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Evaluation of antifungal potency of natural products against stem and root rot of sesame

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Abstract
Stem and root rot is an important disease of sesame. To manage it in natural way, different oils of plant origin and several locally available oil cakes as well as manures were tested at different concentrations *in vitro* using poison food technique. Among different oils, Clove oil registered 100% mycelial inhibition at all concentrations followed by neem oil recording 72.57%, 81.86% and 80.66% and mustard oil registering 71.82%, 77.76% and 88.60% at 2%, 3% and 5% concentrations respectively. Among all organic products tested, extract of neem cake registered maximum growth inhition of 60%, 76.57% and 93.73% followed by Mustard cake (58.20%, 68.89%, 93.73%) and Mahua cake (51.53%,68.49%, 87.13%) at 3%, 4% and 5% concentrations respectively. The effectiveness of oils and organic products were dose dependent.

Keywords: *Macrophomina phaseolina*, methanol, oils, organic products, sesame, stem and root rot

Introduction
Sesame (*Sesamum indicum* L.) is an important edible oilseed crop grown in hotter and drier areas of tropical and subtropical regions. It is valued as quality food, nutrition, edible oil, bio-medicine and health care, all in one. Around 60-65 countries of the world produces sesame seed. China, India, Sudan, Myanmar, Uganda, Nigeria, Bangladesh, Pakistan, Tanzania, Mexico, Thailand and Egypt are major producer of sesame. India is the world leader with the maximum production (25.8%) from the largest area (29.8%) and highest export (40%) in the world. India produces 870 thousand metric tons in fiscal year 2015-2016. Sesame is largely cultivated in the western, southern and eastern parts of India with productivity of 413 kg/ha (Annual report,2016-17, Department of Agriculture, Cooperation & Farmers Welfare Ministry of Agriculture & Farmers Welfare, Government of India) [2]. Now days, area and production of sesame is declining in the traditional areas due to severe biotic stresses such as Bacterial blight, Macrophomina stem and root rot, Phyllody, Fusarium wilt, Powdery mildew, Alternaria leaf spot and Cercospora leaf spot. Among different biotic constraints, stem and root rot disease is a serious problem causing huge loss. Indiscriminate use of fungicides for managing diseases leads to resistance as well as leaves residues in environment and food products. As modern society is becoming more health-conscious, there is a constant need to develop newer and effective alternatives to chemicals. In that line, Oils of plant origin, oil cakes and animal products may act as inputs in organic food production, both in the field and in controlled environments. Present study was carried out to critically analyse the antifungal potency of different oils, oil cakes and manures against stem and root rot of sesame.

Materials and Methods
Pathogen culture
The pathogen inciting stem and root rot disease was isolated from infected sesame plant and identified as *Macrophomina phaseolina* which is further confirmed by ITCC, IARI, New Delhi with ID No. 9811.15. Pathogen was grown in potato dextrose agar media and 5days old fresh culture was used for the studies.

Materials used
For testing efficacy of different oils, seven different oils of plant origin such as karanj, neem, eucalyptus, clove, sesame, olive and mustard oil were collected and tested *in vitro*. Different commonly available oilcake such as mustard cake, neem cake, sesame cake, karanj cake and mahua cake were collected from oil mills as well as from market. Poultry manure was collected from poultry farm and goat manure was collected from nearby Goat farm. The experiment was undertaken in Department of Plant Pathology, College of Agriculture, OUAT, Bhubaneswar.

