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Antifungal activity of cow urine distillates of local botanicals against major pathogens of bell pepper

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Cow urine distillates of botanicals *viz, Ranunculus muricatus, Melia azedarach, Vitex negundo, Eupatorium* sp. and *Murraya koenigii* and cow urine alone were evaluated at 0.5, 2.0, 4.0, 6.0, 8.0 and 10.0% concentrations under *in vitro* conditions against bell pepper pathogens namely *Rhizoctonia solani, Sclerotinia sclerotiorum, Sclerotium rolfsii, Phytophthora nicotianae, Fusarium oxysporum* f.sp. *capsici, Fusarium solani* and *Colletotrichum capsici*. *M. koenigii* and *V. negundo* provided the complete inhibition in mycelial growth of *C. capsici, Rhizoctonia solani, Phytophthora nicotianae, Sclerotinia sclerotiorum, Fusarium solani* and *S. rolfsii* at 10% concentration. *R. muricatus* and *Murraya azedarach* caused 100% inhibition of *R. solani, S. sclerotiorum* and *S. rolfsii* whereas more than 98% inhibition in mycelial growth of *F. solani, P. nicotianae, R. solani* and *C. capsici* was observed at 10% concentration. *Eupatorium* sp. showed 63.05 to 99.69% inhibition against all the test pathogens. Inhibition in rest of the concentrations ranged between 85.06 to 98.97%. Cow urine distillate exhibited more than 98% inhibition in mycelial growth of *P. nicotianae* and *S. sclerotiorum* and 93% inhibition of *C. capsici, R. solani, F. solani, F. oxysporum* f.sp. *capsici* and *S. rolfsii* at 10% concentration and inhibition in rest of concentrations was observed to the tune of 59.70 to 96.77%. Cow urine distillates of botanicals were found more inhibitory to pathogens than cow urine distillate alone.

Key words: Antifungal activity, cow urine distillate, *Ranunculus muricatus*, *Melia azedarach*, *Vitex negundo*, *Eupatorium* sp., *Murraya koenigii*.

INTRODUCTION

Bell pepper (*Capsicum annuum* L. var. *grossum* Sendt.), is one of the most important off-season vegetable of Himachal Pradesh and offers potential for boosting economy of farmers of hilly regions. It is a warm season and chilling sensitive crop. It is affected by various diseases *viz.,* anthracnose (*Colletotrichum capsici* (Synd.) Butler and Bisby), fruit rot (*Phytophthora nicotianae* Breda de Haan), powdery mildew (*Leveillula taurica* (Lev.) Arn.), stem rot (*Sclerotium rolfsii* Sacc.), wilt (*Fusarium oxysporum* (Schlect.) emend. Synd. and Hans. f.sp. *capsici* Riv.) and root rot (*Rhizoctonia solani* Kuhn) which affect the yield and quality of the produce, thereby reducing the profit margin of producers (Ochigbo and Harris, 1989). Anthracnose is one of the most important diseases of bell pepper causing yield losses upto 50% followed by phytophthora blight and fusarium wilt that also cause huge reduction in fruit yield (Erwin and Ribeiro, 1996; Pakdeevaraparn et al., 2005).

Although, a large number of fungicides have been reported effective against these pathogens globally, but recurrent and inadequate use of specific chemicals over the years has led to the development of resistant strains,

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destruction of natural predators and parasites, appearance of new toxicant tolerant pathogen races (Corke, 1980), environmental contamination and health hazards (Okigbo and Ogbonnaya, 2006). To overcome these problems, it becomes necessary to adopt the ecofriendly management strategies such as the use of biological control agents or the use of extracted antagonistic natural plant-derived compounds (Pal and Gardener, 2006).

