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Research Article

Antimycotic Efficacy of Organic Amendments against *Pythium aphanidermatum* (Edson) Fitzpatrick

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Abstract

The present study was conducted to exploit the antimycotic potential of 8 organic amendments (plant oil cakes: Mustard, Neem, Groundnut, Mahua, Cotton, Sesame, Castor, Pungam) against *Pythium aphanidermatum*. *P. aphanidermatum* was isolated from infected ginger procured from Jhadol (Udaipur dist.). Organic amendments were tested by Poisoned food Technique at 100% concentrations. 100% cow dung (binder) was also assayed to know the antimycotic efficacy. Standard fungicides Thiram, Mancozeb and Bavistin were also tested against the same. A control containing only PDA was also maintained with all the experimental studies.

Among the used organic amendments the best result was found of Neem oil cake i.e. 87.76% inhibition of test fungi followed by Mahua, Pungam, Mustard, Castor, Cotton and Groundnut respectively. The least inhibition among the taken oil cakes was showed by Sesame oil cake i.e. 38.87%. Cow dung (binder) showed the 46.08% inhibition of test fungi.

Standard fungicides Mancozeb, Thiram and Bavistin were also evaluated. Among the three taken fungicides Mancozeb and Thiram showed 100% inhibition whereas Bavistin showed 90.14% inhibition against the test fungus *Pythium aphanidermatum.*

Keywords: *Soft Rot; Pythium aphanidermatum; Organic Amendments; Ginger; Poisoned Food Technique*

General Introduction

Ginger belongs to family Zingiberaceae is one of the important cash crops of India. It is a commercial crop grown for its aromatic rhizomes which are used as an important spice as well as medicine [1]. Ginger also has a great potential health benefits including its chemopreventive, blood pressure-lowering, cholesterol-lowering and antiplatelet aggregation properties. Ginger has important medicinal properties such as antimicrobial, antioxidant, anti-inflammatory, antioxidative, hypoglycemic, hepatoprotective, diuretic, and anticancerous properties [2].

Ginger is also an important cash crop that earns a sizeable amount of foreign exchange for the country [3]. India is the largest producer and exporter of ginger. Demand of ginger is increasing continuously at global level. As the demand is increasing day by day the production as well as the quality of ginger should have to be increased, but ginger rot/ rhizome rot which is one of the most destructive diseases of ginger worldwide [4] causes yield losses up to 50 - 90%. Rhizome rot of ginger caused by *Pythium* species among which *Pythium aphanidermatum* is a major constraints for the production of healthy rhizome and associated with soft rot disease of ginger sometimes it causes total failure of crop [5].

The disease is both soil as well as seed-borne, *Pythium* infection can occur at all stages in ginger plant during growth even during storage, transportation and marketing. Roots, developing rhizome, buds, and collar regions are mainly affected [6]. The fungus multiplies with

the availability of water and soil moisture. Younger sprouts are most susceptible to the pathogen. The infection starts at the collar region of the pseudostem appears as light yellow in colour which gradually spreads down to the leaf blade and leaf sheath along the margin, in the early stages, the middle portion of the lamina remains green while the margins become yellow, the yellowing spreads to all the leaves of plant from bottom upwards and is followed by drooping, withering and drying, the collar region of the pseudo- stem shows pale translucent brown colour which becomes water-soaked, due to destruction of parenchymatous tissues the infection from the collar spreads to the rhizome gradually [4].

The disease spreads either through diseased rhizomes or through the spores present in the soil. The infected plant debris remaining in the field is an important source of infection. Spread of the disease from one field to another through rain water has also been reported [7]. Cellulolytic and pectinolytic enzymes produced by *Pythium* are responsible for the tissue disintegration as well as maceration which lead to successful infection. It also infects a large range of different hosts, causes damping off of many economically important crops resulting in multibillion dollar losses worldwide [8].

The species of *Pythium* pose an unspecific wide range of hosts [9]. This makes *Pythium* species more harmful for harvest, as the crop rotation itself is not enough to eradicate the disease. Lying fallow is not either an efficient control practice, because *Pythium* is saprophytic and survives for a long time within vegetal organic matter in decomposition.

