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ANTIFUNGAL EFFICACY OF AQUEOUS EXTRACTS OF NEEM CAKE, KARANJ CAKE AND VERMICOMPOST AGAINST SOME PHYTOPATHOGENIC FUNGI

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ABSTRACT
Several agro-based waste and byproducts are known and cited to play an important role in the management of plant diseases in ancient texts. They act directly or indirectly on plant pathogens to inhibit the growth and multiplication or by inducing resistance in crop plants. In the present experiments, aqueous extracts of neem cake, karanj cake and vermicompost were tested against some important phytopathogenic fungi viz., *Helminthosporium pennisetti*, *Curvularia lunata* and *Colletotrichum gloeosporioides* f. sp. *mangiferae* for their antifungal activities. *H. pennisetti* was found to be most sensitive one followed by *C. gloeosporioides* f. sp. *mangiferae* and *C. lunata* against all the tested drugs. Against *C. gloeosporioides* f. sp. *mangiferae*, aqueous extract of karanj cake was most effective where GI_{50} was found to be 0.41% drug concentration followed by neem cake (0.46%) and vermicompost (0.86%). In case of *C. lunata* and *H. pennisetti,* neem cake extract was most effective with GI_{50} value of 0.27% and 0.11% respectively. The GI_{50} values noted for *C. lunata* with extracts of karanj cake (0.70%), vermicompost (0.88%) and for *H. pennisetti* were (0.20%) and (0.22%) respectively.

INTRODUCTION

The indiscriminate use of synthetic chemicals for the control of pests and diseases of crop plants has posed serious threat to human health and environment leading to disturbed biodiversity, outbreaks of secondary pests, resurgence development of resistance in the pathogens and contamination of food chain in the ecosystem. Fungal diseases of crop plants have always been one of the major constraints in successful crop production which causes severe yield loss every year. Injudicious use of synthetic fungicides for controlling plant diseases have given rise to negative effects on human and animal health and agro-ecosystem. However, the researchers are optimistic in developing alternatives to chemical fungicides. Eco-friendly systems involving plant products and biological agents, which act directly on the pathogens or indirectly by inducing resistance in plants (Mishra and Raja, 1999), have gained considerable importance as an alternative to synthetic fungicides.

Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environment impact and danger to consumers in contrast to the synthetic pesticides (Varma and Dubey, 1999). Active principles from medicinal plants are being tried as replacements of synthetic fungicides in management plant diseases in organic farming system. Plant extracts have unique antimicrobial properties, which act in holistic mode. Various workers have reported that plant extracts and their secondary metabolites; alkaloids, terpenoid, glycosides and phenolic acids have a number of medicinal properties and affect biological functions at very low concentrations; some of them also possess antimicrobial activity (Srivastava *et al*., 1994; Singh *et al*., 1999).

The Meliaceae specially neem (*Azardirachta indic*a. A. Juss.), contains at least 35 biologically active principles of which nimbin and azadirachtin (Pennington *et al*., 1975) are the most active insecticidal ingredients and are present predominantly in the seeds, leaves and other parts of the neem tree. *A. indica*, a common medicinal plant that could be exploited as the source of a potent biocide that has immense fungicidal effect against several fungal pathogens including *Aspergillus* and *Rhizopus* (Mondali, 2009). Besides its therapeutic efficacy, neem has already established its potential as a source of naturally occurring agrochemicals. It has been previously reported that the active ingredients of neem constitute mostly of triterpenoides, *e.g.*, nimbin, nimbicidine, azadirachtin etc. (Brahmachari, 2004). Karanj (*Pongamia pinnata* L.), which belongs to family Fabaceae, and is inhabitants of India, Sri Lanka, Malaysia, North Australia and Indonesia, exhibited outstanding antifungal activity against the soil-borne phytophagus fungus *Sclerotium rolfsii* (Sacc.). Kumar *et al*. (2003) covered a review on the chemical composition of *P. pinnata*, and the insecticidal, antimicrobial, nematicidal and medicinal properties of compounds isolated from *P. pinnata*. Aqueous extract of vermicompost has shown to depress soilborne pathogens and pests (Szczech *et al*.,

1993; Nakasone *et al*., 1999; Rodríguez *et al*., 2000; Zaller, 2006; Reddy *et al*., 2012). Extracts from thermophilic compost proved to be effective against various fungal diseases of leaves and fruits especially when applied prophylactically (Weltzien, 1989; Scheuerell and Mahaffee, 2002).

