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Screening of Medicinal Plant Leaf Extract in the Control of Seed Borne *Fusarium graminearum* and *Fusarium moniliforme* Conidial Germination under *in vitro* Condition

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Abstract: *Fusarium graminearum* schwabe and *Fusarium moniliforme* sheld, conidia germination were controlled by using medicinal plant leaf extracts under *in vitro* condition. Totally 46 plant leaf extracts were used for the screening purpose. Among them, zimmu leaf extracts recorded minimum of 15 and 18% germination of *F. graminearum* and *F. moniliforme* conidia. The screened top four plant leaf extracts were tested against the seed-borne *Fusarium* sp. by poison food technique under different concentrations (0.1, 0.2 and 0.5%). Among the four plant extracts 0.2% zimmu leaf extract recorded maximum inhibition of seed-borne *Fusarium* sp. mycelial growth, both ethanol and methanol extracts. Among the different concentrations 100% recorded the maximum inhibition area of 6.160 cm^2 against *F. graminearum* and *F. moniliforme*. It was for determining best concentration to further development of Emulsifiable Concentration (EC) formulation for field trial.

Key words: Zimmu, leaf extract, *Fusarium*, macroconidia, microconidia

INTRODUCTION

Sowing cereal seed that is free of seed-borne (both internal and external) pathogens is the primary means of limiting the introduction of pathogens, into a field, especially new pathogens. Ear blight and scab of wheat causing *F. graminearum* first reported in India from Arunachal Pradesh and from Wellington, Tamil Nadu (Saharan *et al*., 2004). Wheat grain yield losses of 15.1-29.0% have been reported due to ear blight incidence on different wheat varieties from Arunachal Pradesh (Chaudhary *et al*., 1991). In Punjab, maximum yield loss of 21.6% was recorded in wheat variety PBW 222 due to *Fusarium* head blight (Kaur *et al*., 2000). With the increasing pesticide residues concerns in agricultural products and environment as well as the incidence of resistance in plant pathogens not in favor of chemical pesticides, the use of non-chemical and eco-friendly methods including natural metabolites have assumed greater significance. From past decades the look for plant extracts and essential oils with biocontrol activity was carried out in an increasing scale. Maruzzella and Baltes (1959) tested 100 essential oils out of 119 spice oils which recorded antimicrobial activity against at least 12 phytopathogenic fungi and 50 of these compounds showed broad spectrum activity against all fungi tested. A major challenge facing crop production is the need to provide field disease control measures that may help maintain higher quality plant products. The present investigation attempted to bridge these research gaps, through the following objects: (1) Screening best performed antifungal activity of medicinal plant leaves extracts against *F. graminearum* macroconidia and *F. moniliforme* microconidia, (2) To determine the best concentration to develop the EC formulation for field spray.

MATERIALS AND METHODS

F. graminearum and *F. moniliforme* were isolated from wheat and sorghum seeds respectively and detected through dry seed examination (Enikuomehin, 2005), blotter method (ISTA, 2008) and deep freezing method. The fungi were purified by single spore isolation method (Riker and Riker, 1936) and identified to species level based on synoptic keys by Nelson *et al*. (1983).

Preparation of plant leaf extracts: Totally 46 leaf sample of the plants was collected from the herbal garden at the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. Fresh plant leaves were used for extraction. The leaves were first washed with distilled water and finally with sterile water. Ground in a pestle and mortar by adding sterile water at the rate of 1:1 (w/v), the macerate was squeezed using cotton wool to get the extract, the extract was strained through two

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layers of muslin cloth and finally through Whatman No. 1 filter paper. This formed the standard plant extract solution (100%). This was further diluted either with the medium or sterile distilled water (v/v) to have the required concentration prior to plating the plant extracts prepared were heated in a water bath at 40-50°C for 10 min to avoid contamination (Jaganathan, 1984).

