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Review Article

A Eco-friendly management of seed borne mycoflora of Mustard (Brassica sp.) through botanicals and determination of antifungal activity by SEM.

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ABSTRACT

The study was planned to investigate the efficiency of Plant extract of Moringa oleifera against, Datura stramonium , Azadirachta indica and Corton bonplandianum against some seed born fungi (Aspergillus sp., Rhizopus sp., Fusarium sp., Alternaria sp., Cochleobolus sp., Rhizoctonia sp.) isolated from oil seed (Jhumi) using standard microbiological procedures and to investigate effectiveness of plant extract on seed borne mycoflora by SEM study. The process well diffusion method is used to study the efficacy of Plant extract. The extracts were poured into the wells at different concentrations like 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml. After incubation zones of inhibition was carried out by agar plate observed. As the concentrations of extracts increased the activity also increased and thus the zones of inhibition too increase. Hence Moringa oleifera, Datura stramonium, Azadirachta indica and Corton bonplandianum can be used against various seed born fungal disease.

Keywords: Mycoflora, Seed treatment, Aspergillus sp., Rhizopus sp., Fusarium sp., Alternaria sp., Cochleobolus sp., Rhizoctonia sp., Antifungal activity

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INTRODUCTION

Oilseeds are raised in almost all the parts of the country. Interestingly, in some regions of the country, they are considered as important oil-seeds. Oilseeds are the source of oil-cake as well as vegetable oil. However, the export of oilseeds has been curtailed to meet the increasing demands of the country. The oilseed accounts for 13% of the Gross Cropped Area, 3% of the Gross National Product and 10% value of all agricultural commodities. This sector has recorded annual growth rate of area, production and yield @ 2.44%, 5.47% and 2.96% respectively during last decade (1999-2009). During the last few years, the domestic consumption of edible oils has increased substantially and has touched the level of 18.90 million tonnes in 2011-12 and is likely to increase further. With per capita consumption of vegetable oils at the rate of 16kg/year/person for a projected population of 1276 million, the total vegetable oils demand is likely to touch 20.4 million tonnes by 2017. This oil-seed is generally cultivated in light soils and are grown as a Rabi crop. It is grown in Gujarat and Andhra Pradesh. The oil extracted from these seeds is used in the manufacture of hydrogenated oil. India is of the biggest producers of this particular oil seed. Thus, it can be said that India is a huge producer of oil seeds and most of its states produce one form of oil seed or the other. Despite the rapid spread of the crop, a disheartening trend is that the productivity is going down in recent years. Several diseases are known to cause yield loss in the oilseed crops and many of these diseases are caused by seed borne mycoflora *viz., Aspergillus niger, Rhizopus sp., Fusarium sp., Alternaria sp., Colletotrichum sp., Rhizoctonia sp. Among these, Alternaria sp., Aspergillus sp.* has been considered as a potentially destructive on many oilseed crops in different countries. The loss in yield varies from 25- 60percent depending on the stage and the extent of infection [1]. Hence, it is imperative that the seeds must be tested before they are sown in the field. The uses and expectations of seed treatments with chemicals are greater today but due to the impact of environment regulations they have either banned or restricted the use of older chemicals like organo mercurial fungicides, because of their residual toxicity. Bio protectants applied to seeds may not only protect the seeds but also may colonize and protect roots and increase the plant growth. However, biological agents have tended to be somewhat less effective and more variable than chemical seed treatments. The study was planned to investigate the efficiency of Plant extract of *Moringa oleifera*, *Datura stramonium, Azadirachta indica* and *Corton bonplandianum* against some seed born fungi (*Aspergillus sp., Rhizopus sp., Fusarium sp., Alternaria sp., Cochleobolus sp., Rhizoctonia sp.*) isolated from oil seed (Jhumi) using standard microbiological procedures and to investigate effectiveness of plant extract on seed borne mycoflora by SEM study.