Several reports indicated that foliar spray of aqueous extract of neem cake showed antifungal efficacy against powdery mildew of balsam and also have ability to influence the secondary metabolites in soil borne phytopathogenic fungus *Sclerotium rolfsii*. Similarly aqueous extract of vermicompost have potential to inhibit powdery mildew and ability to induce systemic resistance in pea and balsam (Singh *et al*. 2003; Singh *et al*., 2003; Singh *et al*., 2010a, 2010b). Maurya *et al*. (2004) reported that several plant extracts like cashew nut (*Anacardium occidentale* L.) shell, rhizome of ginger (*Zingiber officinale* Roscoe.) and motha grass (*Cyperus rotundus* L.), leaves of butter tree (*Madhuca indica* J. F. Gmel.) and tulsi (*Ocimum sanctum* L.) have shown antifungal properties against powdery mildew of pea under field condition. Singh *et al*. (2003) reported that the plant alkaloids of *Corydalis longipes* DC. Showed potential antifungal property against some phytopathogenic fungi. Sahni *et al*. (2005) reported that the plant alkaloid non-securinine show strong antifungal activity against some phytopathogenic fungi. Keeping these views in mind, the experiments were designed to see the efficacy of aqueous extracts oil cakes of neem and karanj and of the vermicompost against some phytopathogenic fungi.

MATERIALS AND METHODS

Isolation and purification of plant pathogenic fungi

The phytopathogenic fungi were isolated from their respective hosts which were collected from the experimental farm of the ICAR-Research Complex for Eastern Region, Research Centre, Ranchi, Jharkhand on potato dextrose agar (peeled potato 250g, dextrose 20g, agar 15g, distilled water; 1 l). The cultures were further purified by single spore isolation /hyphal tip isolation techniques and maintained at 25 ± 2ºC on PDA slants for the further experimentation.

Preparation of aqueous extracts

Aqueous extract (w/v) of neem cake, karanj cake and vermicompost were obtained.

Preparation of stock solution and test concentrations

Stock solution of 10mL was prepared by dissolving separately 10mg of extracted drug in 1mL in sterilized distilled water. Then the test concentrations (0.2 to 1.0%) were prepared from the stock solution by diluting with sterilized distilled water.

Bioassay of aqueous extract of different of cakes and vermicompost

One drop (30- 40μL) of test concentration was placed on grease – free glass slides. A loop full fungal spore (200-300) were picked up with the help of a sterile inoculation needle from 7 -10 days old growing cultures and mixed in the test concentration. The slides were later placed in moist chambers made by placing two sterile moist filter papers on the lid and base of petri dishes. The spores were then incubated at 25 ± 2ºC for 24h. Spores germination were observed after staining with Cotton Blue prepared in lactophenol under Phase Contrast microscope (type 020–519.503 LB 30T, Leica, Germany); spores mixed in sterile distilled water served as control. All the experiments were conducted in triplicate. Spore germination inhibition was calculated by using following formula:

$$\% \text{ Inhibition of spore germination} = \frac{\text{Number of germinated spores in control} - \text{Number of germinated spores in the treatment}}{\text{Total spores germinated in control}} \times 100$$

Statistical analysis

The data for percent spore germination inhibition thus obtained was used to analyze GI_{50} (drug concentration where 50% pathogen spore germination inhibited) using the computer package program SPSS (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Antimicrobial efficacy of aqueous extracts of neem cake, karanj cake and vermicompost were observed for inhibition of spore germination against three sporulating fungi, *C. lunata*, *H. pennisetti* and *C. gloeosporioides* f. sp. *mangiferae* (Table 1) and comparative growth inhibitory activity (GI_{50}) of various aqueous extracts against phytopathogenic fungi were analyzed (Table 2). Among different phytopathogens *H. pennisetti* was found to be most sensitive one followed by *C. gloeosporioides* f. sp. *mangiferae* and *C. lunata*. Neem cake extract was found most effective against *H. pennisetti* where 100% growth inhibition was observed at 0.6% drug concentration (GI_{50} - 0.11%) followed by *C. gloeosporioides* f. sp. *mangiferae* where 100% growth inhibition at 0.9% drug concentration and *C. lunata* where 100% growth inhibition was at highest drug concentration used *i.e.* 1.0%, but the GI_{50} value of neem cake extract against these two fungi were observed to be 0.46% and 0.27% respectively. With karanj cake extract, 100% growth inhibition in *H. pennisetti* was observed at 0.6% drug concentration (GI_{50} - 0.2%). The same was observed at 0.9% drug concentration for *C. gloeosporioides* f. sp. *mangiferae* (GI_{50}-0.41%) and for C. *lunata,* only 83.81% inhibition was observed at 1.0% drug concentration (GI_{50}-0.70%). Vermicompost extract was least effective of all with 100% growth inhibition against only one of the test pathogen, *H. pennisetti* at 0.9 % drug concentration (GI_{50}-0.22%). The GI_{50} value of the same drug was 0.7% against *C. lunata* and 0.86% against *C. gloeosporioides* f. sp. *mangiferae*.