Effect of plant extracts on the conidial germination (Montgomery and Moore, 1938): Seven day old culture of the *Fusarium* was used for the experiment. The spores were obtained by flooding with sterile water. The spore suspension (7×10^6 spores mL^{-1}) was prepared and centrifuged at 2000 rpm for 10 min to remove the mycelial fragments. Ten percent concentration of the plant extract was prepared using sterile distilled water and placed on cavity slides separately and allowed to evaporate. A drop of the spore suspension was placed over the dried extract and incubated in a moist chamber at room temperature (28+2°C). A control was maintained using sterile distilled water instead of plant extracts. The percent spore germination was recorded each 3 h interval and expressed as percent inhibition over control (Vincent, 1947):

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

Where:

I = Percent inhibition of spore germination/mycelial growth
C = Spore germination/mycelial growth in control
T = Spore germination/mycelial growth in treatment

Effect of screened plant extracts extracted in different solvents on the mycelial growth of *Fusarium* sp. by poisoned food technique (Schmitz, 1930): Fresh leaves (100 g) of the four most effective plant species were washed in distilled water and were separately ground in 100 mL of ethanol and methanol at 1:1 (w/v) ratio. The extracts were filtered through muslin cloth and finally through Whatman No. 1 filter paper and centrifuged at 3000 rpm for 20 min. The supernatant fluid was concentrated under laboratory condition to complete dryness after discarding the pellet. The residue of the extracts were made up to 100 mL (100%) with distilled water and used for different concentration viz., 0.1, 0.2 and 0.5%. The inhibitory effects on mycelial growth (cm) were determined after preparation.

Effect of zimmu leaf extracts (different concentrations) against *Fusarium* sp. under *in vitro* condition (paper disc method): The study disc (No. 2 Whatmann) was cut into small disc (9 mm diameter) and sterilized at 180°C for 30 min in hot air oven and soaked in methanol extract of zimmu plants leaf extract at different concentrations (25, 50, 75 and 100%). The discs were placed at the centre of 9 cm diameter petri dishes containing Potato Dextrose Agar (PDA) amended with spore suspension (1×10^3 conidia mL^{-1}). The petri dishes kept in incubator with 25°C. Filter paper disc soaked in distilled water served as control. Three replications were maintained. The diameter of inhibition zone was measured after 24, 48 and 72 h of incubation and inhibition area was calculated.

Statistical analysis: The statistical analysis of the experimental data was carried out by adopting the standard method (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Among the 46 plant extracts many were found to inhibitory effect on conidial germination of *F. graminearum* and *F. moniliforme*. The zimmu leaf extract recorded maximum inhibition of *F. graminearum* macroconidial germination of 15% (Table 1 and Fig. 1). The same leaf extract reported 18% inhibition of *F. moniliforme* microconidial germination, because the characteristic *F. moniliforme* produces more microconidia than macroconidia (Table 2 and Fig. 1). The similar studies were carried by Shekhawat and Prasada (1971). They studied the antifungal properties of forty one plant species and reported that the spore germination of *A. tenuis* was completely inhibited by cold water leaf extract of *Prosopis spicigera*, *Allium sativum* and *Acacia loculata*. Menaka *et al.* (2003) reported that *Allium sativum*, *Allium cepa*, *Adenocalyma alliaceum*, *Prosopis juliflora* and *Azadirachta indica* recorded minimum fungal growth of *Fusarium* and *Curvularia*. The extract of zimmu was most effective against *A. niger*, *A. flavus* which recorded highest reduction in biomass production. Shekhawat and Prasada (1971) reported that spore germination of *A. tenuis* was inhibited by cold water leaf extract of *Allium cepa* L., *Allium sativum* L. and the spore germination of *Helminthosporium* sp. was inhibited by leaf extracts of *Allium cepa* L. and *Allium sativum* L. Valluvaparidasan (1994) reported that the leaf extracts of *Prosopis juliflora*, *P. cubeba*, *Allium aliaceum*, *Lawsonia inermis*, *Eucalyptus globules*, *Terminalia chebula*, *Andrographis paniculatus* reduced the mycelial growth of *H. oryzae* in rice seed. *In vitro* tests revealed that the leaf extracts of *A. indica*, *Mentha arvensis*, *Aegle marmelos*, *C. roseus*, *L. camara*, *Pongamia pinnata*, *V. negundo* and *Nerium odorum* and flower extracts of *C. roseus* inhibited mycelial growth and spore germination of

Table 1: Screening of plant leaf extracts against *F. graminearum* spore germination under *in vitro* condition