Higher plants are a treasure house of phytochemicals, which serve as valuable drugs that have helped to combat several fatal diseases the world over. Higher plants are known to posses' fungitoxicity against spore germination and mycelial growth of phytopathogenic fungi (Varma and Dubey, 1999; Kiran and Raveesha, 2006). Extracts of *Ranunculus muricatus, Vitex negundo, Murraya koenigii, Melia azedarach* and *Eupatorium* species have been reported to be highly effective against different plant pathogens (Hassanein et al., 2008). Cow urine has a unique place in Ayurveda and has been described in 'Sushrita Sumhita' and Ashtanga Sangraha' to be the most effective substance secretion of animal origin with innumerable therapeutic values. As per Ayurvedic literature, cow urine possesses many medicinal properties (Krishnamurthi et al., 2004; Chauhan et al., 2001). Different preparations of cow urine like cow urine distillate, photoactivated cow urine, fresh cow urine have antimicrobial properties against human pathogens (Sathasivam et al., 2010). Therefore, the present study was carried out to prepare cow urine distillate of botanicals and to determine the antifungal activities against pathogens of bell pepper.

MATERIALS AND METHODS

Collection and preservation of botanicals

Five botanicals namely *R. muricatus, V. negundo, M. koenigii, M. azedarach* and *Eupatorium* species were used in the present study. Leaves of all the botanicals were collected from upper parts of stem during spring from the surroundings of Palampur. *R. muricatus* was 60 days old and other botanicals were two years old when their leaves collected. The leaves were oven dried by spreading them on the shelves of hot air oven over two to three layered blotting sheets at 50°C for 5 to 6 h for two to three days. After drying, the respective plant material was ground in a blender to obtain fine dry powder. Sufficient powdery biomass of all the botanicals was stored in paper bags (Tassel bags) at room temperature for further use.

Collection of cow urine

Fresh cow urine was collected in sterilized vessels from desi cows maintained at Organic Farm, CSK HPKV, Palampur. Cow urine was stored in earthen pots. 15 days old cow urine was used for further studies.

Cow urine distillate preparation

Cow urine was distilled at 100°C using temperature controlled distillation apparatus. The distillate was stored in sterile glass flask at 4°C in refrigerator for further studies (Sathasivam et al., 2010).

Preparation of distillate of botanicals

Powder of all five botanicals was soaked in cow urine over night and distilled at 50 to 60°C using distillation apparatus. The distillate thus obtained was collected in conical flasks and stored at 4°C.

Test pathogens

Test pathogens *viz., S. rolfsii, Fusarium solani*, *F. oxysporum* f.sp. *capsici, Sclerotinia sclerotiorum, C. capsici, P. nicotianae* and *R. solani* were isolated from diseased samples of bell pepper using standard methodology on PDA medium. Small bits of infected tissues were surface sterilized by dipping in a solution of 0.1% mercuric chloride for 10 to 15 s and washed thrice in sterilized water under laminar air flow. The bits were dried under two folds of sterilized filter papers and transferred to PDA slants. The tubes were incubated at 25 ± 1°C for 7 to 8 days and purified by single spore/hyphal tip method. Pure cultures were maintained on PDA slants.

Evaluation of antifungal potential

Distillates of cow urine alone and cow urine + botanicals were evaluated for their antifungal activity against *S. rolfsii, F. solani*, *F. oxysporum* f.sp. *capsici, S. sclerotiorum, C. capsici, P. nicotianae* and *R. solani* by poisoned food technique (Falck, 1907) with and without autoclaving. Double strength PDA medium was amended with equal quantity of cow urine distillate of botanicals and cow urine distillate alone at different concentrations ranging from 0.5, 2.0, 4.0, 6.0, 8.0 and 10.0% and aseptically poured in sterilized plates. Medium amended with cow urine distillate and sterilized distilled water served as control. Seven days old mycelial bits (5 mm) were placed in the centre of plate and plates were incubated at 25 ± 1°C. When control plates were covered with mycelial growth of pathogens, percent inhibition in mycelial growth was determined following McKinney (1923)

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

where, I = % inhibition, C = mycelial growth in control, T = mycelial growth in treatment .

RESULTS

Cow urine distillates of five botanicals were tested for their antifungal activity against pathogens of capsicum at different concentrations under *in vitro* conditions. Among these botanicals, *M. koenigii* was found most effective botanical against the pathogens. It caused maximum (100%) mycelial inhibition of *S. rolfsii, F. solani*, *S. sclerotiorum, C. capsici, P. nicotianae* and *R. solani* at 10% concentration and more than 99% inhibition at 8% concentration, respectively. While at lower concentrations, inhibition was 88 to 99% (Table 1). *V. negundo* was equally effective and exhibited complete inhibition of all the test pathogens except *P. nicotianae*

Table 1. Antifungal activity of cow urine distillate of *Murraya koenigii* against capsicum pathogens.

