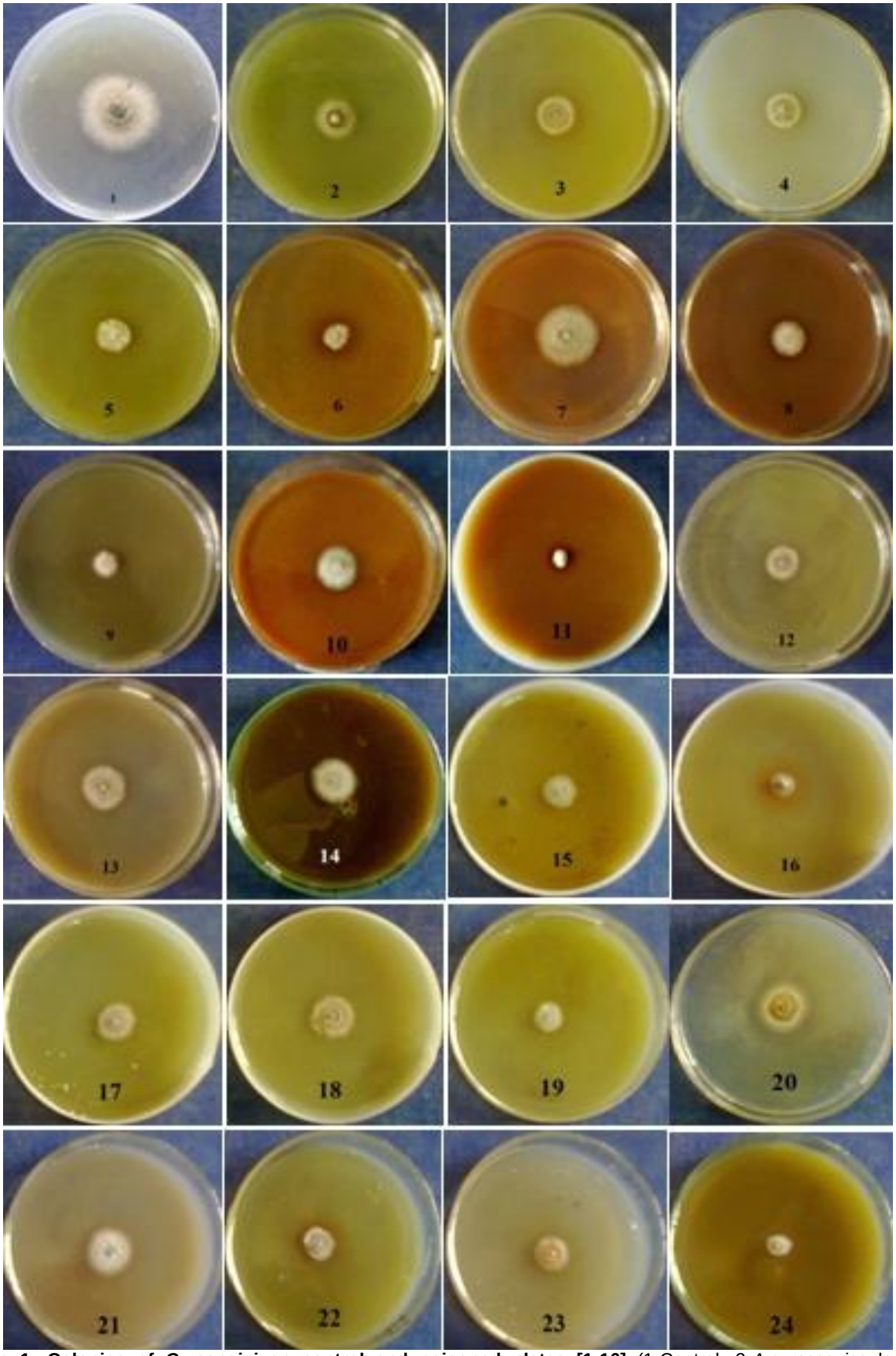

Figure 1: Colonies of *C. capsici* on control and poisoned plates [1-16] (1-Control; 2-*A.curassavica* leaf; 3-*A.curassavica* flower; 4-*A.curassavica* root; 5-*F.zeylanica* leaf; 6-*F.zeylanica* bark; 7-*P.macrantha* leaf; 8-*P.macrantha* bark; 9-*L.roxburghii*; 10-*P.dioica* bark; 11- *P.dioica* leaf; 12-*A.muricata*; 13-*D.buxfolia*; 14-*D.montana* leaf; 15- *D.montana* bark; 16-*R.tetraphylla*; 17-*T.heyneana* leaf; 18-*T.heyneana* flower; 19-*C.odorata* leaf; 20-*C.odorata* inflorescence; 21-*A.roxburghiana*; 22-*O.dioica*; 23-*J.arborescens*; 24-*M.indica*)