Bioassay of different oils on mycelial growth of the pathogen *in vitro*

In order to study effect of different oils of plant origin, seven different oils such as karanj, neem, eucalyptus, clove, sesame, olive and mustard were selected and tested at 2%, 3% and 5% concentrations *in vitro* using poison food technique (Nene and Thapliyal, 1973) [14]. The oils were individually added in melted, cooled and sterilized PDA at desired concentrations at the time of pouring in the petriplates. Plates were incubated at room temperature after placing 5 mm disc of actively growing 5 days old pure culture of *M. phaseolina.* One set of plates were maintained without any oils which serves as control. Each treatment was replicated thrice following completely randomized design. Observations were taken after 7 days and per cent mycelia growth was calculated using Vincent formulae (Vincent, 1947) [16].

$$I = \frac{(C-T)}{C} x100$$

I = Per cent inhibition of mycelium
C = Growth of mycelium in control
T = Growth of mycelium in treatment

Bioassay of different organic products on mycelia growth of *Macrophomina phaseolina in vitro*

The efficacy of seven locally available organic products such as mustard cake, neem cake, sesame cake, karanj cake, mahua cake, poultry manure and goat manure were studied at three concentrations i.e. 3%, 4% and 5% *in vitro* using poison food technique for this experiment methanolic extract was used. Hundred grams of organic product was crushed and dissolved in 100 ml of methanol and kept overnight. In the next day morning it is filtered through filter paper. The filtrate was used as stock. From this stock, 3%, 4% and 5% concentrations were prepared and plated. The plates were then inoculated with 5 mm mycelia disc. Each treatment was replicated thrice following completely randomized block design. Plates with no organic products were treated as control. All the inoculated plates were kept at room temperature till the control plate was fully covered the plate (5 days). The radial diameters were measured and per cent inhibition of test fungus over control was calculated using Vincent formulae.

Result and discussion

Bioassay of different oils against mycelial inhibition of *M. phaseolina in vitro*

All the oils tested against *M. phaseolina in vitro* at different concentrations recorded good mycelial inhibition over control. At 2% concentration, clove oil recorded maximum mycelial inhibition of 100% followed by neem oil (72.57%) and mustard oil (71.82%) which were significantly different from each other. Least effective was olive oil which recorded 10.07% growth inhibitionation. At 3% concentration again clove oil was found highly effective witnessing 100% mycelial inhibition followed by neem oil (81.86%) and mustard oil (77.76%). Olive oil was again least effective at 3% concentration. Clove oil registered 100% inhibition followed by neem oil (88.60%) at 4% concentration. Least effectiveness was recorded in olive oil (57.46%). Clove oil proved efficacious among all the oil tested followed by neem oil.

Bioassay of different organic products against *Macrophomina phaseolina*

The methanolic extracts of different oil cakes were evaluated for their inhibitory effect against *M. phaseolina*. All the organic extracts gave significant inhibitory effect on the fungal growth. Among all the organic extracts, maximum growth inhition was recorded from the extract of neem cake (60%) followed by Mustard cake (58.20%) and Mahua cake (51.53%) at 3% concentration which were significantly different from each other. Poultry manure recorded least effective at 3% concentration. At 4% concentration, neem cake recorded maximum mycelial inhibition of 76.57% followed by mustard cake (68.89%) and mahua cake (68.49%). Both neem cake and mustard cake recorded maximum mycelia inhibition of 93.73% followed by mahua cake (87.13%) at 5% concentration. Neem cake was proved efficacious among all.

Clove oil was found more efficacious followed by neem oil and mustard oil *in vitro* which also reported by earlier workers. Murthy and Amonkar (1973) [13] reported oil of garlic (*Allium sativum* L.) in natural and synthetic forms suppress the activity of many air and soil-borne fungi. Kazmi *et al.* (1995) [8] and Alice *et al*. (1996) [1] reported neem oil to be more effective than benomyl and carbendazimn in *in vitro* studies**.** Effectiveness of neem extracts and oil as a fungicide has earlier been reported by several workers (Ilyas *et al*., 1997 [6]; Sharma and Basandrai, 1997 [15] and Lokhande *et al*., 1998 [11]). Dubey and Kumar (2003) [4] observed that all the sclerotia lost their viability after 96h of treatment with neem oil, mancozeb and bavistin. Kumari *et al* (2012) [7] found palmarosa oil to be the most effective as seed dresser in reducing root rot incidence. These reports of earlier workers corroborate the present finding.