Conc. (%)	*Sclerotium rolfsii*		*Fusarium solani*		*F. oxysporum* f.sp. *capsici*		*Sclerotinia sclerotiorum*		*Colletotrichum capsici*		*Phytophthora nicotianae*		*Rhizoctonia solani*	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
0.5	7.07	88.89	3.41	94.64	4.09	93.56	4.34	93.18	1.97	96.91	3.60	94.32	5.17	91.87
2.0	1.97	96.91	2.22	96.50	2.18	96.57	0.27	99.58	1.43	97.75	1.97	96.84	1.43	97.75
4.0	0.57	99.11	1.65	97.40	1.93	96.97	0.20	99.69	0.81	98.72	1.54	96.97	0.48	99.24
6.0	0.21	99.67	0.98	98.46	0.66	98.96	0.00	100.00	0.65	98.99	0.66	98.77	0.37	99.42
8.0	0.00	100.00	0.20	99.69	0.57	99.11	0.00	100.00	0.00	100.00	0.57	99.11	0.00	100.00
10.0	0.00	100.00	0.00	100.00	0.30	99.53	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00
Control	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.58	0.00	63.59	0.00
CD (p=0.05)	0.63	-	0.79	-	0.81	-	0.85	-	0.60	-	0.60	-	0.88	-

a - Mycelial growth (cm^2), b - % Inhibition; 1. Mycelial growth measured were same in all the fungi 2. Area is 3.14 x radius of fungi x radius of fungi 3. CD means: Dependence of Confidence. Test of significance gives p value at 5% level of significance.

Table 2. Antifungal activity of cow urine distillate of *Vitex negundo* against capsicum pathogens.

Conc. (%)	*Sclerotium rolfsii*		*Fusarium solani*		*F. oxysporum* f.sp. *capsici*		*Sclerotinia sclerotiorum*		*Colletotrichum capsici*		*Phytophthora nicotianae*		*Rhizoctonia solani*	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
0.5	23.17	63.55	9.70	84.74	8.65	86.39	16.72	73.70	9.50	85.06	8.50	86.63	22.95	63.90
2.0	18.85	70.36	7.98	87.45	7.80	87.73	1.93	96.97	5.01	92.12	6.50	89.77	12.46	80.41
4.0	1.69	97.34	6.40	89.93	5.02	92.10	1.16	98.17	3.69	94.20	3.46	94.56	4.84	92.39
6.0	0.59	99.07	5.10	91.97	2.67	95.80	0.00	100.00	3.41	94.64	2.50	96.07	1.73	97.28
8.0	0.00	100.00	0.81	98.72	0.96	98.49	0.00	100.00	1.12	98.24	1.54	97.58	0.00	100.00
10.0	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	1.07	98.32	0.00	100.00
Control	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00
CD (p=0.05)	0.94	-	0.12	-	0.11	-	0.57	-	0.12	-	1.00	-	0.96	-

a - Mycelial growth (cm^2), b - % Inhibition.

(98%) at 10% concentration and at 8% concentration, its distillate provided 100% mycelial inhibition of *S. rolfsii, S. sclerotiorum* and *R. solani* and more than 97% inhibition of rest of the pathogens. While at concentrations ranging from 0.5 to 6.0%, inhibition was observed from 63 to 98% (Table 2).

Distillates of *M. azedarach* and *Eupatorium* sp. showed strong antifungal activity against pathogens of bell pepper (Tables 3 and 4). They provided 100% mycelial inhibition of *S. rolfsii, S. sclerotiorum, F. oxysporum* f.sp. *capsici* and *R. solani* and 98% inhibition of *F. solani*, *C. capsici* and *P. nicotianae* at 10% concentration. While at lower concentrations, inhibition ranged between 64 to 98%. *R. muricatus* distillate was found comparatively less effective against the test pathogens. It showed more than 98% inhibition in mycelial growth of all the pathogens at highest concentration whereas at 8% and lower concentrations, inhibition ranged between 77 to 99% (Table 5). Minimum mycelial inhibition was caused by cow urine distillate at all the tested concentrations. Its distillate exhibited less than 98% inhibition of all the pathogens of bell pepper (Table 6).

