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A study on mycoflora associated with selected seeds and the effect of three medicinal plant extract on seed viability and seedling growth in various concentration

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Abstract
The present investigation determines seed mycoflora associated with *Arachis hypogea L. and Brassica nigra L.* and their biocontrol by aqueous medicinal plant extract. The experiment was done in the seeds collected from Shakthan market. The main aim of this study is to isolate fungi associated with *Arachis hypogaea L. and Brassica nigra L.* by agar plate method and blotter plate method. The fungi were isolated from sterilized seeds and control seeds and identified based on their morphological structure and characters. Three fungal species isolated from the seeds were *Aspergillus niger, A. flavus and A. fumigatus*. Percentage of fungal incidents on seeds and their impact on germination rate and growth is also studied using agar and blotter plate method. The seed-borne fungi are capable of reducing the germination rate and further development of the plant. In the view of this, the present study has been undertaken to screen some medicinal plant extracts against these seed-borne fungi. The medicinal plants screened for their antifungal activity were *Azadirachta indica, Allium sativum and Piper betle*. All seeds were treated with aqueous extract of fresh samples. Of these *Allium sativum and Acadiachata indica* exhibited the highest antifungal activity in both plants tested. This study suggest that medicinal plant extracts can be used as an antifungal agent.

Keywords: Seed mycoflora, Medicinal plant, fungi, seeds, crops

Introduction

India is an agriculturally based country where more than 50% of population is depend on agriculture. India is the world's largest producer of pulses, rice, wheat, spice product. These structures the main sources of income emergency purposes and widely consumed due to its ability to compensate for the nutrient deficiencies of rice such as lack of vitamins and minerals. Seed is a miniature plant and is considered as a basic input for production. About 90% of the world's food crops are sown using seed. Seed plays vital role in the total biological yield per unit time and unit plant surface (*Patekar M. A. et.al., 2017*). The importance of quality seeds has been recognized from the time immemorial. Seed quality has been treated as sacred, being an important factor in the improvement of agriculture andagrarian societies. Oil seed crops are grown for the purpose of nutrition of oil, which is contain in their seeds. Seed oils are used for industrial as well as edible purposes. Major world oil seed crops are soyabean, sunflower, brassica, rapseed, peanuts, rice and cotton. The divorce agroeconomic conditions in the country are favorable for growing 9 annual oil seed crops, which include 7 edible oil seeds. Oil seed crops are mainly used for the production of oil, a constituent of our diet, being an important source of fatty acids. A lower production of oil seeds is a major problem that could be
associated with in that of variousbiotic and abiotic stresses. The peanut, is also known as the groundnut, and taxonomically classified as *Arachis hypogaea*, is a legume crop grown mainly for its edible seeds. It is widely grown in the tropics and subtropics, being important to both small and large commercial producers. It is classified as both a grain legume and, due to its high oil content, an oil crop World annual production of shelled peanuts was 44 million tones in 2016, led by China with 38% of the world total. Atypically among legume crop plants, peanut pods develop underground (geocarpy) rather than above ground. With this characteristic in mind, the botanist Carl Linnaeus gave peanuts the specific epithet hypogaea, which means "under the earth". The mustard plant is any one of several plant species in the genera *Brassica* and Sinapis in the family Brassicaceae (the mustard family). Mustard seed is used as a spice.

Grinding and mixing the seeds with water, vinegar, or other liquids creates the yellow condiment known as prepared mustard. The seeds can also be pressed to makemustard oil, and the edible leaves can be eaten as mustard greens. Many vegetables are cultivated, varieties of mustard. plants; domestication may have begun 6,000 years ago.

Mustard required cool and dry weather and a fair supply of soil moisture during the growing period and a dry clear weather at the time of maturity. In India they are grown in Rabi season from September-October toFebruary-March. Some plant diseases are spread through the actual seeds themselves. When customers buy our seeds, we do our best to ensure that these seeds are disease-free. Seed borne disease refers to the particular plant diseases that are transmitted the by seed. Diseases of plants are caused primarily by three types of pathogens: bacteria, 3fungi, and viruses. Despite those fungi comprise the largest group of pathogens, the bulk ofseed-specific diseases are caused by bacteria or viruses. This is due to the fact that bacteria and viruses are more adept at entering and then travelling through the veins of the plant, a phenomenon known as 'systemic infection'. Fungi, in contrast, tend to be restricted to the outer layers of the plant, where they initiate infection by means of air-borne spores and then proceed to spread by attacking nearby cells of the outer layers.