Historical background of the pathogen:

Alternaria blight is a destructive disease of rapeseed-mustard throughout the world and appears each year in crop fields. The disease is caused by different species of *Alternaria viz. A. brassicae* (Berk) Sacc, *A. brassicicola* (Schw.), *A. raphani* Grows and Skolo and *A.alternata* (Fr) Keisskr. Among than *A. brassicae* is much more destructive and occurs more frequently than *A. brassicicola* on *B. Juncea* [2]. The literature on the disease is voluminous, however, in the present review, only those aspects which have a direct bearing on the investigation, have been included. The genus *Alternaria* was first described by Neesin 1817 with type species *A. tenius*; which was later renamed as *A. alternata*. Berkeley, noticed fungal infection on plant belonging to the family Brassicaceae and indentified the fungus as *Macrosporium brassicae* (Berk) which was later renamed as *A. Brassicae* (Berk) Sacc by Saccardo in 1886. In India, *Altenaria* blight was first observed on sarson from Tirhoot in 1901 but the fungus was thought to be new and described as *Sporodochium brassicae*. Later Mason, first observed the *Alternaria* species from aherbarium material of sarson from Pusa (Bihar) India.

Mechanism of seed infection by fungi:

Fungal infection of seed borne pathogens may reach the ovule of the seed at any stage from the initiation of ovule formation to the mature seed. The fungal plant pathogens vary in their modes of multiplication and attack on the lost plant [3,4,5,6] fungal propagules / spores germinate and the growing hyphae determine the entry of the pathogens in plant tissue including the fruit and seed. The physiological and biochemical factors inside the fruit and seed further control the establishment of successful infection. The mechanism of transmission of infection into the seed [7, 8, 9, 10].

MATERIALS AND METHOD:

Site of experiment:

The experiments were carried out in the department of Biotechnology, NIT Durgapur, Burdwan, W.B, India.

Collection of oil seed sample:

For studying mycoflora associated with oil seed (variety-Jhumi) are collected from various region of oil seed grower. Mainly seeds are collected from different Indo-Gangatic areas (Burdwan, Hooghly, Howrah) of West Bengal. The collected seed samples are shade dried and stored in paper bags at room temperature for further studies.

Detection of seed Mycoflora:

International Seed Testing Association (ISTA) techniques (1966, 1973, and 1976) were used for the detection and isolation of seed-borne mycoflora of oil seed (Jhumi). The methods used for seed health testing were: Inspection of dry seeds or visual examination; Incubation method, rolled paper towel method, standard blotter method, Agar Plate method; Deep freeze method, pathogenicity test. Brief account of the methods employed during the course of the present investigation is given below.

Surface Sterilization of Seed:

Surface sterilization of the seeds are done by one percent sodium hypochlorite for 30 seconds. The seeds are then washed with three changes of sterile water.

Standard blotter method:

As per available literature reports three pieces of filter paper [11, 12] should be properly soaked in sterilized water and are placed at the bottom of a 9 cm well labelled plastic Petri dishes. Generally Twenty Five (25) seeds per Petri dish are placed using a pair of forceps and making sure that seeds are placed equidistantly under aseptic conditions. The lids of each Petri dish should be held in place with gummy cello tape. The Petri dishes containing seeds are incubated at room temperature ($25^{o}\pm 2^{o}C$) for 7 days under alternating cycles of light and darkness of 12 hours each.

Fig no: 1 Standard blotter method

Agar plate method:

As described in the literature in the agar plate method [13] 20 ml of potato dextrose agar are distributed to each of the sterile Petri plates under aseptic conditions. After cooling, crop seed samples are transferred [14] to the agar plate. Twenty Five seeds per Petri plate are to kept at equidistance in a circle and incubated at room temperature ($25^{o}\pm 2^{o}$ C) under 12 hours alternating cycles of light and darkness for 7 days and has to be observed everyday for the growth of fungi [15].