Among all the botanical distillates tested, cow urine distillates of *M. koenigii* and *V. negundo* showed strong antifungal properties against all the pathogens at 8% and above concentrations followed by *M. azedarach, Eupatorium* sp. and *R. muricatus*. However distillate of cow urine alone was least effective against the test pathogens (Figure 1a to g). With the addition of cow urine, the antifungal efficacy of botanicals enhanced.

DISCUSSION

All the cow urine distillates of botanicals were found effective against the test pathogens but *V. negundo* and *M. koenigii* found most inhibitory than cow urine distillate. There does not appear to be any report on antifungal activity of cow urine distillate of *R. muricatus, Eupatorium* sp., *M. azedarach, V. negundo* and *M. koenigii* against pathogens of bell pepper. However, the antifungal activity of cow urine distillate has been reported by

Table 3. Antifungal activity of cow urine distillate of *Melia azedarach* against capsicum pathogens.

Conc. (%)	*Sclerotium rolfsii*		*Fusarium solani*		*F. oxysporum* f.sp. *capsici*		*Sclerotinia sclerotiorum*		*Colletotrichum capsici*		*Phytophthora nicotianae*		*Rhizoctonia solani*	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
0.5	22.89	64.00	10.46	83.55	8.90	86.00	17.50	72.47	8.50	86.63	8.70	86.31	23.00	63.83
2.0	16.61	73.88	9.16	85.59	5.50	91.35	11.24	82.33	7.87	87.62	7.12	88.80	19.11	69.95
4.0	3.69	94.20	7.10	88.83	4.10	93.55	1.29	97.97	4.34	93.18	4.60	92.76	14.40	77.35
6.0	0.79	98.77	6.41	89.91	3.80	94.02	0.22	99.65	3.31	94.79	3.25	94.88	4.60	92.76
8.0	0.20	99.69	4.50	92.92	3.50	94.49	0.00	100.00	1.93	96.97	3.03	95.23	0.00	100.00
10.0	0.00	100.00	0.20	99.68	0.00	100.00	0.00	100.00	0.42	99.34	0.27	99.58	0.00	100.00
Control	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00
CD (p=0.05)	0.10	-	0.15	-	0.13	-	0.88	-	0.14	-	0.37	-	0.99	-

a - Mycelial growth (cm^2), b - % Inhibition.

Table 4. Antifungal activity of cow urine distillate of *Eupatorium* sp. against capsicum pathogens.

Conc. (%)	*Sclerotium rolfsii*		*Fusarium solani*		*F. oxysporum* f.sp. *capsici*		*Sclerotinia sclerotiorum*		*Colletotrichum capsici*		*Phytophthora nicotianae*		*Rhizoctonia solani*	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
0.5	24.13	63.05	16.00	74.83	9.60	84.90	15.50	75.46	9.55	84.98	4.46	92.99	23.00	63.83
2.0	19.5	69.33	14.91	76.55	5.78	90.91	12.00	81.12	8.39	86.80	2.54	96.00	22.01	65.39
4.0	16.85	73.50	7.32	88.48	3.45	94.57	5.40	91.50	5.05	92.05	1.89	97.03	12.10	80.97
6.0	0.81	98.72	6.60	89.62	1.50	97.64	1.93	96.97	4.50	92.92	1.47	97.69	4.91	92.27
8.0	0.20	99.69	5.41	91.49	0.00	100.00	0.53	99.16	4.12	93.52	1.33	97.91	3.70	94.18
10.0	0.00	100.00	3.30	94.81	0.00	100.00	0.00	100.00	0.51	99.19	0.59	99.07	0.00	100.00
Control	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00
CD (p=0.05)	0.88	-	0.12	-	0.79	-	0.81	-	0.15	-	0.99	-	0.13	-

a - Mycelial growth (cm^2), b - % Inhibition.