Review and Literature

Oil seed crops are mainly used for the production of oil, a constituent of our diet, being an important source of fatty

acids. A lower production of oil seeds is a major problem that could be associated with the impact of various biotic and abiotic stresses. Diener and Davis
(1969) observed groundnuts contaminated with mycotoxins to be highly toxic to all domestic and laboratory animals. Ground nut (*Arachis hypogea L.)* is one of the most important and widely cultivated oil seed crop. The seeds of ground nut have found to carry a number of fungi. The most common cause of spoiled ground nut seed in storage is due to the growth of fungi. storage fungi infect the seeds as the seed is transferred into the storage and can spread rapidly throughout the bulk under favorable conditions. The fungi grow on the ground nut seed; they become visible and can kill the seed and produce and undesirable odour, taste and some time the seeds are not suitable for human consumption due to the production of mycotoxic substance by the seed fungi accompanied by change in the chemical nature of the seed. Seed
deterioration due to various mycoflora is common feature leading to loss of viability and numerous fungi develop on stored seed (*Lalithakumari et al. 1970*).
Mustard plants belong to the commonly known mustard family Brassicaceae of the order Brassicales (previously denoted as Capparales) including over 330 genera and over 3700 species that are distributed worldwide. Notable characteristics of this family are the four sepals in median position of the flowers followed by four alternating petals that are arranged in a crossform referring to the old family name Cruciferae. The presence of organosulphur compounds is also a unique characteristic of this plant family.
Aspergillus niger is a phytopathogenic fungus responsible for the plant disease called "black mold", and it is considered the most versatile microorganism. This fungus causes the "black mold" disease and it is the most common contaminant of grains and crops worldwide.
Aspergillus, one sizeable genus belonging to *Aspergillaceae* family.A. niger acquired a great economic importance when James Currie (1917).
Aspergillus flavus and A. parasiticus infect and produce aflatoxins in groundnuts. Groundnut (*Arachis hypogaea L.)* is one of the most important oilseed crops in world agricultural trade.13th most important food crop worldwide (Reddy, Sudhakar, & Reddy, 2011).
Allium sativum L. (Garlic) belongs to the family Amaryllidaceae, has been originated in Asia, and is also widely cultivated in Egypt, Mexico, China, and Europe. It is essential for microbial pathogen infections by its organosulfur compounds (*Lawson, 1988*).
Azadirachta indica belogs to family Meliaceae. Neem (*Azadirachta indica*) A. Juss is the mostversatile and useful medicinal plant ever found. Its every part is rich in bioactive compounds, which have traditionally been used to treat different ailments including infectious diseases. neem was first medicinal plant found a place in the Siddha system (Kumar and Navaratnam2013).
Piper betle (L) commonly known as betel vine belongs to the family Piperaceae.*Piper betle (L)* is a popular medicinal plant in Asia. Plant leaves have been used to medicinal plant. In this paper, a review of the literature was conducted to display recent studies (published in 2010–2020) on the antibacterial and antifungal properties of betel leaf.

Material and method

1. Collection of seed samples: Some stored seeds of economically important crops were collected from some markets in Groundnut (*Arachis hypogea* and Mustard (*Brassica nigra).*
a. Common Name: Groundnut Binomial Name*: Arachis hypogaea* Family: Fabaceae
b. Common AME: Mustard
Binomia Name: Brassica nigra
Family: Brassicaceae

2. Detection and identification of seed born fungi of stored seeds: The samples of *Arachis hypogaea L. and Brassica nigra L*. were assayed for the presence of seed born fungi using (A) Agar plate method (B) Blotter methods recommended by ISTA (1966), De Tempe (1970), Neergaard (1977) and Agarwal (1981).

3. Fumigation of culture laboratory

1. It is important to evacuate persons from the room or area before starting fumigation.
2. One should be very careful to avoid accidental entry into the room while fumigation in progress.
3. Beware of splashing as the reagents react. The reaction generates heat.
4. Wear protective clothing, gloves, mask and safety glasses.
5. Avoid inhalation of the fumes.

4. Sterilization of Laminar air flow chamber and glass wares

a. Sterilization of Laminar air flow chamber: The laminar air flow chamber is a device which is used for sterilization. This reduces the possibility if contamination while working with cultures of fungi inside a laminar air flow chamber. The working area was cleaned by using 100% alcohol and cotton.
b. After this, UV radiation is privided for 15 minutes for sterilizing environment.
   Sterilization of glass ware: Glass wares such as test tubes, petri-dishes etc. Were sterilized using hot air oven, electric hot air sterilizing oven as turned on and pre-heated in to160°C. using a soft, non-abrasive cloth, antibacterial dish soap and warm water gently clean the petri-dishes and dried with a soft non-abrasive dry cloth. Then the petri-dishes were rapped using clean papers and they were kept in the oven at 160°C for two hours to be sterilized.

5. Surfacesterilization.seeds: Stored seeds were surface sterilized using 0.1%HgCl2 (Mercury chloride) for four minutes, rinsed with distilled water for 3 - 4 times and dried between sterilized filter papers.

6. Preparation of Potato Dextrose Agar (PDA): General purpose medium Composition:

1. Potato - 200g/l 2. Agar - 15g/l
3. Dextrose - 20g/l. 4. Distilled water - 1000ml

7. Preparation of Potato Infusion

Steps involved in potato infusion preparation

1. Take 200 gm of potato for 1L of PDA media preparation.
2. Wash the potato to remove dirt.

3. Peel off the skin and dice them.
4. Add the pieces to 1L of distilled water.
5. Boil for 20-25 min on a hot plate.
6. Collect the extract through the muslin cloth.

- The preparation of the media by using the above raw materials is rather tedious. Hence in recent times, the infused form of potato is being replaced with commercially available potato starch/extract powder.4 gm of the potato extract powder is equivalent to 200 gm of potato infusion.