Singh *et al*. (2010a) reported that foliar spray of aqueous extract of neem cake show antifungal efficacy against powdery mildew of balsam. Singh *et al*. (2010 b) reported that the neem cake has ability to influence the secondary metabolites in soil borne phytopathogenic fungus *Sclerotium rolfsii*. Bhonde *et al*. (1999) have reported that ccommercially available neem formulations had shown antifungal activity against several plant pathogenic fungi *viz*., *Fusarium oxysporum, Alternaria solani, C. lunata, Helminthosporium* sp. and *Sclerotium rolfsii*. Singh *et al*. (2003) reported that the aqueous extract of vermicompost has potential to inhibit powdery mildew and have ability to reduce systemic resistance in pea and balsam. It is evident from the several reports that vermicompost extracts are effective

Table 1: Growth inhibition% of different phytopathogenic fungi against various aqueous extracts

Drug concentration (%)	*Colletotrichum gloeosporioides* f. sp. *mangiferae*	*Curvularia lunata*	*Helminthosporium pennisetti*
	% inhibition by Neem cake extract		
0.20	27.90 ± 1.86	43.27 ± 0.24	70.35 ± 0.35
0.30	31.07 ± 1.57	49.86 ± 0.23	79.69 ± 0.47
0.40	34.63 ± 2.37	59.20 ± 0.44	85.14 ± 0.55
0.50	39.13 ± 4.55	74.23 ± 1.63	90.94 ± 0.41
0.60	54.62 ± 2.66	77.21 ± 1.48	100
0.70	70.30 ± 0.28	81.29 ± 1.49	100
0.80	81.68 ± 1.50	86.82 ± 0.96	100
0.90	100	93.17 ± 1.11	100
1.00	100	100	100
	% inhibition by Karanj cake extract		
0.20	29.35 ± 0.31	5.02 ± 1.85	54.33 ± 0.34
0.30	35.71 ± 0.63	12.46 ± 3.94	57.26 ± 0.46
0.40	40.70 ± 1.26	22.18 ± 4.58	65.88 ± 0.30
0.50	50.51 ± 0.44	30.81 ± 3.99	83.64 ± 0.34
0.60	61.66 ± 1.05	35.74 ± 1.36	100
0.70	73.28 ± 0.77	41.21 ± 1.95	100
0.80	91.42 ± 1.17	50.17 ± 4.01	100
0.90	100	64.58 ± 0.87	100
1.00	100	83.81 ± 2.80	100
	% inhibition by Vermicompost extract		
0.20	25.36 ± 0.32	7.79 ± 0.23	49.14 ± 2.12
0.30	28.46 ± 0.64	12.76 ± 0.45	53.89 ± 1.53
0.40	31.65 ± 0.24	21.25 ± 0.23	58.09 ± 1.69
0.50	34.65 ± 0.69	25.00 ± 1.61	65.07 ± 2.29
0.60	38.60 ± 0.50	32.67 ± 1.67	66.90 ± 1.48
0.70	44.22 ± 1.25	36.40 ± 2.01	71.26 ± 1.22
0.80	51.03 ± 1.17	41.85 ± 1.52	83.88 ± 2.42
0.90	56.36 ± 1.09	52.90 ± 2.75	100
1.00	64.24 ± 1.58	62.75 ± 2.57	100

± standard error of mean

Table 2: Comparative growth inhibitory activity (GI_{50}) of various aqueous extracts against phytopathogenic fungi.