Synthetic fungicides

Synthetic fungicides are used to control the disease but most of the synthetic fungicides not only impose adverse effects on ecosystems, but have also created a possible carcinogenic risk and toxicological problems and cause deleterious effects on human health as well [10]. Moreover, resistance by pathogens to fungicides has rendered certain fungicides ineffective [11]. Folman., *et al*. 2004 [12] reported that chemical fight against this aggressive pathogen is not significant. Thus, there is a need to search for an environmentally safe and economically viable strategy for the control of diseases and to reduce the dependence on the synthetic agrochemicals.

To overcome the circumstances, the present study was conducted to exploit the antifungal potential of eight different organic amendments against *Pythium aphanidermatum* under *in vitro* conditions. Cow dung (a binder) is also studied along with standard fungicides Mancozeb, Thiram and Bavistin which are mostly used against *Pythium aphanidermatum.*

The soil fertility in the natural farming can be maintained by the application of organic amendments. Various biological content as well as physical and chemical properties of the soil can be enhanced during the decomposition of organic amendments and hence improving the soil biotic conditions, texture and structure [13]. Organic amendments are very effective and are available easily at very affordable cost. They are the excellent source of nitrogen and phosphorus responsible for increase in yield and quality maximization in sustainable crop production. Organic amendment in agriculture are beneficial as they reduce crop losses they are cheaper, eco-friendly and easily bio-degradable.

Oil seed cakes refer to the by-products after the oil extraction from the oilseeds. The global demand of oilseeds is increasing every year. The oil cakes very often inherit high amount of biochemical constituents, including fungitoxic biomolecules. Oil cakes not only reduce the disease severity but also enhance the antagonists, soil fertility and crop yield to a significant level. It also contains terpenoids and other volatile compounds which can released through the process of hydrolysis [14]. The oilseed cakes also having good quantity of secondary metabolites, such as phenolic acids and flavonoids [15]. Due to presence of various secondary metabolites these oil cakes can improve the soil structure, water holding capacity and cation exchange capacity and hence promotes the plant growth.

Sources of oil cakes

Oilseed cakes refer to the by-products after the oil extraction from the oilseed. The oilseed cakes are generally used as fodder due to their high protein and energy contents.

Oil cakes	Nutrient Contents %		
Non Edible Oil cakes	**N**	**P2O5**	**K2O**
Castor cake	4.3	1.8	1.3
Cotton seed cake	3.9	1.8	1.6
Karanj cake	3.9	0.9	1.2
Musturd	4.0	1.0	1.0
Mahua cake	2.5	0.8	1.2
Pungam	0.19	1.3	1.6-2
Neem cake	2.4	0.7	1.0
Safflower cake	4.9	1.4	1.2
Edible oil-cakes			
Coconut cake	3.0	1.9	1.8
Cotton seed cake (decorticated)	6.4	2.9	2. 2
Groundnut cake	7.3	1.5	1.3
Linseed cake	4.9	1.4	1.3
Niger cake	4.7	1.8	1.3
Sesamum cake	6.2	2.0	1.2
Safflower cake (decorticated)	7.9	2.2	1.9
Rape seed cake	5.2	1.8	1.2

Table 1: *Various types of Oil cakes and their Nutrient Contents.*

Types of oil cakes:

1. Edible oil cakes are safe for livestocks to fed; like: Groundnut cake, corn cake, Sesame cake, Coconut cake etc.

2. Non edible oil cakes are generally not fit for feeding livestock; e.g.: Castor cake, Neem cake, mustard cake, Pungam cake, Mahua cake etc.

Both edible and non-edible oil cakes can be used as manures, they are good for soil constitution. Edible oil cakes are generally fed to cattle and non-edible oil cakes are used as manures. These cakes showed the antifungal and antibacterial activity in diseased horticultural crops. Nutrient value of oil cakes are also good. It need to be well powdered before use and application in the farms for even distribution and easy decomposition.

In this study 100% concentrations of organic amendments were applied using Poisoned food technique against *Pythium aphanidermatum* for antimycotic activity.

Out of the eight organic amendments tested, neem oil cake extract exhibited the highest mycelial reduction of 87.76% followed by Mahua oil cake i.e. 83.55% then Pungam oil cake 74.23%, this was succeeded by other oil cakes taken for antimycotic activity. The least inhibition among the taken oil cakes was showed by Sesame oil cake i.e. 38.87%. Cow dung showed the 46.08% inhibition against test fungi.