8. Agar plate method (PDA): 25ml of sterilized PDA medium was poured in a pre-sterilized borosil glass petri-dishes. The petri - plates were allowed to cool at room temperature 33+_°C; then 1gm of sterilized seeds were plated at equal distances in triplicates and unsterilized seeds were kept 17as control under aseptic condition. Plates were incubated at room temperature for seven days. On eighth day the seeds were examined under microscope for preliminary determination of seed mycoflora. The seed-born fungi found on each and every seed were isolated and identified, brought in to pure culture and maintained on PDA plates.

9. Moist blotter plate method (BPT): In moist blotter plate; the seeds were placed on water soaked here layered white blotter papers of 8.0 cm diameter and placed in pre sterilized borosil glass petridishes.1gm sterilized seeds were placed at equal distance in duplicates and unsterilized seeds kept as control in moist blotter paper under microscope for the preliminary determination of seed mycoflora. The seed born fungi found on each and every seed were isolated and identified, brought in to pure culture and maintained on PDA plates. In both Agar plate method and Moist blotter method examine the frequency of seed-borne fungi during incubation. The result was expressed in percentage
•Frequency of occurrence % = No. of seeds on which fungal species occurs × 100 /Total.no.of.seeds

10. Identification of fungi: Generally, identification of fungi species is based on morphological characters of the colony and microscopic examinations. The colony which includes the circumstances of the colony, the presence or absence or aerial mycelium, the colour, wrinkles, furrows and any other pigment production were the macro morphological characters were evaluated. Although more rapidly available microscopy and culture remain commonly used and essential tools for identification of fungal species.

11. Staining technique for fungi: needles were flamed over the burning Bunson burner. Then using a needle, a small portion of the growth on the culture plate was transferred on the culture plate, unto it a drop of Lacto phenol in cotton blue on the slide. TInoculatinghe specimen was teased carefully using inoculating wire loops to avoid squashing and overcrowding of the mycelium. The specimen is observed under the microscope for microscopic identification.

Preparation of medicinal plant leaf extracts: Samples used in the leaf extracts

a. Medicinal Plants Name: Neem Binomial Name: *Azadirect indica L.*
   Family: Meliaceae
b. Medicinal Plants Name: Garlic Binomial Name: *Allium sativum L.*
   Family: Amaryllidaceae
c. Medicinal Plants Name: Betle Binomial Name: *Piper betle L.* Family: Piperaceae

Effect of medicinal plant extracts on incidence of fungi in various concentration

After preparing various concentration (100%,75%,50%,25%) soaking in the sterilized petri-dishes on three layers of blotting paper water soaked in distilled water and each petri-plates containing 1gm seed. Then the petri-plates will keep in the sterilized incubation chamber at 33+-°C and data will be recorded seven days after sowing. The percentage of fungal infection and their effects on growth were observed.

Seed germination method: In seed germination method, evaluate the impact of seed-brone fungi on percentage in seed germination. Effects of seed-brone fungi on rate of seed germination were observed in all these methods.

a. Agar plate method: In this method 1gm of surface sterilized seeds were placed at equal distance in triplicate and unsterilized seed as control in PDA containing petri-plates. Observe the effects of seed-brone fungi on the percentage of seed germination of both sterilized seeds for seven days and the details were recorded.

b. Moist blotter method: In order to evaluate the effect of seed brone-fungi on the process of seed germination in BPT method, seeds were incubated in the moist blotters at room temperature for seven days. After incubation period the rate of seed germination of sterilized and unsterilized seeds were recorded.

Medicinal plant leaf extract method: Seeds were treated in all four medicinal plant leaf extract for half an hour, they were placed in sterilized petri-plates on three layered blotting paper soaked in distilled water. The petri-plates will be kept in sterilized incubation. Observes and recorded the data after seven days of incubation. The percentage of seed germination of each were recorded. In all these cases, examine the germination rate after incidents of fungi during incubation. The result was expressed in percentage. Frequency of germination % = No. of seeds germinated afterincidents of fungi ×100. Total no. of seeds

Growth parameters: Observe the radicle, plumule length by using scale and takaverage and count number of leaves and add in the table.

Results and Conclusions

Identification of seed borne fungi: The seed samples of Groundnut and Mustard were collected and tested initially, by employing agar plate method (PDA) and standard blotter plate technique (BPT) as described in ‘materials and methods. Seed mycoflora of stored seeds of commercially important crops were observed. Three fungi were isolated. The present work reveals the dominance of *Aspergillus sps.* The dominant species among two seed varieties were isolated. They were *Aspergillus niger, A. flavus and A. fumigates.*

Table 1: Fungal isolates of stored seeds.

SEED	Archis hypogea L.	Brassica Niger L.
Aspergillus niger	✓	✓
Aspergillus flavus	x	✓
Aspergillus	✓	✓

The dominant fungi isolated from the seeds were *Aspergillus niger, A. flavus and A. fumigatus.* Fungal infection is more in PDA method than BPT method. *A. niger, A. flavus andA. fumigatus* is present in PDA method and A. niger, A. flavus and A. fumigatus is present in BPT method. PDA method is more susceptible to fungal infection because a continuousnutrient is present in this method.

Table 2: Fugal species present in both PDA and BPT method.