Pathogen	Source of drugs	Heterogeneity		Regression Equation (y = a + bx)	b ± SE	GI_{50}	Fiducial limits (%)	
		χ^2	df				Min	max
Colletotrichum gloeosporioides f. sp. *mangiferae*	Neem Cake	26.071	5	8.14462 + .81932	.81932 ± .10060	0.46	0.32931	0.65943
	Karanj Cake	22.267	5	9.88218 + 1.02110	1.02110 ± .10333	0.41	0.29014	0.52271
	Vermicompost	3.773	6	1.05401 + .08377	.08377 ± .07947	0.86	0.70133	1.20991
Curvularia lunata	Neem Cake	5.984	6	14.01605 + 1.32222	1.32222 ± .09434	0.27	0.31002	0.23413
	Karanj Cake	16.998	7	7.03629 + .50968	.50968 ± .07244	0.70	0.62496	0.81225
	Vermicompost	4.882	7	1.95836 + .13661	.13661 ± .06976	0.88	0.79484	1.00013
Helminthosporium pennisetti	Neem Cake	.263	2	7.03498 + 1.85183	1.85183 ± .26323	0.11	0.03493	0.15793
	Karanj Cake	5.953	2	5.79508 + 1.31816	1.31816 ± .22746	0.20	-	-
	Vermicompost	.339	3	4.15297 + .64997	.64997 ± .15651	0.22	0.07807	0.30016

Y = Probit Kill, x = log concentration, GI_{50} = Concentration calculated to give 50% spore germination inhibition, a = Intercept, b = Slope, SE = Standard Error

antimicrobial agents against soil-borne pathogens (Szczech *et al*., 1993; Rodriguez *et al*., 2000; Szczech and Smolinska, 2001; Edwards and Arancon, 2004; Zaller, 2006) and do not produce any residual effects. The underlying mechanisms are not clearly understood, but involvement of induced resistance is considered (Fokkema, 1993). These bioagents are non-polluting, cost effective, non-hazardous and can be prepared with available materials in the field. It does not disturb soil ecological balance. Singh *et al*. (2003) reported that the plant alkaloids of *Corydalis longipes* showing potential antifungal property against some phytopathogenic fungi. Plant extracts of many higher plants like neem have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials (Satish *et al*., 1999). The presence or absence of spore pigment did not seem to affect the activity of the drug used. Hyaline spores of *Colletotrichum* species as well as pigmented spores of *Curvularia* and *Helminthosporium* species were sensitive nearly in the same range. Similar results were reported by Maurya *et al*. (2002). In contrast, Singh *et al*. (1990) found that hyaline spores were more sensitive as compared to pigmented spores. To conclude the results, neem cake extracts may be used to control *H. pennisetti* and *C.*

lunata and karanj cake extracts to control *C. gloeosporioides* f. sp. *mangiferae*. On the basis of the results obtained during the experiment and reports of success of botanicals on controlling plant pathogenic fungi, the test organic aqueous extracts hold promise for the organic and ecofriendly, management of foliar diseases caused by the pathogens under field conditions.

REFERENCES

Bhonde, S. B., Deshpande, S. G. and Sharma, R. N. 1999. *In vitro* evaluation on inhibitory nature of some neem formulations against plant pathogenic fungi. *Hindustan Antibiot Bull*. **41:** 22-24.

Brahmachari, G. 2004. Neem-An Omnipotent Plant: A Retrospection. *Chem Bio Chem* (WileyVCH). **5:** 408-421.

Edwards, C. A. and Arancon, N. 2004. Vermicompost suppress plant pests and disease Attacks. In: *REDNOVA NEWS*: http://www.rednova.com/display/?id =55938.

Fokkema, N. J. 1993. Opportunities and problems of control of foliar pathogens with microorganisms. *Pesticide Science*. **37:** 411–416.

Kumar, S., Meera, B. and Kalidhar, S. B. 2003. A review of the chemistry and biological activity of *Pongamia pinnata*. *J. Medicinal and Aromatic Plant Sci.* **25(2):** 411-465.

Maurya, S., Singh, D. P., Srivastava, J. S. and Singh, U. P. 2004. Effect of some plant extracts on pea powdery mildew (*Erysiphe pisi*). *Ann. Pl. Protec. Sci.* **12(2):** 296: 200.

Maurya, S., Srivastava, J. S., Jha, R. N., Pandey V. B. and Singh, U. P. 2002. Efficacy of Alkaloid (-)-Corypalmine against Spore Germination of Some Fungi. *Folia Microbiol*. **47(3):** 287-290.

Mishra, S. K. and Raja, R. 1999. Systemic acquired resistance: *a review*. *Ann. Agric. Res.* **20:** 249–259.

Mondali, N. K., Mojumdar, A., Chatterje, S. K., Banerjee, A., Datta, J. K. and Gupta, S. 2009. Antifungal activities and chemical characterization of Neem leaf extracts on the growth of some selected fungal species in vitro culture medium. *J. Appl. Sci. Environ. Mgmt.* Vol. **13(1):** 49 – 53.