Table 5. Antifungal activity of cow urine distillate of *Ranunculus muricatus* against capsicum pathogens.

Conc. (%)	*Sclerotium rolfsii*		*Fusarium solani*		*F. oxysporum* f.sp. *capsici*		*Sclerotinia sclerotiorum*		*Colletotrichum capsici*		*Phytophthora nicotianae*		*Rhizoctonia solani*	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
0.5	21.83	65.67	9.62	84.88	6.60	89.62	12.45	80.42	3.80	94.03	5.31	91.65	15.90	75.00
2.0	14.40	77.35	6.23	90.21	5.44	91.44	6.91	89.13	3.30	94.81	4.91	92.28	10.75	83.10
4.0	4.40	93.08	4.64	92.71	3.74	94.12	2.40	96.22	2.71	95.74	3.04	95.23	6.30	90.09
6.0	2.54	96.00	3.80	94.03	3.41	94.64	1.89	97.03	2.10	96.69	2.45	96.15	4.55	92.84
8.0	0.60	99.06	3.19	94.98	3.19	94.98	0.57	99.11	2.27	96.43	1.33	97.91	1.93	96.97
10.0	0.47	99.27	0.95	98.51	1.43	97.75	0.30	99.53	0.29	99.55	1.50	97.64	0.00	100.00
Control	63.58	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00
CD (p=0.05)	0.23	-	0.13	-	0.15	-	0.14	-	0.94	-	0.99	-	0.13	-

a - Mycelial growth (cm^2), b - % Inhibition.

Shah et al. 2011) against human pathogens *viz., Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus*. These observations are likely to be the result of the presence of certain volatile and non volatile components present in urine and formation of more cations and nitrosoamines. Sathasivam et al. (2010) analyzed the antibacterial and antifungal activity of cow urine distillate against some clinical pathogens such as *Salmonella typhii*, *K. pneumonia* and *Aspergillus niger* and increase in antimicrobial properties of cow urine distillate may be attributed to the acidic pH after distillation.

Although, antifungal activity of cow urine distillate of botanicals has not been reported earlier against pathogens of capsicum but some researchers report the antifungal activity of water distillates of *Artemisia sieberi* and *Eupatorium odoratum* against *R. solani, F. solani* and *C. capsici* (Puttawong and Wongroung, 2009) and

Table 6. Antifungal activity of cow urine distillate against capsicum pathogens.

Conc. (%)	*Sclerotium rolfsii*		*Fusarium solani*		*F. oxysporum* f.sp. *capsici*		*Sclerotinia sclerotiorum*		*Colletotrichum capsici*		*Phytophthora nicotianae*		*Rhizoctonia solani*	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
0.5	25.62	59.70	16.13	74.63	11.24	82.33	18.21	71.36	9.87	84.47	9.08	85.73	23.29	63.38
2.0	20.29	68.10	15.20	76.10	9.53	85.02	12.35	80.58	8.50	86.63	7.30	88.52	23.00	63.82
4.0	10.08	84.15	7.46	88.26	5.24	91.76	6.53	89.74	5.24	91.76	5.87	90.78	15.20	76.10
6.0	7.30	88.52	6.91	89.13	5.12	91.95	2.05	96.77	4.91	92.28	3.35	94.73	5.04	92.08
8.0	5.51	91.33	5.58	91.22	5.10	91.97	0.61	99.04	4.30	93.23	3.19	94.98	4.34	93.18
10.0	3.40	94.65	3.41	93.71	5.02	92.10	0.46	99.27	4.15	93.47	2.74	95.70	2.70	95.75
Control	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00	63.59	0.00
CD (p=0.05)	0.11	-	0.91	-	0.97	-	0.12	-	0.88	-	0.10	-	0.13	-

a - Mycelial growth (cm^2), b - % Inhibition.