Seed sample	Pda method	Bpt method
Archis hypogea L.	Aspergillus flavus	Aspergillus niger
	Aspergillus fumigates	
Brassica Niger L.	Aspergillus fumigates	Aspergillus fumigates
	Aspergillus flavus	Aspergillus flavus
	Aspergillus niger	

Scientific Classification

A. Aspergillus niger

- Kingdom: Fungi
- Phylum: Ascomycota
- Class: Eurotiomycetes
- Order: Eurotiales
- Family: Trichocomaceae
- Genus: *Aspergillus*
- Species*: A. niger*
- Subspecies: *A. niger*
- Scientific Name*: Aspergillus niger.*

a. Morphological characteristic features

1. *A. niger*, the fungal species which occur in almost all Petri-plates.
2. *A. niger* is also known as the black mold and causes infection in seed borne fungi.

B. Aspergillus flavus

- Scientific classification:
- Domain: Eukaryota
- Kingdom: Fungi
- Division: Ascomycota
- Class: Eurotiomycetes
- Order: Eurotiales
- Family: Aspergillaceae
- Genus: *Aspergillus*
- Species: *A. flavus*
- Binomial name*: Aspergillus flavus*

Morphological characteristic features

1. The Aspergillus flavus group of fungi.
2. Young the conidia of A. flavus appear yellow green in colour.

C. Aspergillus fumigates

- Kingdom: Fungi
- Division: Ascomycota
- Class: Eurotiomycete
- Order: Eurotiales
- Family: Trichocomaceae
- Genus: *Aspergillus*
- Species: *fumigates*

Morphological characteristic features

1. The fungi can survive at 37 degrees Celsius while the conidia can survive temperatures ofup to 70 degrees Celsius.
2. They produce spores of between 200 and 400 mm

Comparative incidents of seed mycoflora by two different culture method.

The incidents ofseed-borne fungi were determined on two different methods carriedout simultaneously account ofseed borne mycoflora by two different culture methods. (Plate 5, Table 3 and Graph 1).

Table 3: Comparative incidents of seed borne fungi on different seed inPDA and BPT culturemethod in percentages.

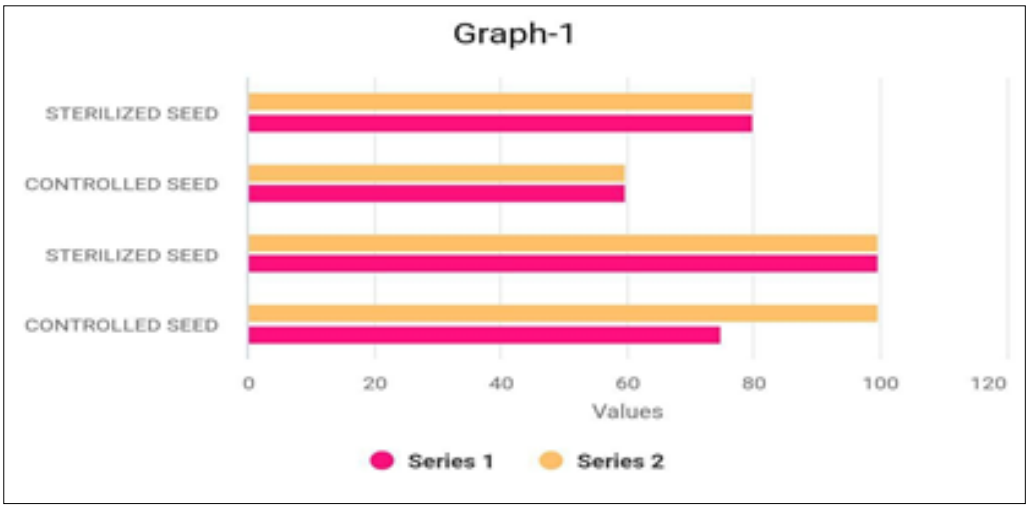
Sr No	Seed sample	Incident of seed borne fungi %			
		Agar plate	Agar plate	Blotter plate	Blotter plate
		Sterilzed seed	Controlle d seed	Sterilzed seed	Controlle d seed
1	*Arachis hypogea L.*	70%	100%	60%	80%
2	*Brassica nigra L.*	100%	100%	60%	80%

Dominant three fungal types were observed on both culture methods. Those were *Aspergillus niger, A. flavus and A. fumigatus*. In the present study, the incidents of seed mycoflora were more in controlled seeds than in the sterile seeds in both methods. In thecase of sterilized seeds, the maximum incidence of fungi in PDA method was observed in mustard (100%) but in Groudnut (70%) minimum was observed.