Materials and Methods

Collection and isolation of test pathogen from infected ginger

Diseased seedlings expressing the typical soft rot symptoms produced by *Pythium aphanidermatum* were gathered and brought to the aseptic laboratory conditions. The gathered samples were rinsed in a tap water to get rid off the debris and sand.

For isolation of *Pythium* spp. 1.5 cm blocks of infected rhizomes were washed in running tap water, surface-sterilised in 0.5% sodium hypochlorite for 3 minutes, rinsed in sterile distilled water, blotted dry on sterile filter paper, and placed onto different selective media by following the method as suggested by Eckert and Tsao 1962 [16]; Jeffers and Martin 2010 [17]. The plates were then incubated in the dark at 25 ± 1°C for 2 days and examined for the presence of *Pythium* colonies. The characteristic feature of *Pythium* species is development of white cottony growth which grows faster than other fungal species. Actively growing hyphal tips from periphery of the plate extending from ginger block were transferred to fresh PDA medium. They were also transferred to different media (CMA, PCA, PDA, WA, V8JA) to study the colony morphology and incubated at 28°C.

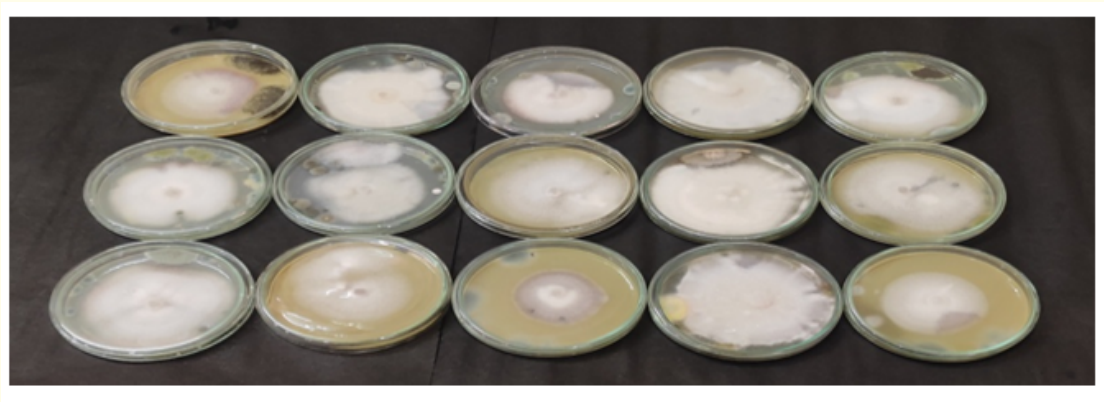


Figure 1: *Isolated fungal complex from infected ginger.*

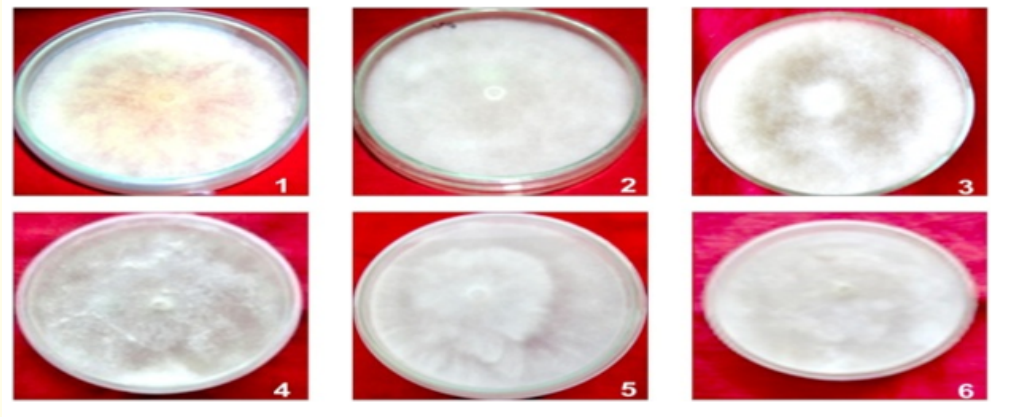


Figure 2: *Pythium aphanidermatum (Colony on different media 1.PDA, 2.V8JA, 3.WA, 4.PCA, 5.CMA, 6.OMA).*