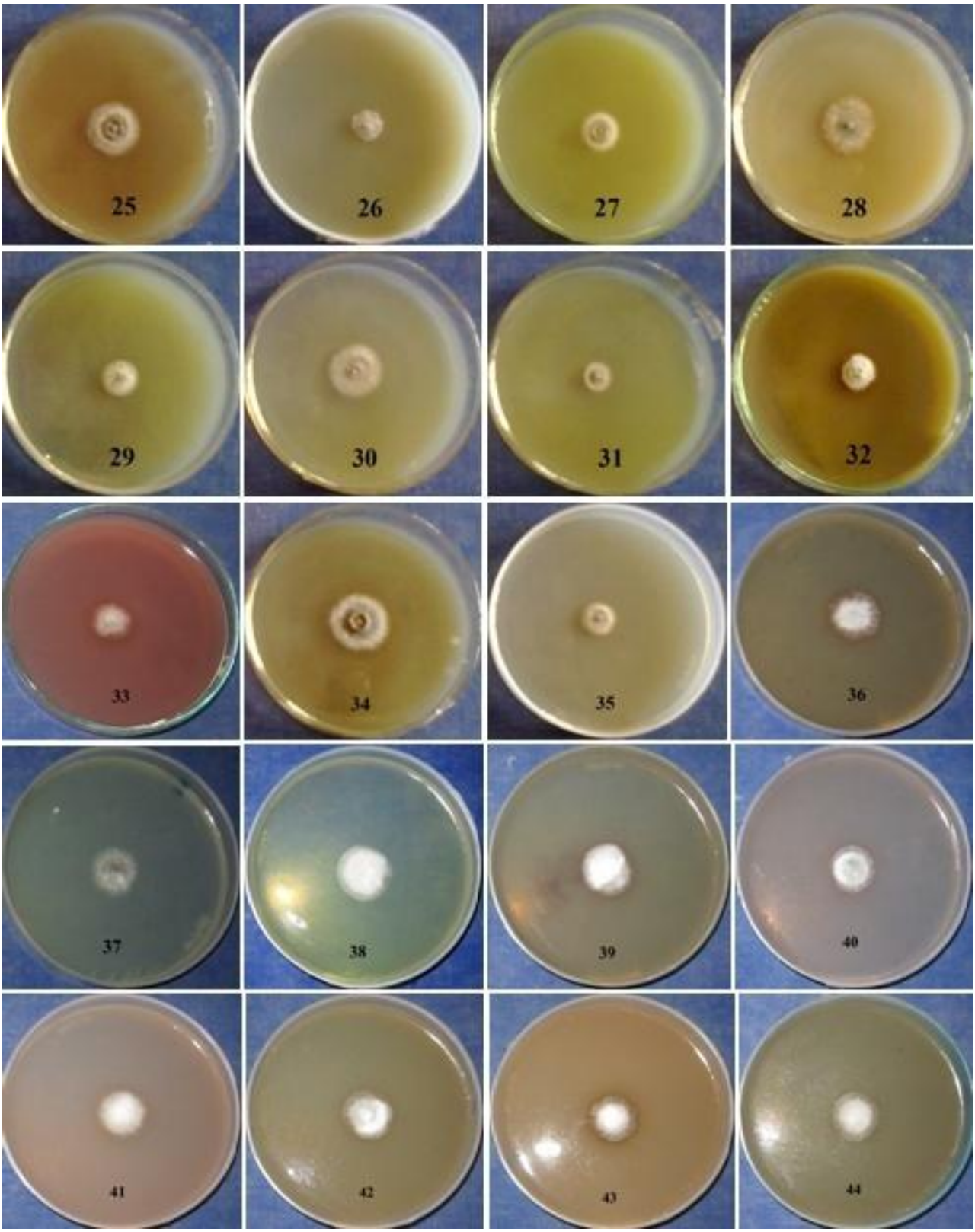

Figure 2: Colonies of *C. capsici* on control and poisoned plates [25-44] (25-*P.nigra*; 26-*F.montana*; 27-*E.kologa*; 28-*C.dicoccum*; 29-*C.roxburghii*; 30-*R.cordifolia*; 31-*P.scandens*;32-*A.lakoocha*; 33-*H.indicus*; 34-*M.pruriens*; 35-*A.indica*; 36-*D.regia* leaf; 37-*Z.mauritiana*; 38-*C.frutescens*; 39-*A.galanga* leaf; 40-*A.galanga* rhizome; 41-*P.ferrugineum* flower; 42-*P.ferrugineum* leaf; 43-*C.pulcherrima* flower; 44-*C.pulcherrima* leaf)

CONCLUSIONS

The use of fungicides of plant origin has been shown an effective alternative to synthetic chemicals in order to counter the potential hazardous effect on the environment as well as consumer. In the present study, the extracts of all 29 plants collected at different regions of Western Ghats of Shivamogga district, Karnataka displayed inhibitory activity against chilli anthracnose causing fungus in terms of inhibition of mycelial growth. These plants can be exploited as natural fungicides for the control of chilli anthracnose. The study made here is an *in vitro* study and further experiments fields is required to ascertain the possible application of these botanicals for the management of disease.

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