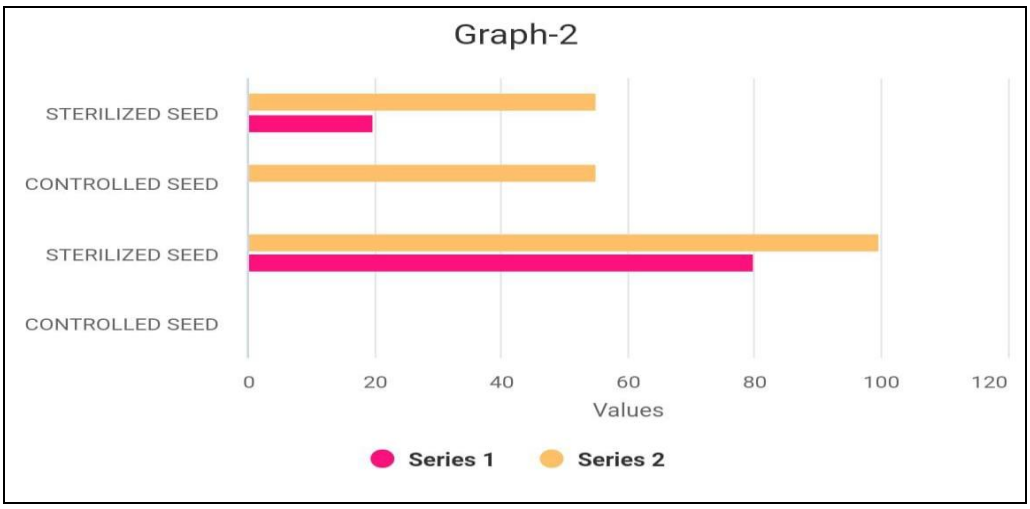
Table 4: Comparative rate of germination in test seeds by two different culturesmethods inpercentages.

Sl. No	Seed sample	Rate germination of seeds %			
		Agar plate	Agar plate	Blotter plate	Blotter plate
		Sterilzed seed	Controlle d seed	Sterilzed seed	Controle d seed
1	*Arachis hypogea L.*	80%	_	20%	_
2	*Brassica nigra L.*	100%	50%	50%	_

In PDA method Groundnut showed 80% germination after the fungal incidents, but Mustard show 100% germination. The germination studies of BPT culture method reveals maximum germination occurred in Mustard (50%). Both Groundnut and Mustard



Graph 1: Comparative incidents of seed borne fungi on different seed inPDA and BPT culturemethod in percentage



Graph 2: Comparative rate of germination in test seeds by two different cultures methods in percentages.

Antifungal activity of medicinal plant extract water extract of fresh samples (Neem, Garlic and Betel) were prepared and seeds were treated in it as described in 'materials andmethods'. Observe frequency of fungal infection and germination rate after medicinal plantextract treatment after medicinal plant extract treatment by both PDA and BPT method *Archis hypogea L.*

Table 5: Frequency of fungal incidents and germination rate after medicinal plant extract treatment.

Sl.no	Concen tration	Neem	Neem	Garlic	Garlic	Betel	Betel
		Frequenc y of fungal infection	Germinati on Rate	Frequenc y of fungal infection	Germinati on Rate	Frequenc y of fungal infection	Germinati on Rate
1	100%	10%	80%	10%	90%	15%	80%
2	75%	20%	50%	15%	60%	30%	50%
3	50%	25%	30%	20%	40%	40%	30%
4	25%	40%	10%	35%	20%	50%	20%

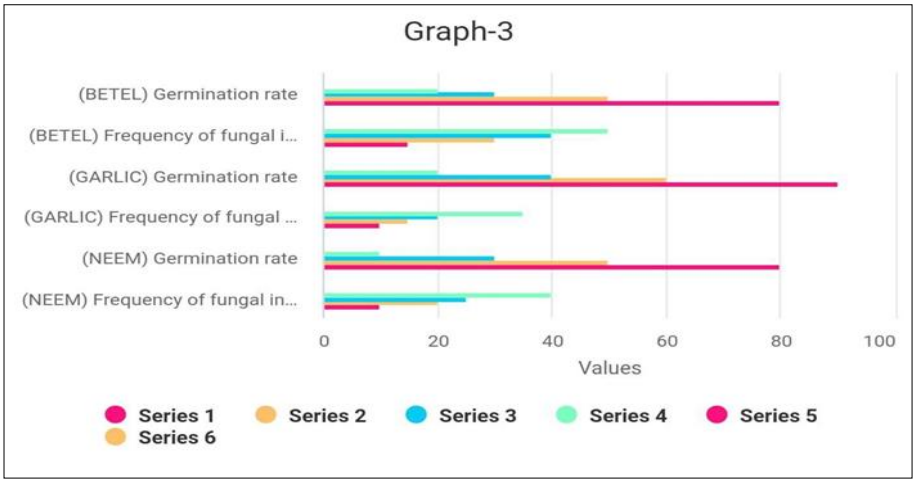
Brassica nigra L.

Table 6: Frequency of fungal incidents and germination rate after medicinal planted ex tract treatment.

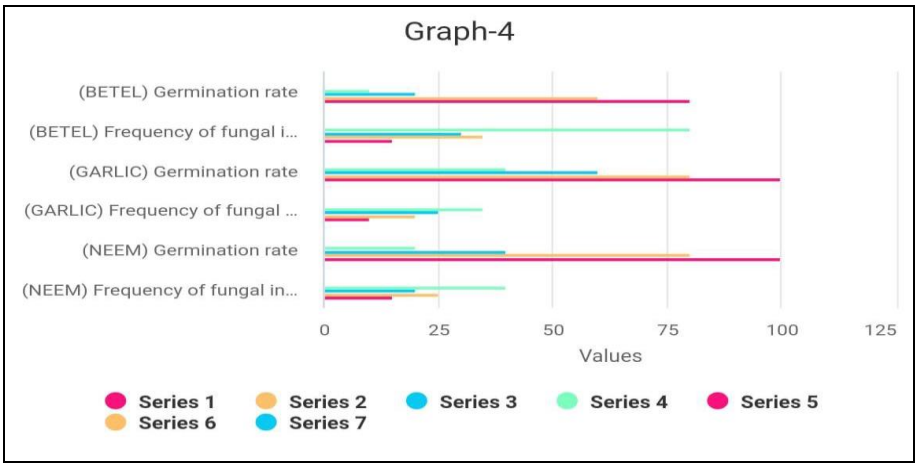
Sl.no	Concen tration	Neem	Neem	Garlic	Garlic	Betel	Betel
		Frequenc y of fungal infection	Germinati on Rate	Frequenc y of fungal infection	Germinati on Rate	Frequenc y of fungal infection	Germinati on Rate
1	100%	15%	100%	10%	100%	15%	80%
2	75%	25%	80%	20%	80%	35%	60%
3	50%	20%	40%	25%	60%	30%	20%