Treatments	Spore germination (%)			
	3 h	6 h	9 h	12 h
Adhatoda	2 (08.13)	6 (14.18)	12 (20.27)	24 (29.33)
Agapana toa	17 (24.35)	66 (54.33)	78 (62.03)	97 (80.03)
Agave	12 (20.27)	41(39.82)	52 (46.15)	92 (73.57)
Aloe vera	20 (26.57)	86 (68.03)	91 (72.54)	100 (89.79)
Alternanthera	22 (27.97)	92 (73.57)	98 (81.87)	100 (89.79)
Aswagandha	21 (27.28)	90 (71.57)	94 (75.82)	100 (89.79)
Blepharis	21 (27.28)	89 (70.63)	98 (81.87)	100 (89.79)
Brahmi	17 (24.35)	78 (62.03)	91 (72.54)	100 (89.79)
Cissus	18 (25.10)	73 (58.70)	79 (62.73)	97(80.03)
Coleus	19 (25.84)	76 (60.67)	90 (71.57)	98 (81.87)
Coleus	27 (31.31)	91 (72.54)	96 (78.46)	100 (89.79)
Curry leaf	16 (23.57)	78 (62.03)	92 (73.57)	100 (89.79)
Gymnema	20 (26.56)	83 (65.65)	94 (75.82)	100 (89.79)
Henna	2 (08.13)	6 (14.18)	11 (19.37)	29 (32.58)
Hibiscus	20 (26.57)	79 (62.73)	91 (72.54)	99 (84.26)
Indian ipecac	18 (25.10)	79 (62.72)	92 (73.57)	100 (89.79)
Insulin plant	0 (00.21)	6 (14.18)	12 (20.27)	17 (24.35)
Ixora	22 (27.97)	89 (70.63)	98 (81.87)	100 (89.79)
Kallimudaiyan	21 (27.28)	84 (66.42)	94 (75.82)	99 (84.26)
Karbakarasi	22 (27.97)	90 (71.57)	96 (78.47)	100 (89.79)
Kayyantakarai	20 (26.57)	91 (72.54)	96 (78.46)	100 (89.79)
Kesavarthini	18 (25.10)	77 (61.34)	89 (70.63)	98 (81.87)
Kodikkalli	21 (27.28)	86 (68.03)	91 (72.54)	100 (89.79)
Koriveli	29 (32.58)	91(72.54)	94 (75.83)	99 (84.26)
Lemon grass	21 (27.28)	92 (73.57)	97 (80.03)	100 (89.79)
Lippia	21 (27.28)	91 (72.54)	97 (80.03)	100 (89.79)
Masipathiri	20 (26.57)	86 (68.03)	93 (74.66)	99 (84.26)
Nagamalli	3 (09.97)	10 (18.44)	48 (43.85)	85 (67.22)
Nagathanthi	19 (25.84)	82 (64.90)	96 (78.47)	100 (89.79)
Nirmulli	20 (26.57)	86 (68.03)	91 (72.54)	99 (84.26)
Ocimum	21 (27.28)	85 (67.22)	98 (81.87)	100 (89.79)
Ocimum	23 (28.67)	85 (67.22)	97 (80.03)	100 (89.79)
Pepper mint	19 (25.84)	79 (62.73)	91 (72.54)	99 (84.26)
Periwinkle	22 (27.97)	88 (69.73)	96 (78.47)	100 (89.79)
Ponnanganni	26 (30.66)	91 (72.54)	98 (81.87)	100 (89.79)
Porndhi	27 (31.31)	90 (71.57)	92 (73.57)	99 (84.26)
Psyllium	2 (08.13)	6 (14.18)	14 (21.97)	70 (56.79)
Senna	21 (27.28)	83 (65.65)	93 (74.66)	99 (84.26)
Solanum	22 (27.97)	81 (64.16)	93 (74.66)	99 (84.26)
Spear mint	21 (27.28)	89 (70.63)	95 (77.08)	100 (89.79)
Sweet basil	20 (26.57)	79 (62.73)	91 (72.54)	100 (89.79)
Sweet flag	23 (28.65)	91 (72.54)	98 (81.87)	100 (89.79)
Thorn apple	20 (26.57)	88 (69.73)	96 (78.47)	100 (89.79)
Tylophora	19 (25.84)	87 (68.86)	96 (78.47)	100 (89.79)
Vettiver	20 (26.57)	78 (62.03)	89 (70.63)	99 (84.26)
Zimmu	1 (5.74)	4 (11.54)	9 (17.46)	15 (22.79)
Control	37 (37.47)	92 (73.57)	98 (81.87)	100 (89.79)