Fig no: 2 Agar plate method

Rolled towel method:

It is another process described by researchers to study the germinability of the seeds in the laboratory at room temperature (30º±2ºC). A total of 200 seeds are recommended to be randomly taken from each variety and 50 seeds are placed between a pair of moist paper towels [16]. The towels are rolled and the ends are closed by robber band and covered by polyethylene paper to prevent drying. First observation is recommended to take after 5 days and final count has to be taken after 14 days of incubation period pertaining to (a) % germination, (b) non germinated seed (hard seed and rotten seed), (c) shoot length and, (d) root length. For determination of seed mycoflora the fungal growth on infected seed are taken with the needle and observed under compound microscope.[17].

Deep freeze method:

This method is developed by scientists to detect slow growing pathogens. According to researchers three hundred seeds by moderately infected pathogens, are generally placed at the rate of 25 seeds per plate on moistened blotters in the way as described under Standard blotter method. The Petri plates are incubated at 20º± 2ºC for 24 h under alternate cycles of 12 h NUV light and darkness, for next 24 hours the plates are incubated at –20ºC in dark and then kept back under original conditions for next five days. After eight days of incubation, the seeds are examined under stereobinocular microscope [30]. The frequency of the fungus is calculated by the following formula: No. of seeds containing a particular fungus

$$\frac{}{\text{Total seeds used}} \times 100$$

METHODOLOGY FOR MANAGEMENT STUDIES:

Evaluation of Seed Priming:

Seed priming is a process to increase the germination rate of crops. Priming is one of the most important physiological methods which improves the seed performance and provides faster and synchronized germination. This technique helps in the germination rate by giving some biological and physiological advantage such as enzyme activation which help in rapid synthesis of nucleic acid and any other essential component of cell division. There are various methods of seed priming such as osmopriming, halopriming, hydropriming, hormonalpriming etc. Seed priming is a process where seeds are soaking in priming agents before its germination. Thus the germination rate of the seed will be increased.

Technique of seed priming	Priming agent
Halopriming	Solution of inorganic salts i.e. NaCl, KNO3 CaCl2, CaSO4
Osmopriming	Solutions of sugar, polyethylene glycol (PEG), glycerol, sorbitol, or mannitol
Hormonalpriming	salicylic acid, ascorbate, kinetin

Table no: 1 -List of Seed priming agent.

EVALUATION OF PLANT EXTRACT:

Four plant are used to study the evaluation of plant extract on seed borne mycoflora.Those are Neem, Datura, Sajne, and Bon Tulsi. Several concentration are made (400 mg/ml; 200mg/ml; 100mg/ml; and 50mg/ml) to study the efficacy of plant extract.

Solvents used for Plant extract:

I. Hexane
II. Ethyl acetate
III. Benzene
IV. Chloroform

Figure 3: Different plant extracts

Preparation of different plant extract:

There are four types of plant extract prepared to evolution of plant extract are 1.Benzene extract 2.Hexane extract 3.Chloroform extract 4.Ethyl acetate extract. 5gm of air-dried powder of leaves was mixed with 25ml of benzene in a conical flask and then kept on a rotary shaker for 10mints. Then they were bind with tissue paper and rubber band.

Some holes were made so that air can pass through it and then take room temperature for 3-5 days for evaporate.

Table no:2 -List of plant extract and recommended doses.

Sl. No	Common name	Plant part used	Half Recommended dose	Recommended dose
1	Bon Tulsi	Leaf	5%	10%
2	Neem	Leaf	5%	10%
3	Sajne	Leaf	5%	10%
4	Datura	Leaf	5%	10%

Evaluation of Bio-agents :

Three bio- agents i.e. *Trichoderma viride* (Parbham) and *Pseudomonas fluorescens* and *Bacillus subtilis* were used to control the seed borne mycoflora associated with the oilseed cultivars on PDA medium. Three species of seven days old cultures of *Trichoderma viride* (Parbham) and *Pseudomonas fluorescens* and *Bacillus subtilis* were used for seed treatment of oilseed cultivars. Every 5 and 10 gm. *Trichoderma* cultures were mixed with 100 gm (W/W) fine charcoal powder to increase total volume and adhesive vortex was added for better sticking with the oilseeds. The oilseed seeds were moist with sterilized distilled water and coated with *Trichoderma viride* (Parbham) and *Pseudomonas fluorescens* culture. Plating of oilseeds was done on agar plates. Observations regarding mycoflora associated with the seeds and seed germination per cent were recorded after seven days.