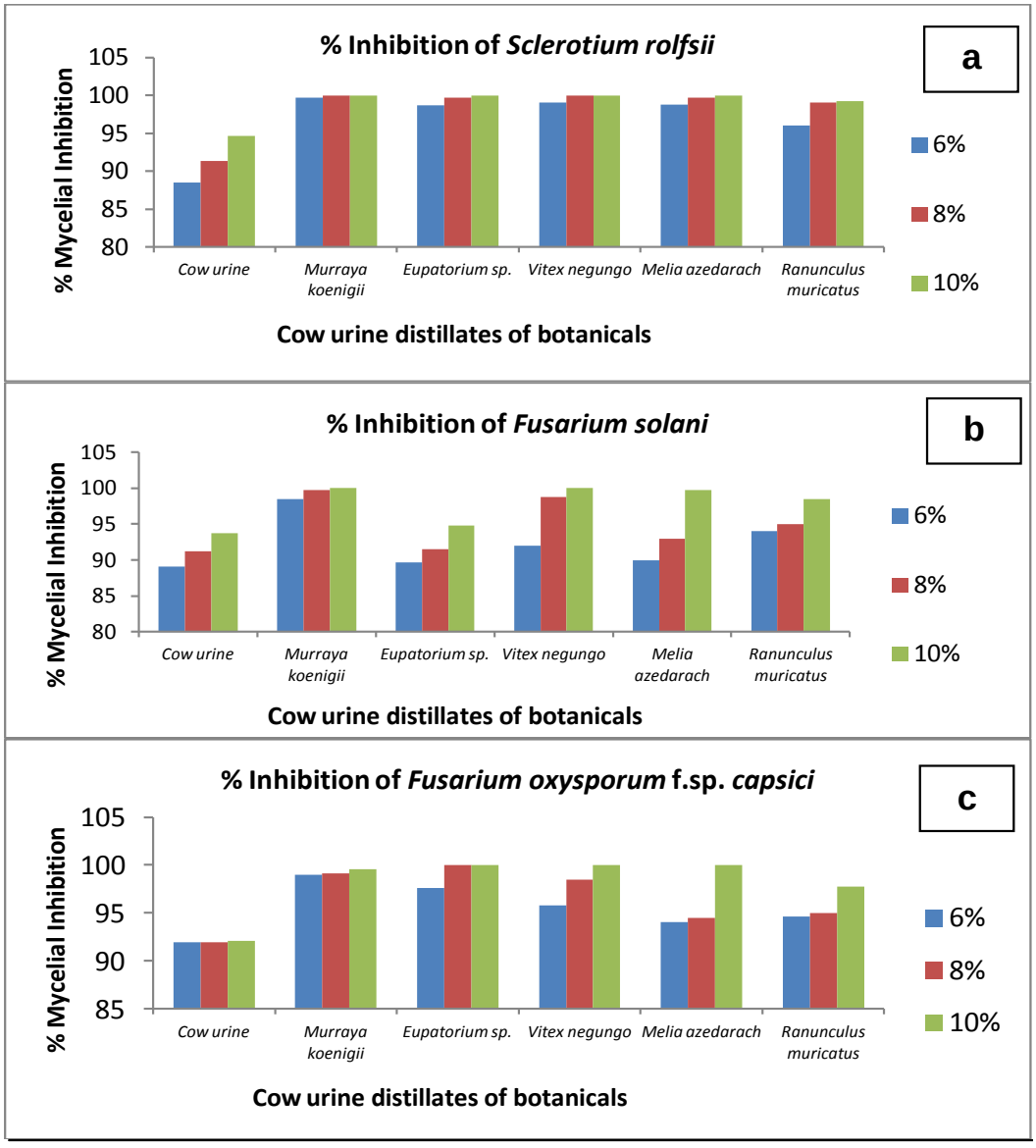

Figure 1. Antifungal activity of cow urine distillate of botanicals against pathogens of capsicum [a – *Sclerotium rolfsii*, b – *Fusarium solani*, c – *Fusarium oxysporum* f.sp. *capsici*, d – *Sclerotinia sclerotiorum*, e –*Colletotrichum capsici*, f – *Phytophthora nicotianae*, g - *Rhizoctonia solani*].