Values in the parentheses are arc sine transformed values

Table 2: Screening of plant leaf extracts against *F. moniliforme* spore germination under *in vitro* condition

Treatments	Spore germination (%)			
	6 h	12 h	18 h	24 h
Adhatoda	0 (00.21)	4 (11.54)	10 (18.44)	19 (25.84)
Agapana toa	11 (19.37)	33 (35.06)	51 (45.57)	79 (62.73)
Agave	19 (25.84)	60 (50.77)	85 (67.21)	100 (89.79)
Aloe vera	19 (25.84)	69 (56.17)	92 (73.57)	100 (89.79)
Alternanthera	20 (26.57)	68 (55.55)	91 (72.54)	100(89.79)
Aswagandha	10 (18.43)	27 (31.31)	36 (36.87)	81 (64.16)
Blepharis	19 (25.84)	69 (56.17)	91 (72.54)	100 (89.79)
Brahmi	3 (09.97)	11 (19.37)	19 (25.84)	53 (46.72)
Cissus	14 (21.94)	57 (49.03)	78 (62.03)	97 (80.03)
Coleus	14 (21.97)	48 (43.86)	67 (54.94)	88 (69.73)
Coleus	19 (25.84)	71 (57.42)	83 (65.65)	99 (84.26)
Curry leaf	19 (25.84)	67 (54.94)	90 (71.57)	100 (89.79)
Gymnema	20 (26.57)	70 (56.79)	89 (70.63)	100 (89.79)
Henna	0 (00.21)	0 (00.21)	12 (20.27)	21 (27.28)
Hibiscus	4 (11.54)	12 (20.27)	19 (25.84)	71 (57.42)
Indian ipecac	20 (26.57)	72 (58.05)	92 (73.57)	100 (89.79)
Insulin plant	12 (20.27)	31 (33.83)	41 (39.82)	89 (70.63)
Ixora	20 (26.57)	69 (56.17)	92 (73.57)	100 (89.79)
Kallimudaiyan	13 (21.13)	32 (34.45)	59 (50.19)	90 (71.57)
Karbakarasi	21 (27.28)	63 (52.54)	89 (70.63)	100 (89.79)
Kayyantakarai	20 (26.57)	69 (56.17)	82 (64.90)	100 (89.79)
Kesavarthini	7 (15.34)	18 (25.10)	21 (27.28)	72 (58.05)
Kodikkalli	13 (21.13)	29 (32.58)	45 (42.13)	89 (70.63)
Koriveli	20 (26.57)	53 (46.72)	83 (65.65)	98 (81.87)
Lemon grass	21 (27.28)	68 (55.55)	92 (73.57)	100 (89.79)
Lippia	21 (27.28)	69 (56.17)	95 (77.08)	100 (89.79)
Masipathiri	13 (21.13)	49 (44.43)	62 (51.94)	79 (62.73)
Nagamalli	1 (05.73)	3 (09.97)	6 (14.18)	51 (45.57)
Nagathanthi	3 (09.97)	11 (19.37)	18 (25.10)	59 (50.18)
Nirmulli	20 (26.57)	60 (50.77)	75 (60.00)	98 (81.87)
Ocimum	14 (21.94)	41 (39.82)	64 (53.13)	81 (64.16)
Ocimum	11 (19.37)	49 (44.43)	66 (54.33)	95 (77.08)
Pepper mint	20 (26.57)	72 (58.05)	94 (75.82)	100 (89.79)
Periwinkle	14 (21.94)	43 (40.98)	61 (51.36)	80 (63.44)
Ponnanganni	19 (25.84)	61 (51.36)	89 (70.63)	96 (78.47)
Porndhi	21 (27.28)	68 (55.55)	96 (78.47)	100 (89.79)
Psyllium	3 (09.97)	7 (15.34)	15 (22.79)	39 (38.65)
Senna	21 (27.28)	71 (57.42)	89 (70.63)	100 (89.79)
Solanum	19 (25.84)	69 (56.17)	96 (78.47)	100 (89.79)
Spear mint	19 (25.84)	71 (57.42)	93 (74.66)	100 (89.79)
Sweet basil	21 (27.28)	61 (51.36)	84 (66.42)	99 (84.26)
Sweet flag	19 (25.84)	68 (55.55)	92 (73.57)	100 (89.79)
Thorn apple	21 (27.28)	69 (56.17)	91 (72.54)	100 (89.79)
Tylophora	19 (25.84)	71 (57.42)	89 (70.63)	98 (81.87)
Vettiver	4 (11.54)	13 (21.13)	19 (25.84)	61 (51.36)
Zimmu	0 (00.21)	9 (17.46)	11 (19.37)	18 (25.10)
Control	24 (29.33)	74 (59.34)	96 (78.47)	100 (89.79)