Isolation of test pathogens from infected soil by baiting and serial dilutions

Isolation of test pathogens were done from infected soil by spreading 100 gm of soil wetted by 10 ml of water onto a paper towel placed in sterilized plastic trays (37 cm x 27 cm). About 10 - 15 pea seeds were placed on top of the soil. The set up was placed in dark for 4 - 5 days at 25 ± 1°C. After 4 - 5 days, pea seeds (loaded with test pathogens) showing presence of white fluffy mycelium characteristic of *Pythium* were placed on selective medium PPP agar (0.10g of pimaricin, 0.05g of penicillin and 0.05g of polymyxin per liter in corn meal agar) and PARP agar (0.005g of pimaricin, 0.25 mg of ampicillin, 0.01g of rifampicin, and 0.10g of PCNB pentachloronitrobenzene per liter in corn meal agar) according to Eckert and Tsao 1962; Jeffers and Martin, 2010 respectively for isolation of *Pythium* species. Plates were incubated in the dark at 25 ± 1°C for 2 days and examined for the presence of *Pythium.* The actively growing hyphal tips from baits were transferred to new PDA media and were selected on the basis of colony characteristics in PDA. Then they were studied microscopically to confirm the presence of test pathogens.

The images are shown below.

Figure 3: *Infected Soil samples collected from different sites at Jhadol (Udaipur).*

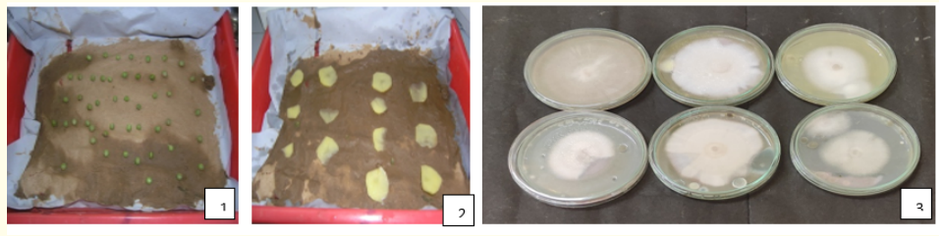


Figure 4: *Isolation of Pythium from infected soil using 1.Pea seeds, 2.Potato slice, 3. Isolated fungal complex.*

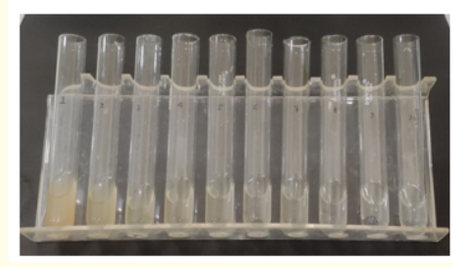


Figure 5: *Serial dilution of Soil.*

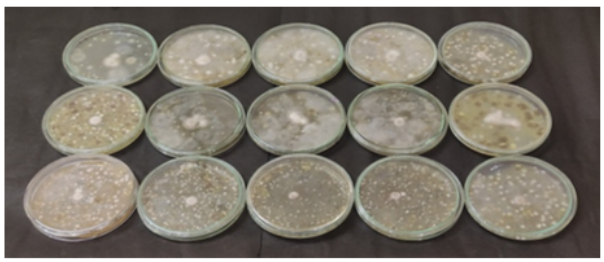


Figure 6: *Isolated fungal complex from soil sample.*

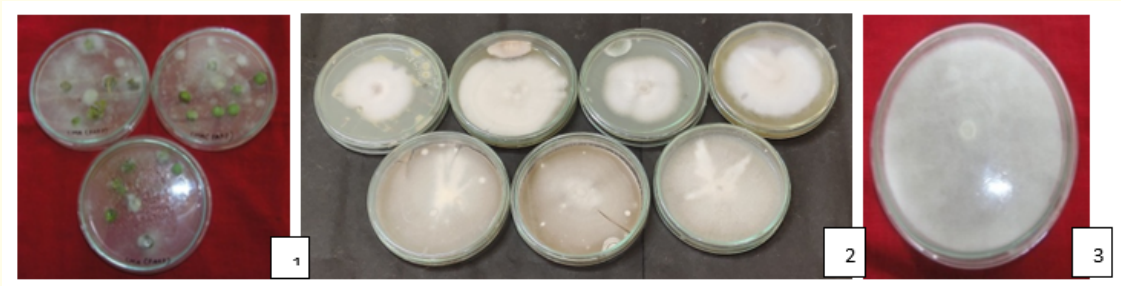


Figure 7: *Isolation of Pythium from infected water using 1.Pea seeds, 2.Isolated fungal complex, 3. Purified culture of P. aphanidermatum on CMA.*