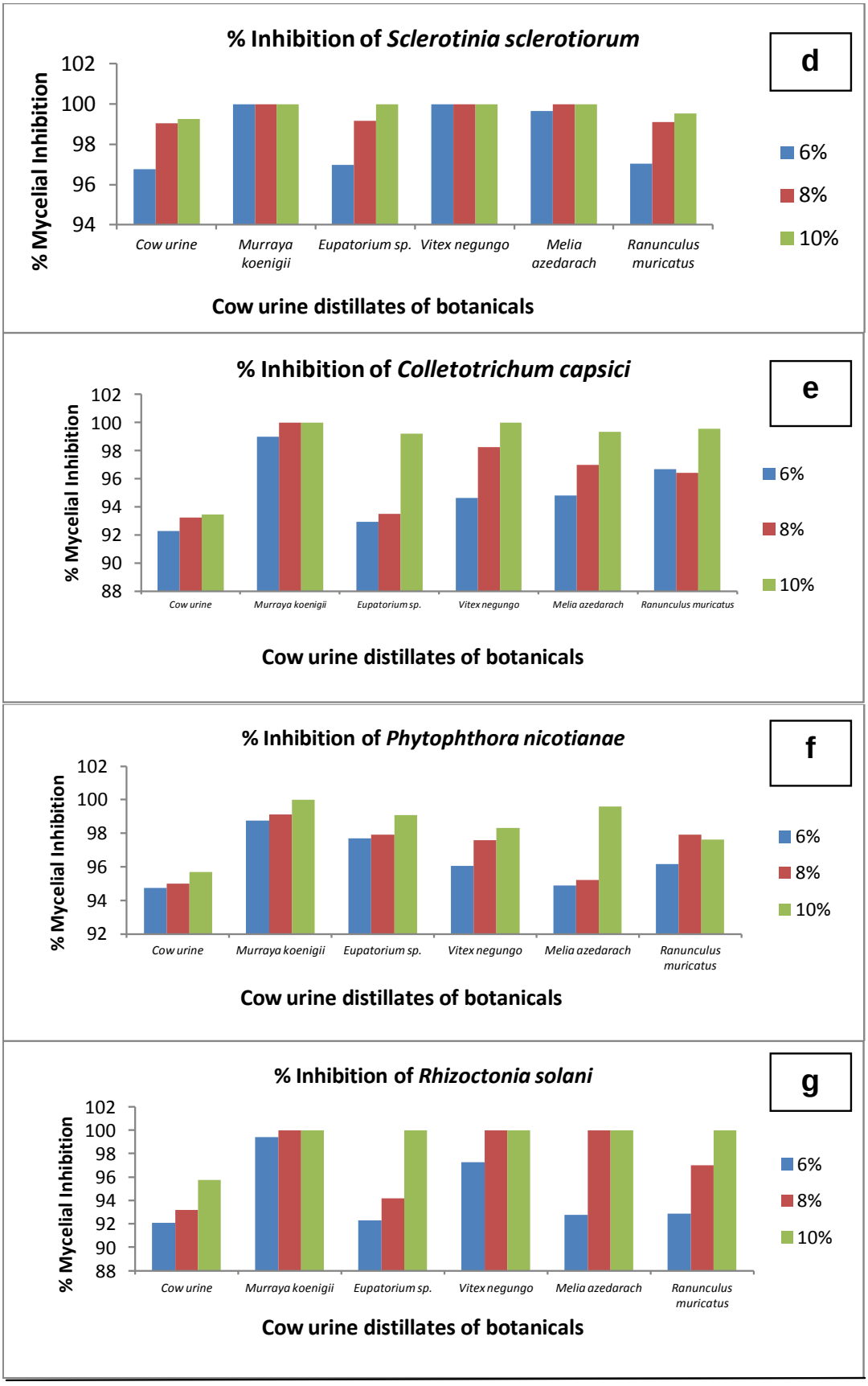

Figure 1. Contd.

Ocimum sanctum and *M. koenigii* against *R. solani, F. solani* and *Helminthosporium solani* (Subudhi et al., 2010), respectively. Water distillate of *Eupatorium* sp. has been reported to contain antifungal compounds such as nepodin, parietin and chrysophenol (Bhattarai and Shrestha, 2009). Laboratory screening of plant extracts in the present study has given encouraging results, indicating their potential use in the management of bell pepper diseases and other soil borne pathogens. Cow urine distillation of botanicals enhanced their antifungal activity against the pathogens of bell pepper.