Values in the parentheses are arc sine transformed values

seed-borne mycoflora of sorghum including *A. tenuis*, *A. flavus*, *C. lunata* and *F. moniliforme* (Meena, 1989). Khallil (2001) reported that extracts of *Allium cepa* bulbs and *Eucalyptus rostrata* leaves inhibited *A. solani*.

The screened plants, Zimmu, Adhathoda, Henna and Insulin plants extract were further evolved by using methanol and ethanol solvent extracts. Totally 4 plant leaf extracts were tested against the seed-borne *Fusarium* sp. by poison food technique under different concentrations (0.1, 0.2 and 0.5%). Among the 4 plant extracts 0.2% zimmu leaf extract recorded maximum inhibition of *F. graminearum* mycelial growth, both ethanol and methanol extracts. Between the solvents methanol extract recorded maximum inhibition (77.22%) over control. It's the best treatment; followed by 0.2% zimmu leaf (ethanol) extract (Table 3). The same leaf extracts (0.2%) treatment recorded maximum inhibition (2.21 cm) of *F. moniliforme* growth under *in vitro* condition, followed by 0.2% zimmu leaf (ethanol) extract (Table 4 and Fig. 2). The germination of spores of

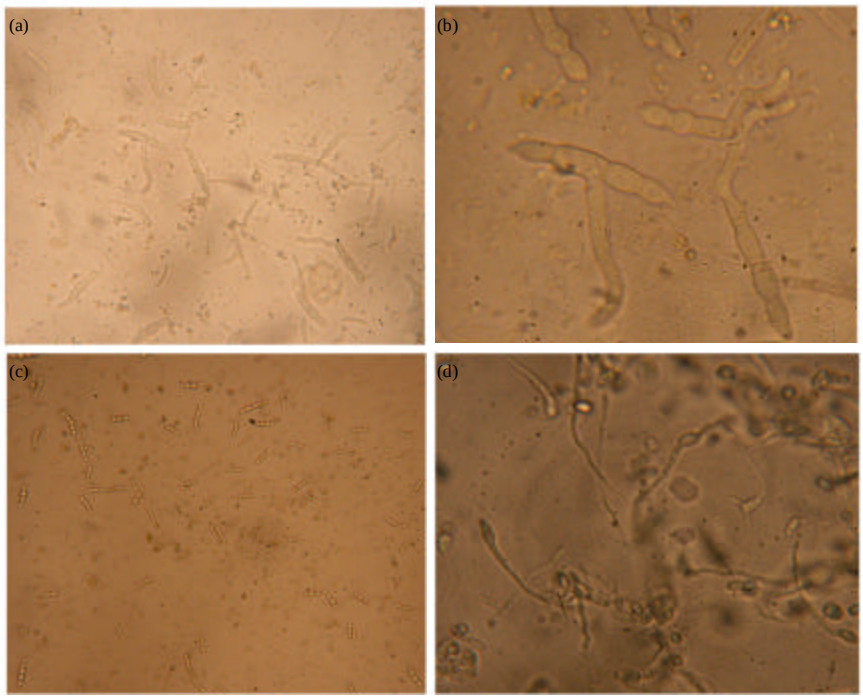

Fig. 1(a-d): *In vitro* screening of plant leaf extracts against *Fusarium* sp. by spore germination assay, (a) Macroconidial germination of *F. graminearum* in Adhatoda leaf extract treated, (b) Control, (c) Macroconidial germination of *F. graminearum* in zimmu leaf extract treated, (d) Microconidial germination of *F. moniliforme* zimmu leaf extract treated