Identification of test pathogen

According to Middleton 1943 [18]; Waterhouse, 1968 [19]; Van der Plaats-Niterink, 1981 [20] and Dick, 1990 [21], identification of *Pythium* species is generally based on morphology, hence in the present study both of the test fungi *P. aphanidermatum* and *P. myriotylum* were identified on the basis of morphological structures using standard keys [20]. Test pathogens *P. aphanidermatum* and *P. myriotylum* were also grown on different medium to observe cultural characteristics in various medium (PDA, V8JA, WA, PCA, CMA, OMA) respectively and incubated at room temperature 28 - 30°C [20-22]. Test pathogens were microscopically identified up to the species level on the basis of sexual and asexual structures according to standard keys given by Waterhouse [19] and Plaats-Niterink [20] by using Olympus microscope (Olympus, Japan, Model No. BX51). The formation of reproductive organs as well as mycelial growth of test fungi is good on oat meal agar (OMA), corn meal agar (CMA) and potato dextrose agar (PDA) but specially abundant on CMA. Hence, myceliums from corn meal agar were taken to observe sexual and asexual organs. Sporangia were induced according to the method described by Dick [21], Abdelzaher., *et al* [23]. For sporangial production, autoclaved grass blades, 1 - 2 cm in length, were placed in contact with V-8 juice agar where the test pathogens were pre- cultured at 25°C incubated at room temperature for 1 - 2 days. Then grass blades loaded with test pathogens were transferred into petriplates containing sterile distilled water. Abundant sporangia produced in the plates were then observed under the microscope. Sexual structures along with sporangia were also induced by following the method as described by Khalaf et al. [24] According to this method rectangular pieces of agar culture (20 mm square) from 3 days old cultures of test pathogens growing on potato carrot agar (PCA) were sub cultured into sterile Petri plates and flooded with 20 ml sterile distilled water.

The dishes were incubated at 4°C for 1 - 3 hours then at room temperature (25 ± 1°C), water was changed for first three hours and then sporangia as well as sexual structures were observed microscopically.