Nakasone, A. K., Bettiol, W. and de Souza, R. M. 1999. The effect of water extracts of organic matter on plant pathogens. *Summa Phytopathologica*. **25:** 330–335.

Pennington, T. D. and Styles, B. T. 1975. A generic monograph of the Meliaceae. *Blumea*. **22:** 419–540.

Reddy, S. A., Bagyaraj, D. J. and Kale R. D. 2012. Management of tomato bacterial spot caused by *Xanthomonas campestris* using vermicompost. *J. Biopest.* **5(1):** 10-13.

Rodríguez, J. A., Zavaleta, E., Sanchez, P. and Gonzalez, H. 2000. The effect of vermicompost on plant nutrition, yield and incidence of root and crown rot of gerbera (*Gerbera jamesonii* H Bolus). *Fitopatologia*. **35:** 66–79.

Sahni, S., Maurya, S., Singh, U. P., Singh, A. K., Singh, V. P. and Pandey, V. B. 2005. Antifungal Activity of Nor-securinine Against Some Phytopathogenic Fungi. *Mycobiology*. **33(2):** 97-103.

Satish. S., Raveesha, K. A. and Janardhana, G. R. 1999. Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars. *Letter in Applied Microbiology*. **28:** 145–147.

Scheuerell, S. and Mahaffee, W. 2002. Compost tea: Principles and prospects for plant disease control. *Compost Science and Utilization*. **10:** 313–338.

Singh, N. V., Azmi, S., Maurya, S., Singh, U. P., Jha, R. N. and Pandey, V. B. 2003. Two Plant alkaloids isolated from *Corydalis longipes* as potential antifungal agents. *Folia Microbiol*. **48:** 605-609.

Singh, S. K., Sarma, B. K., Srivastava, J. S., Singh, U. P. and Ray, A. B. 1999. Antifungal activity of ¢3-Alstovenine, a plant alkaloid isolated from *Alstonia venenata*. *Folia Microbiol*. **44:** 510-512.

Singh, U. P., Maurya, S. Singh, A. and Gohain, L. 2010a. Foliar spray of aqueous extract of neem cake to control balsam powdery mildew. *Archives of phytopathology and plants protection*. **43(11)**:1056-1063.

Singh, U. P., Gohain, L., Singh, A., Maurya, S. and Sahani, S. 2010b. Phenolic acid change in mycelia of *Sclerotium rolfsii* as influenced by neem cake and *Zephyarenthes citrine* bulb. *Archives of phytopathology and plants protection*. **43(2):** 160-167.

Singh, U. P., Maurya, S. and Singh, D. P. 2003. Antifungal activity and induced resistance in pea by aqueous extract of vermicompost and for control of powdery mildew of pea and balsam. *Journal of plant disease and protection*. **110(6):** 544-553.

Singh, U. P., Pandey, V. N., Wagner, K. G. and Singh, K. P. 1990. Antifungal activity of ajoene, a constituent of garlic (*Allium sativum*). *Can. J. Bot.* **68:** 1354-1356.

Srivastava, B. P., Singh, K. P., Singh, U. P. and Pandey, V. B. 1994. Effect of some naturally occuring alkaloids on conidial germination of *Botrytis cinerea*. *Bioved*. **5:** 69-72.

Szczech, M. and Smolinska, U. 2001. Comparison of suppressiveness of vermicomposts produced from animal manures sewage sludge against *Phytophthora* var. *nicotianae*. *Phytopath*ology. **149(2):** 77-82.

Szczech, M., Rondomanski, W., Brzeski, M. W., Smolinska, U. and Kotowski, J. F. 1993. Suppressive effect of a commercial earthworm compost on some root infecting pathogens of cabbage and tomato. *Biological Agriculture and Horticulture*. **10:** 47–52.

Varma, J. and Dubey, N. K. 1999. Prospectives of botanical and microbial products as pesticides of tomorrow. *Curr. Sci*. **76(2):** 172–179.

Weltzien, H. C. 1989. Some effects of composted organic materials on plant health. *Agriculture Ecosystems and Environment*. **27:**439–446.

Zaller, J. G. 2006. Foliar Spraying of Vermicompost Extracts: Effects on Fruit Quality and Indications of Late-Blight Suppression of Field-Grown Tomatoes. *Biological Agriculture and Horticulture*. **24:** 165–180.