Table 3: Screening of plant leaf extracts (different solvents) against *F. graminearum* under *in vitro* condition (poison food technique)

		Ethanol		Methanol	
Plants	Concentration (%)	Mean mycelial growth (cm)*	Decrease over control (%)	Mean mycelial growth (cm)*	Decrease over control (%)
Zimmu	0.1	3.68	59.11 (50.25)	3.40	62.22 (52.07)
	0.2	2.25	75.00 (60.00)	2.05	77.22 (61.49)
	0.5	2.97	67.00 (54.94)	2.57	71.44 (57.70)
Adhatoda	0.1	4.02	55.33 (48.06)	3.02	66.44 (54.60)
	0.2	3.81	57.67 (49.41)	2.80	68.89 (56.10)
	0.5	3.61	59.89 (50.71)	2.86	68.22 (55.69)
Henna	0.1	4.45	50.56 (45.32)	4.10	54.44 (47.55)
	0.2	4.06	54.89 (47.81)	3.65	59.44 (50.44)
	0.5	3.86	57.11 (49.09)	3.36	62.67 (52.34)
Insulin plant	0.1	4.15	53.89 (47.23)	4.15	53.89 (47.23)
	0.2	4.04	55.11 (47.93)	3.80	57.78 (49.48)
	0.5	4.21	53.22 (46.85)	3.21	64.33 (53.33)
Control		9.00	00.00 (00.74)	9.00	00.00 (00.74)
CD (p = 0.05)		0.3065		0.2666	

*Mean of three replications, Values in the parentheses are arc sine transformed values

Helminthosporium sp. was completely inhibited by acetone extracts of *Allium cepa* and *Allium sativum* leaves whereas, the spore germination of *Curvularia penniseti* was totally inhibited by acetone extract of *Ocimum sanctum* and mint leaves.

The different concentrations of zimmu leaf extract tested. Among them 100% recorded the maximum inhibition area (6.160 cm^2) against *F. graminearum*. However, it is on par with 75 and 50% concentrations. Similar, observations were also recorded against *F. moniliforme* (Table 5). Hence, the EC formulation of zimmu leaf extract was prepared at 50% concentration for field trials is economical than any other. The extract of zimmu (*A. sativum*×*A. cepa)* at 0.5% concentration exhibited strong antifungal activity against *A. niger*, *F. moniliforme*, *A. flavus*, *C. lunata* and