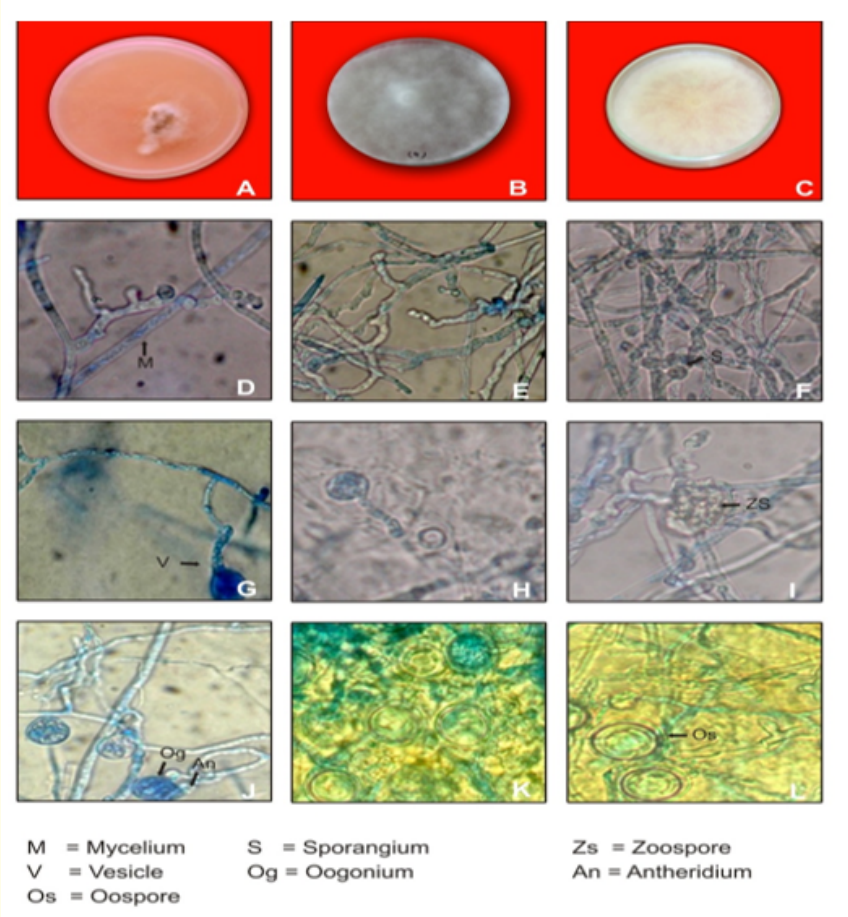


Figure 8: *P. aphanidermatum (A-C: Colony in Plates, D-F: Mycelium and Sporangia, G-I: Vesicles and Bursting of Zoospores, J-L: Oogonium, Antheridium, Oospore).*

Pathogenicity assay

Koch's postulates were used to identify the aggressive, virulent isolate of the pathogen. Pathogenicity testing was done by using randomized complete-block design. Inoculum was prepared from test pathogen which was growing on potato dextrose agar (PDA). Seven days old mycelial mat of test pathogen was harvested by washing with 10 ml of sterile distilled water and then suspending the mycelial mat with 100 ml of tap water in a conical flask. Conical flask was placed on magnetic stirrer for about 1 hour to make the mycelial suspension homogeneous. Then 50 ml of mycelial suspension was used as inoculum to infect one month old ginger plantlets growing in 12 x 15 cm size polythene bags containing standard pot mixture. When the symptoms became apparent, the same were recovered from the infected plants and identified as the test pathogen.

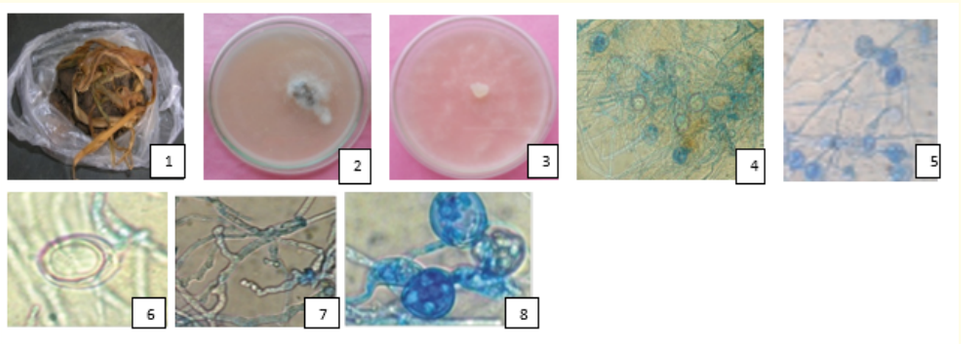


Figure 9: *From left to right: Pathogenicity testing (Pythium aphanidermatum) 1. Plantlet method, 2. Wound Method, 3. Isolated Pythium, 4-5. Microscopic image, 6-7-8. Enlarged view of Oospore, Mycelium, Antheridium and Oogonium).*

Antimycotic efficacy of organic amendments against Pythium aphanidermatum under in-vitro preparation of organic amendments

There are eight types of elicitors i.e. ground nut oil cake, mustard oil cake, cotton oil cake, sesame oil cake, mahua oil cake, coconut oil cake, castor oil cake, neem oil cake, etc. 10 gm of each elicitors were dissolved in 100 ml of autoclaved water for 24h. The mixture was then filtered and the filtrate was the 100% concentration, it was diluted and further used for antifungal activity.

The antifungal activity of each elicitor was tested using poison food technique. 1 ml of each elicitor with 9 ml molten sterile PDA culture medium was poured into pre-sterilized petri- plates (9 cm diameters) and allowed to solidify at room temperature. Thus, prepared petri-plates were inoculated aseptically with 6mm disc of test pathogen's cultures which was placed at the centre of the plate. The Petri-plates were then incubated at 28 ± 2°C for five days. The control plate was maintained without adding the extract of organic amendments containing only PDA was used as control. Antifungal activity of each elicitor was measured as a function of increase in growth diameter of 6 mm disc of inoculums. Three replications were maintained for every treatment in a completely randomized design. The radial growth of the mycelium and Percentage inhibition was reckoned when the control plate attained its utmost full growth or Seven days which one is earlier.