Groundnut and mustard treat with garlic extract became resistant to fungal infection andalso maximum germination rate in both seeds. The seeds of Groundnut and Mustard treatedwith Garlic and Neem extract show high resistivity towards fungal growth. The germinationrate may be increased by changing the concentration if the extract. Neem

extract is good inthe case of Mustard, it has 100% germination rate and it act as good antifungal agent. Neem and Garlic proved to show resistance to fungal infection and showed maximum germinationrate. The seeds treated with Betel extract shows more fungal incidence and low germinationrate, when compared to Neem and Garlic plant extrac.

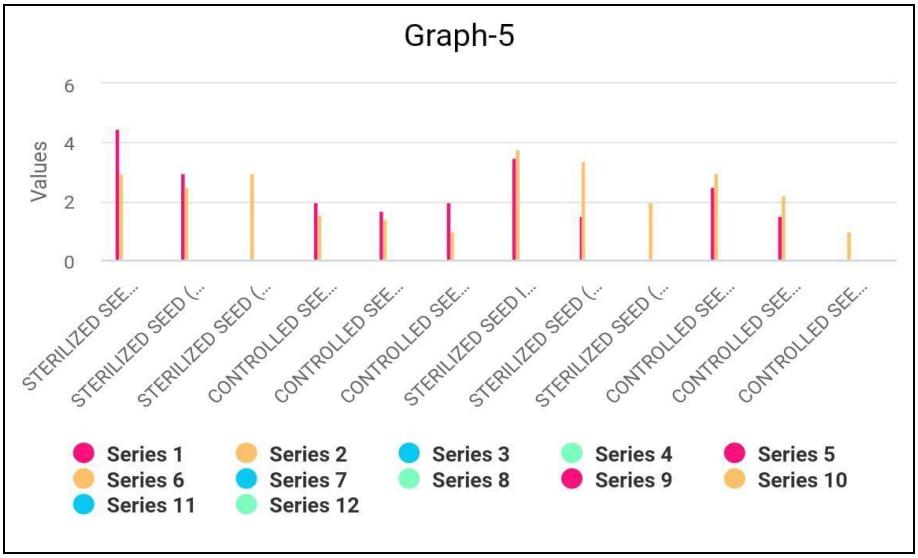


Graph 3: Frequency of fungal incidents and germination rate of Archis hypogea



Graph 4: Frequency of fungal incidents and germination rate of Brassica nigra.

ComparativeStudy of Growth parameters of Groundnut and Mustard by PDA and BPT two different culture methods, carried out simultaneously employing PDA and BPT Method (Table 6, Graph 5).



Graph 5: Comparative study of growth parameters of Groundnut and Mustard by PDAand BPT Method.

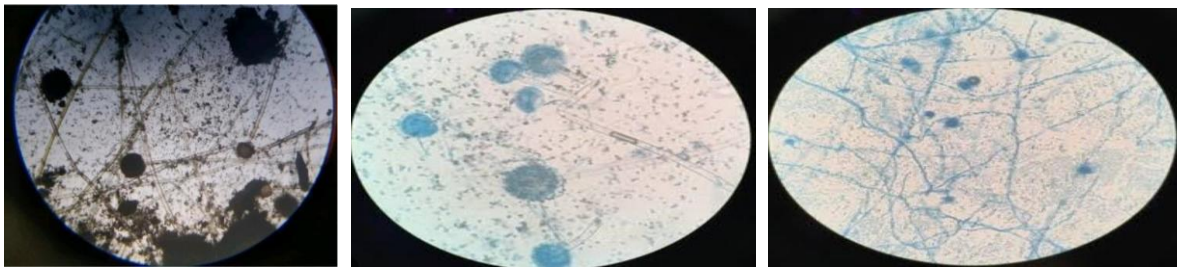
a. Groundnut *b. Mustard*

Plate 1: PD (culture method)

a. Groundnut *b. Mustard*

Plate 2: (BPT (culture method)

Plate 3. (Fungal species present in both (PDA and BPT methods)



a) Aspergillus niger *b) Aspergillus flavus* *c) Aspergillus fumigates*

Plate No 4

a) Neem, Betel, Garlic leaf extract seed soaked medicinal plant

Neem, Garli, Betel extract in control seed borne fungi

Comparative Study of Growth parameters of Groundnut and Mustard by PDA and BPT Method.

An effect of seed borne fungi on growth parameters such as length of radical, lengthof plumule and number of leaves developed were observed on two different culturemethods, carried out simultaneously employing PDA and BPT Method (Table 6, Graph 5).