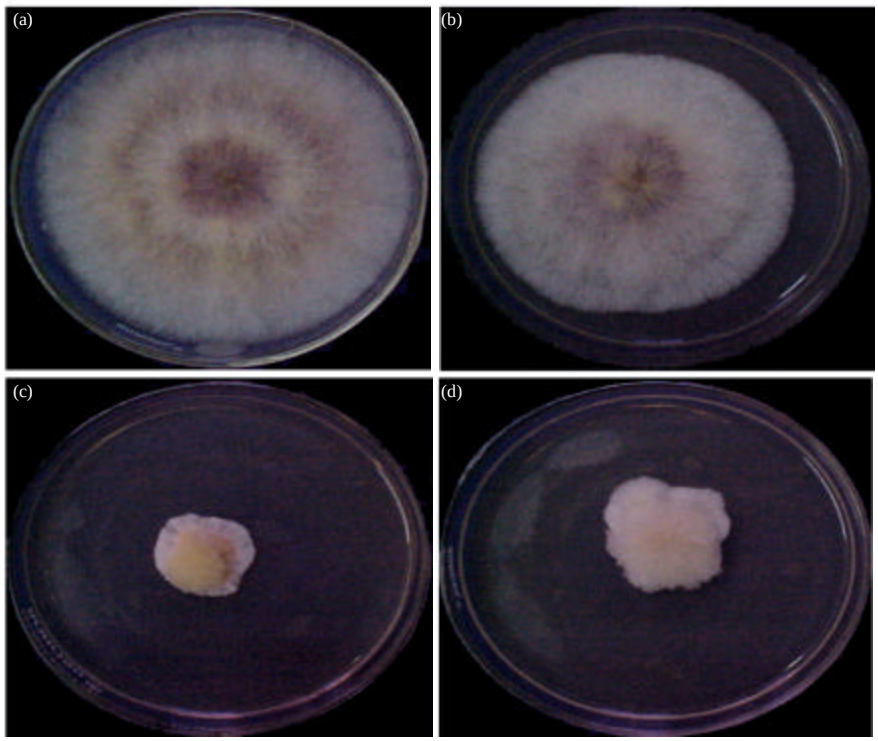

Fig. 2(a-d): Screening of plant leaf extracts against *F. graminearum*, (a) Control plate of *F. graminearum*, (b) 0.1% zimmu leaf extract treated plate, (c) 0.2% zimmu leaf extract treated plate and (d) 0.5 % zimmu leaf extract treated plate

Table 4: Screening of plant leaf extracts (different solvents) against seed borne *Fusarium moniliforme* under *in vitro* condition (poison food technique)

		Ethanol		Methanol	
Plants	Concentration (%)	Mean mycelial growth (cm)*	Decrease over control (%)	Mean mycelial growth (cm)*	Decrease over control (%)
Zimmu	0.1	3.81	57.67 (49.41)	3.39	62.33 (52.14)
	0.2	2.56	71.56 (57.77)	2.21	75.44 (60.29)
	0.5	3.28	63.56 (52.87)	3.05	66.11 (54.40)
Adhatoda	0.1	4.20	53.33 (46.91)	3.02	66.44 (54.60)
	0.2	3.75	58.33 (49.80)	2.80	68.89 (56.10)
	0.5	3.70	58.89 (50.12)	3.10	65.56 (54.07)
Henna	0.1	4.50	50.00 (45.00)	4.15	53.89 (47.23)
	0.2	4.00	55.56 (48.19)	3.40	62.22 (52.07)
	0.5	3.75	58.33 (49.80)	3.45	61.67 (51.75)
Psyllium	0.1	4.25	52.78 (46.60)	4.05	55.00 (47.87)
	0.2	3.94	56.22 (48.57)	3.50	61.11 (51.42)
	0.5	4.25	52.78 (46.60)	3.80	57.78 (49.48)
Control		9.00	00.00 (00.79)	9.00	00.00 (00.79)
CD (p = 0.05)		0.3065		0.2666	

*Mean of three replications, Values in the parentheses are arc sine transformed values

Table 5: Effect of zimmu leaf extracts (different concentration) against seed borne *Fusarium* sp. under *in vitro* condition (paper disc method)

	Fusarium graminearum		*Fusarium moniliforme*	
Concentration (%)	Mycelial growth diameter (cm)*	Inhibition area (cm^2) (πr^2)	Mycelial growth diameter (cm)*	Inhibition area (cm^2) (πr^2)
100	2.80	6.160	3.35	8.818
75	2.75	5.942	3.25	8.299
50	2.70	5.728	3.21	8.046
25	1.60	2.011	2.55	5.109
Control	0.00	-	0.00	-
CD (p = 0.05)	0.1387		0.1437	

*Mean of three replications

H. halodes (Vasanth Baskar, 2007). The antimicrobial activity of zimmu was reported by Sathya (2004) against the phytopathogenic fungi viz., *C. lunata*, *A. solani*, *R. solani* and *A. flavus*. The antifungal activity of *A. cepa* and *A. sativum* against *F. oxysporum*, *R. solani*, *A. flavus*, *A. niger* and *A. parasiticus* were reported by several workers (Wani and Kurucheve, 2004).

CONCLUSION

In conclusion, the present study showed that among the screened medicinal plant leaf extracts under *in vitro* condition, the Zimmu, Adhathoda, Henna and Insulin plants extract exhibit maximum inhibitory against *F. graminearum* and *F. moniliforme* conidia. Among them, 0.2% zimmu leaf extract recorded maximum inhibition of *F. graminearum* and *F. moniliforme* mycelial growth, both in ethanol and methanol extracts. The different concentrations of zimmu leaf extract, 100% recorded the maximum inhibition area of 6.160 cm^2 against *F. graminearum* and 8.818 cm^2 against *F. moniliforme* mycelial growth. Therefore, 0.2% zimmu leaf extract of EC formulation was further screened towards the EC formulation for commercial scale field spray. Besides, safety tests are to be conducted for leaf extract before recommending its use on field conditions.

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