Growth inhibition was calculated by the following formula given below:

% Mycelial growth inhibition = gc-gt/gc× 100

gc = Growth of fungal colony after 7 days incubation period in control set subtracting the diameter of inoculums disc.

gt = Growth of fungal colony after 7 days incubation period.

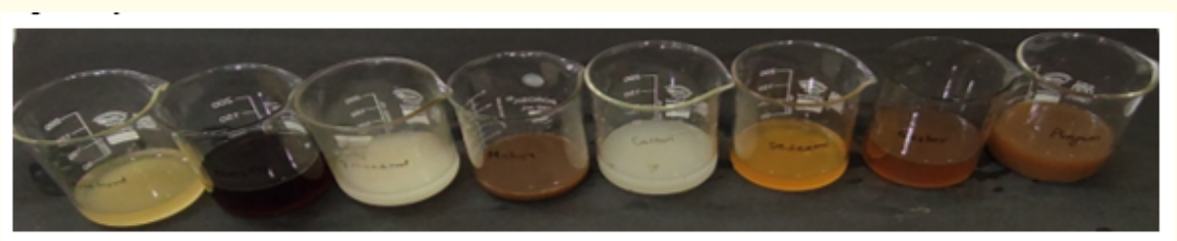


Figure 10: *From left to right: Oil cakes: 1. Mustard, 2. Neem, 3. Groundnut, 4. Mahua, 5. Cotton, 6. Sesame, 7. Castor, 8. Pungum.*

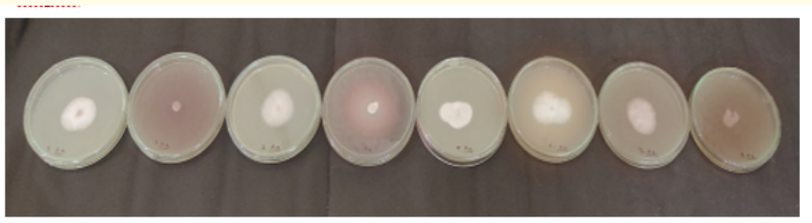


Figure 11: *Antifungal activity of different oil cakes: From Left to Right: 1. Mustard, 2. Neem, 3. Groundnut, 4. Mahua, 5. Cotton, 6. Sesame, 7. Castor, 8. Pungum against Pythium aphanidermatum.*