Methanolic extracts of neem cake recorded maximum growth inhibition followed by mustard cake and mahua cake at all concentrations *in vitro*. Similar findings were also reported by earlier workers. Jha *et al* (2000) [7] who observed mustard cake to inhibit mycelial growth of *M. phaseolina* causing root rot in okra. Gemawat and Verma (1971) [5] tested the effect of formalin, sesamum cake, groundnut cake, mustard cake and farmyard manure against incidence of *Macrophomina phaseolina* of sesame under artificial conditions in pots and found addition of mustard cake to infested soil was most effective giving 16.6% infection as against 83.3% in control. Latha and Rajappan (2001) [10] stated that root rot incidence was significantly reduced by FYM in blackgram. Kumari *et al* (2012) [9] reported vermicompost was most effective and FYM and goat manure proved moderately effective in reducing the root rot incidence under pot conditions. Dhingani *et al* (2013) [3] found extracts of neem cake (59.40%) registered maximum mycelium inhibition against *M. phaseolina in vitro* followed by farm yard manure (42.56%). Next best in order of merit were castor cake and mustard cake. Meena *et al* (2014) [12] reported mustard cake is more effective recording mycelial growth inhibition of 19.42%, 16.64% and 12.20% at 20%,15% and 10% concentration respectively. He also found maximum mycelial growth inhibition of 52.40%, 42.61% and 29.60% with neem cake at concentration of 20%, 15% and 10%, respectively. These reports of earlier workers are in line of confirmation to the present finding.

The results indicated that oils, oil cakes and manure has good mycelial inhibition *in vitro* and their efficacy is dose dependent. Hence, there is necessity of further investigation to isolate and characterize active principle responsible for the

activity and its subsequent exploitation for the purpose of disease management in field conditions. Natural products can be used as alternative to hazardous fungicides as it is cost effective and eco-friendly. They can be exploited for the possible management of deadly pathogen *M. phaseolina*. Accordingly, this is an important proactive measure in preventing the spread of the stem rot disease through a more ecofriendly manner.

Table 1: Bioassay of different oils against mycelia inhibition of *M. phaseolina in vitro*

S. no	Treatments	Mean mycelia inhibition		
		2% conc	3% conc	5% conc
1	Karanj oil	70.50 (57.08)	76.47 (61.50)	85.87 (67.95)
2	Neem oil	72.57 (58.39)	81.86 (64.87)	88.60 (70.28)
3	Eucalyptus oil	34.31 (34.9)	56.05 (48.45)	78.49 (62.34)
4	Clove oil	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
5	Sesame oil	25.98 (30.58)	51.73 (45.97)	73.25 (58.84)
6	Olive oil	10.07 (18.33)	40.77 (39.66)	57.46 (49.27)
7	Mustard oil	71.82 (57.91)	77.94 (62.51)	87.47 (69.25)
8	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SE(m) ±		3.189	2.604	0.733
CD(0.01)		9.642	7.873	2.217

*****Data in parenthesis represents transformed values

Table 2: Bioassay of methanolic extracts of different organic products against *M. phaseolina in vitro*

S. no	Treatments	Mean mycelia inhibition*		
		3% conc	4% conc	5% conc
1	Mustard cake	58.20(49.70)	68.89(56.07)	93.73(75.50)**
2	Neem cake	60.00(50.74)	76.57(61.02)	93.73(75.50)
3	Karanj cake	46.43(42.93)	63.65(59.90)	82.23(65.04)
4	Mahua cake	51.53 (45.86)	68.49(55.83)	87.13(68.95)
5	Sesame cake	46.43(42.93)	65.70(54.12)	83.71(66.16)
6	Poultry manure	43.49(41.24)	52.83(46.60)	72.78(58.53)
7	Goat manure	50.00(44.98)	60.00(50.74)	76.57(61.02)
8	Control	0.00(0.00)	0.00(0.00)	0.00(0.00)
SE(m)±		0.211	0.273	0.534
CD(0.01)		0.647	0.824	1.615

*****Mean of 3 replications ** Data in the paraenthesis represents the transformed value

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