Table 6

Sl. no	Seed sampl E			Growth parameters				
				Agar Plate	Agar Plate	Growth paramet ers	Blotter Plate	Blotter Plate
	Archis hypoge a L.			Sterilize d Seed	Controll ed Seed		Sterilize d Seed	Controll ed Seed
1		A)	Length of radical	4.5	0	Length of radical	2.5	3.5
2		B)	Length of plumule	3	2	Length of plumule	1 .5	1.5
3		C)	No.of.le ves	0	2.7	No.of.le ves	0	0

Table 7: Comparative study of growth parameters of Groundnut and Mustard by PDA and BPT Method.

Sl. no	Seed Sampl E			Growth parameters				
				Agar Plate	Agar Plate	Growth paramet ers	Blotter Plate	Blotter Plate
	Archis hypoge a L.			Sterilize d Seed	Controll ed Seed		Sterilize d Seed	Controll ed Seed
1		A)	Length of radical	3	1.4	Length of radical	3.8	3

In PDA method, Groundnut showed maximum length of radicle and plumule.

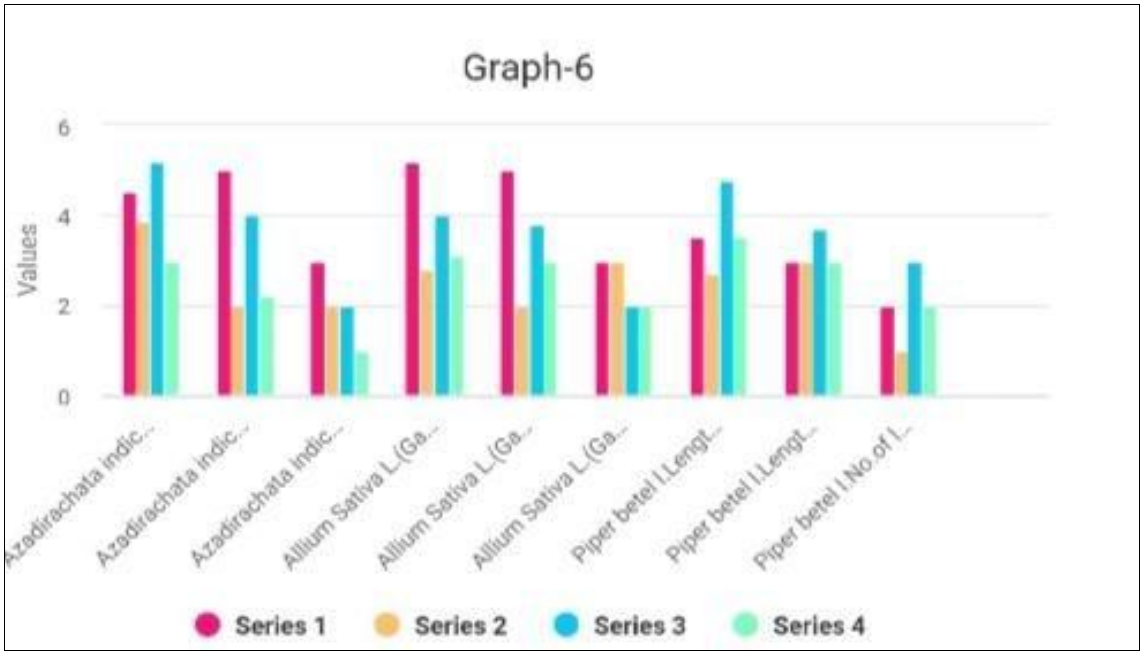
The length of plumule, length of radicle and no. of leaves developed was minimum in Mustard when compared to Groundnut.In BPT method, Groundnut showed minimum length of radicle and plumule and there was minimum number of leaf development in both controlled andsterilized seeds. It was observed that,PDA method was more effective for studying growth parameters of Groundnut and Mustard.

Growth Parameter: Graph 5.5: Comparative study of growth parameters of Groundnut and Mustard by PDA and BPT Method.Comparative study of growth parameters of Groundnut and Mustard after treatmentwith medicinal plant extract

Table 8: Comparative study of growth parameters of Groundnut and Mustard after treatment with medicinal plant extract.

Sl. No	Name of plants	Parameter s	*Archis hypogea L.*	*Archis hypogea L.*	*Brassica niger L.*	*Brassica niger L.*
			Sterilized Seed	Controlled Seed	Sterilized Seed	Controlled Seed
1	*Azadirecta indica (Neem)*	Length of radical	4.5	3.9	5.2	3
		Length of plumule	5	2	4	2.2
		No.of.leave s	3	2	2	1
2	*Allium Sativum (G arlic)*	Length of radical	5.2	2.8	4	3.1
		Length of plumule	5	2	3.8	3
		No.of.leave s	3	3	2	2
3	*Pipper Betel (Betel)*	Length of radical	3.5	2.7	3.8	3.3
		Length of	3	3	3.7	3

Mustard showed maximum length of shoot and radicle after treatment with Neem. Seeds treated with Garlic also showedmore length of shoot and radicle compared to Neem and Betle. The number of leaves developed was not same in all theseeds treated with medicinal plant extract. sterilized seeds when compared to control in both PDA and BPTmethod.