The combination of cow urine and botanical distillates was found to be effective against pathogens of bell pepper. The results indicated that cow urine distillates of *V. negundo* and *M. koenigii* showed complete mycelial inhibition of all the pathogens at 10% concentrations. Thus, cow urine distillates of botanicals which possess antifungal activity can be exploited as biopesticides for the biocontrol of fungal pathogens.

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REFERENCES

Bhattarai N, Shrestha G (2009). Antibacterial and antifungal effect of *Eupatorium adenophorum* Spreng against bacterial and fungal isolates. Nepal J. Sci. Technol. 10:19-95.

Chauhan RS, Singh BP, Singhal LK (2001). Immunomodulation with kamdhenu ark in mice. J. Immunolpathol. 71:89-92.

Corke ATK (1980). Modern developments in the control of plant diseases: Biological Control Methods. Sci. Hortic. 31:54-59.

Erwin DC, Ribeiro OK (1996). *Phytophthora capsici*. In: Erwin DC, Ribeiro OK (ed) *Phytophthora* diseases world-wide. APS Press, St. Paul, NIN, USA. pp. 262-268.

Falck R (1907). Wachstumgesetze, Wachstm-taktorenund temperature Wertder helzerstevenden. *Mycelien* 1:43-154.

Hassanein NM, Abou ZMA, Youssef KA, Mahmoud DA (2008). Efficacy of leaf extracts of neem (*Azadirachta indica*) and chinaberry (*Melia azedarach*) against early blight and wilt diseases of tomato. Au. J. Basic Appl. Sci. 2(3):763-772.

Kiran B, Raveesha KA (2006). Antifungal activity of seed extract of *Psoralea corylifolia* L. Plant Dis. Res. 20:213-215.

Krishnamurthi K, Dutta D, Sivanesan SD, Chakrabarti T (2004). Protective effect of distillate and redistillate of cow's urine in human polymorphonuclear leukocytes challenged with established genotoxic chemicals. Biomed. Environ. Sci. 17:247-256.

McKinney HH (1923). Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. J. Agric. Res. 26(2):195-217.

Ochigbo AA, Harris GP (1989). Effect of film plastic cover on the growth and yield of bush tomato grown in a bed system. J. Hortic. Sci. 64(1):300-302.

Okigbo RN, Ogbonnaya UO (2006). Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on postharvest yam (*Dioscorea* spp.) rot. Afr. J. Biotechnol. 5:727-731.

Pakdeevaraparn P, Wasee S, Taylor PWJ, Mongkolporn O (2005). Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in capsicum. Plant Breed. 124(2):206-208.

Pal KK, Gardener BM (2006). (Eds). The Plant Health Instructor in Biological control of plant pathogens, Constable, London, English. P. 206.

Puttawong S, Wongroung S (2009). Plucao (*Houttuynia cordata*) Thunb. and Sabsua (*Eupatorium odoratum* L.) extracts suppress *Colletotrichum capsici* and *Fusarium oxysporum*. Asian J. Food Agro-Industry Special Issue, pp. 5381-5386.

Sathasivam AK, Muthuselvam M, Rajndran R (2010). Antimicrobial activities of cow urine distillate against some clinical pathogens. Global J. Pharmacol. 4(1):41-44.

Shah CP, Patel DM, Dhami PD, Kakadia J, Bhausar D, Vachhani UD, Trivedi MN, Joshi VJ (2011). *In vitro* screening of antibacterial activity of cow urine against pathogenic human bacterial strains. Int. J. Curr. Pharm. Res. 3(2):91-92.

Subudhi E, Mohanty S, Kuanar A, Panda M (2010). *In vitro* antimicrobial study of plant essential oils and extracts. Int. J. Microbiol. 8(1):1-6.

Varma J, Dubey NK (1999). Prospectives of botanical and microbial products as pesticides of tomorrow. Curr. Sci. 76:172-179.