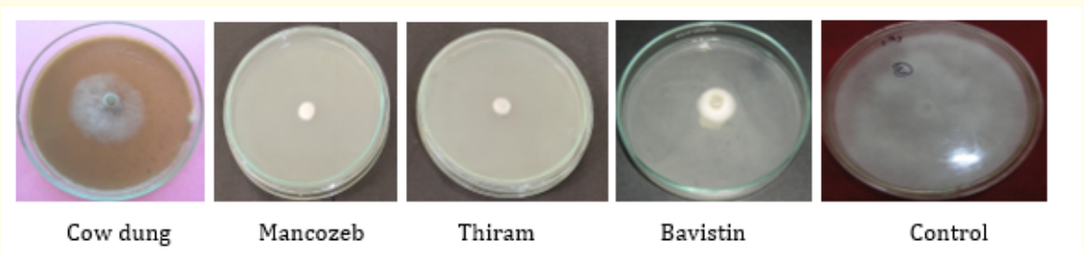


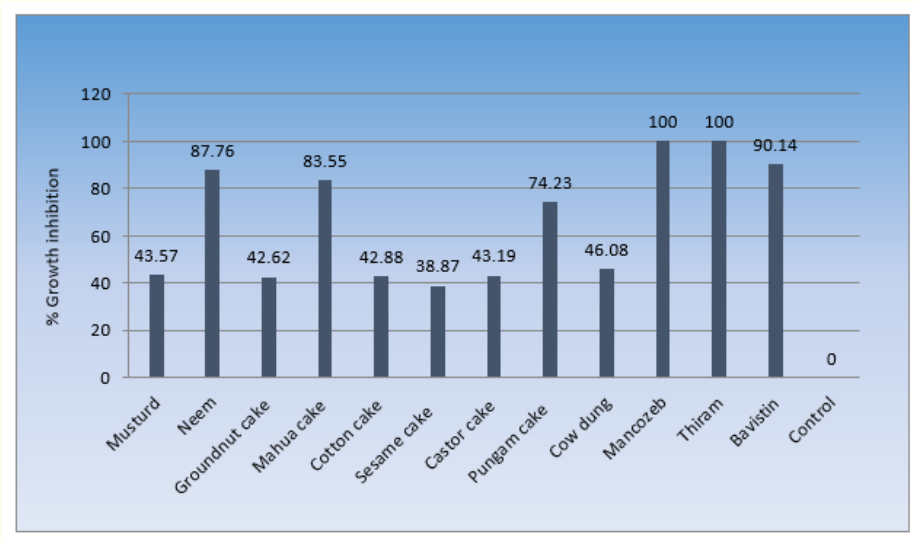
Figure 12

Results and Discussion

From the above experiments we have find that Different oil cakes and cow dung assayed for antifungal activity against *Pythium aphanidermatum* gave inhibitory activity against the same. Among the used oil cakes Neem oil cake (87.76 ± 0.45) gave the significant antifungal activity which is followed by Mahua oil cake (83.55 ± 0.62). Below shown table and graph are representing the results obtained from the above study.

S. No.	Organic Amendment	% Growth Inhibition			Average Mean±SD
		R1	R2	R3	
1.	Musturd	42.80	43.72	44.20	43.57±0.71
2.	Neem	87.34	88.24	87.72	87.76±0.45
3.	Groundnut cake	43.23	42.44	42.21	42.62±0.53
4.	Mahua cake	84.20	83.50	82.96	83.55±0.62
5.	Cotton cake	42.50	43.20	42.95	42.88±0.35
6.	Sesame cake	39.21	38.79	38.62	38.87±0.30
7.	Castor cake	43.44	42.92	43.21	43.19±0.26
8.	Pungam cake	74.55	73.91	74.23	74.23±0.32
9.	Cow dung	46.31	45.76	46.19	46.08±0.28
10.	Mancozeb	NG	NG	NG	100
11.	Thiram	NG	NG	NG	100
12.	Bavistin	89.21	90.10	91.11	90.14±0.95
13.	Control	-	-	-	Full growth

Table 2: *Antimycotic Activity of Organic Amendments (Elicitors), Cow-dung (Binder) and Standard Antifungals against Pythium aphanidermatum.*



Graph 1: *Antimycotic Activity of Organic Amendments (Elicitors), Cow-dung (Binder) and Standard Antifungals against Pythium aphanidermatum.*

Many researchers showed inhibitory activity of various oil cakes against *Pythium aphanidermatum.* Recently, Subharathinam., *et al*. [25] reported that mahua oil cake, pungam oilcake and coconut oilcake @ 50 per cent concentration recorded the good inhibition of mycelial growth of *P. aphanidermatum.*

In the present work 8 oil cakes and Cow dung as well as Thiram, Mancozeb and Bavistin were also assayed for their antifungal activity.

Conclusion

On raising the awareness towards the harmful and deleterious effects of chemical pesticides, people are looking for organically grown agriculture products. Chemical pesticides not only degrading the soil but also causing various health issues including cancer in human. Hence to protect the environment, crops as well as human health the use of organic amendments may be a good initiative. It can be used as a major tool for sustaining the quality of degraded soils due to the intensive use of synthetic chemicals for increasing crop production. The use of oil cakes enhance the good microorganisms as well as the properties of the soil to combat with the soil borne pathogens and hence crops can be protected. On the basis of results obtained it can be concluded that use of oilcakes offers good alternative to traditional applications in the production of environment friendly bioformulation. Cow dung as a binder is also good and eco-friendly option as their availability is very cheaper throughout the year.

We can conclude that on mixing various oilcakes and cow dung we can enhance the soil fertility, yield and quality of crops.

Conflicts of Interest

There are no conflicts of interest. As this is my original research work.

Research Involving Human Participants and/or Animals

In this study no use of any kind of animals for the experimental purpose.

Authors Contribution Statement

Dr. Tahira Parveen perceived the idea, carried out the research study, observed and evaluated the results and drafted the manuscript. Dr. Tripta Jain and Prof. Kanika Sharma guided to Dr. Tahira Parveen in conducting this research study and also reviewed and approved the manuscript.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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