Graph 6: Comparative study of growth parameters of Groundnut and Mustard after treatment with medicinal plant extract.

Discussion

Good seed recognized as an important input in any agricultural production system. One of the importantaspects of good seeds besides high germination and purity is the absence of

seed-borne pathogen. Manypathogenic fungi are seed transmitted, often reduce the germination ability or kill the infected plants orsubstantially reduce the productivity. There for control of

seed-borne fungi is extremely important and thedamaging effects can be relieved through integrated approaches (Diaz *et al*., 1998). This study was conducted todetermine the prevalence of mycoflora associated with some stored seeds of Groundnut and Mustard whichare collected from Shakthan market for the experiment, using PDA and BPT methods. Seed health test in two methods yield three different fungi *Aspergillus niger, A. fumigatus and A. flavus.* A considerable number ofseed- borne fungi belongs to the genera *Aspergillus* (Shazia *et al*., 2004; Tripti *et al*., 2011: Archana andPrakash., 2013) [6]. Comparative incidents of seed mycroflora by two different culture methods reveal threedominant fungal species. These fungal species were present in both PDA and BPT method. There was avariation in percent of occurrence of different fungal species. The incidence of seed mycroflora were present incontrolled and sterilized seed in both PDA and BPT method. Three fungal species were examined. Fungalinfection is more in PDA method than BPT method. *Aspergillus niger, A. fumigatus, A. flavus* is present in both PDA and BPT methods. The nutritional and mycroflora changes of groundnut (Arachis hypogea) wereinvestigated during a storage period of twenty weeks. A total number of seven fungal species were identifiednamely *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Rhizopus sp., Pencillium sp., Mucor sp. andFusarium sp*. (Emmanuel and Olajide., 2012). PDA method is more susceptible to fungal infection because acontinuous nutrient is present in this method.If moisture increases with decrease in temperature was the mostsuitable condition for fungal growth. In PDA culture method the availability of nutrients is continuously alongwith moisture.

Summary

Seed is a small embryonic plant enclosed in a covering called the seed coat, usually withsome store food. Seed is a vital component of the world's diet. Good seeds are the indicationof good plant products. Seed-borne fungal diseases are common in stored seeds. They causeserious problem in human by consumption. Many costly storage problems actually beginduring field exposure, harvesting and conditioning of seed. Excessive harvesting delays, mechanical injuries and improper drying techniques, followed by poor storage conditionscan lead to rapid deterioration of seed and vigor. Seed-borne fungal attack affects thegermination and further developmental processes. The high seed quality is essential in anycrop production venture to attain yield and good quality products. In the present work,significant number of fungi were isolated from these seed samples. It indicated theseed-borne mycoflora of two different samples collected from Shakthan market. It revealedthat seed-borne fungi contaminated both the sterilized and unsterilized seeds. The overallresult revealed that the PDA culture method is more supporting media than BPT culturemethods for isolation of fungi. This is because of the continuous nutrient supply in theculture method. Fungus needs optimum environmental conditions for the growth andspread. Moisture and temperature are the two factor which depends the growth of fungi. Moisture rich food item and seed are more susceptible to the fungal attack. Temperature isinversely proportional to the moisture. In this study *Aspergillus niger, A. flavus and A.fumigatus* were isolated and studied. Fungal infection is more in PDA method than BPTmethod. A. fumigatus are present in both PDA as well as BPT method. Mycotoxins areharmful substances produced by various fungi in foods. Aflatoxin is one kind of mycotoxin. Aflatoxins ate the major poisonous carcinogens that are produced by certain molds whichseen in soil, decaying vegetation and agricultural crops and grains. These aflatoxins areprimarily produced by two closely related fungi which are Aspergillus species. In PDA medium nutrient rich and moisture were present. So, it may be the reason for the reductionin the percentage of fungal

growth in BPT and the increased fungal growth in PDAmethod.More germination rate is obtained in PDA than in BPT method. Nutrient richmedium can be the reason for the germination rate in PDA. The presence of fungal growth inboth the medium can adversely affect the further elongation and developmental processesof the plant. The present study also pointed on a view that medicinal plant extract can beused as a method to control fungal infection. Medicinal plant extract are simple andeco-friendly, so this can be used insted of fungicides. The result shows that Garlic and Neemplant extract proved to be a good antifungal medicine when compared to betel plant extract.

Conclusions

Seeds are of immense biological and economic importance. Seed-borne fungi are a serious problem which adversely affects the seeds and it will hinder germination and further developmental process. There is a need for alternatives to fungicides for the control of seed-borne fungi. Fungal growth can be fastened and due to the moistened environmental condition. Therefore, control of seed-borne fungi is extremely important and the damages can be controlled through different method. A large number of chemicals have been developed for the control of plant diseases. But due to over growing awareness of the side effects of these chemicals, more and more emphasis is being given to the use of biocontrol agents. Now major challenge is felt in the field of plant pathology to introduce some ecofriendly safe alternative control strategies for agriculture, which led researchers to turn their attention to plants and microorganisms as sources of biocontrol agents. Neem, Garlic and Betel are among the important medicinal plant extract have strong antifungal property. This study points on the fungal attack occur oilseeds as Arachis hypogea and Brassica nigra and can be control by aqueous medicinal plant extracts.

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