Table no:3 List of Bio-agent and recommended doses.

Sl No.	Bio-agent	Half Recommended dose	Recommended dose
1	*Trichoderma viride*	0.5%	1.0%
2	*Psudomonas fluorescens*	0.5%	1.0%
3	*Bacillus subtilis*	0.5%	1.0%

Detection of effectiveness of plant extract on seed borne mycoflora by SEM study:

Fungi samples (1 × 1 cm), collected from seedlings, were pre-fixed in 3% glutaraldehyde plus 2% paraformaldehyde in a 0·1 M sodium cacodylate (CAC) buffer (pH 7·2) for 2 h at room temperature and then stored at 4°C until further processing. Samples were washed twice in the CAC buffer for 30 min and then postfixed in 0·1% w/v osmium tetroxide in the same buffer for 2–3 h at room temperature. After fixation, samples were washed (2 × 30 min) with distilled water and dehydrated with a graded ethanol series (20, 40, 60, 70, 80, 90, 95 and 99·5%) consisting of 10 min steps for each ethanol concentration followed by a graded ethanol–acetone series with 30 min steps. Specimens were subsequently dried in a critical-point drier (Agar Scientific Ltd) with liquid CO_2 as the transition fluid, mounted on SEM stubs with double-sided sticky tape and coated. Observations of plant and fungal structures were made using a *ZEISS* Sigma FE scanning electron microscope operated at 15.00 kV and equipped with a camera for digital micrographs.

Statistical Analysis:

Statistical values were analysed using SIGMAPLOT software (Version – 14.0). The 'ANOVA test' were used to analysis of resulting data. The data represents as mean and standard deviation (SD) with Probability value (p).

RESULT:

Morphological Identification of different seed borne mycoflora associated with the oilseed samples by light microscopy:

The identification of fungi was done based on spore morphology and colony character.Ten fungi were noticed on the oilseed samples collected from the different oilseed growing areas of Birbhum district, West Bengal. *Aspergillus niger, Aspergillus flavus* often appeared in many samples along with species of *Rhizopus sp., Fusarium sp., Mucor sp.* and

Alternaria sp. were found mostly. Spore morphology and colony characters are given below.

Aspergillus sp.

Colony of *Aspergillus niger*on seed grows slowly, consisting of a compact to fairy loose white to faintly yellow basal mycelium, which bears abundant erect and initially crowed conidial structures. Conidial heads are typically large and black, compact at first, spherical or split into two or more loose to reasonably well defined columns. Conidiophores are smooth, hyaline or faintly brownish near the apex. Two series of conidia bearing the cells (supporting cells and phialides) are produced but in some heads only phialides are present. Conidia are typically spherical at maturity. Often very rough or spiny, mostly 4-5 µm diameter and very dark in colour or with conspicuous longitudinal striations.

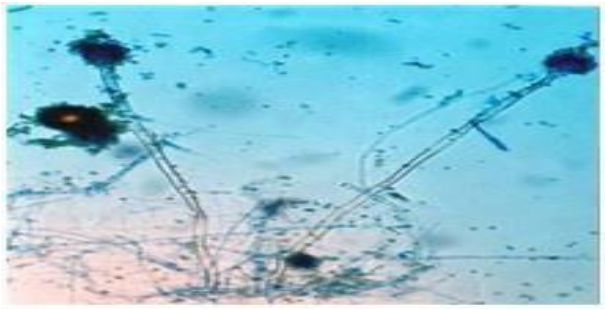


Plate 1: *Aspergillus sp*

Fusarium sp.

The fungus produces abundant loose, aerial white mycelium on incubated seed. In this mycelium several shiny, hyaline, transparent to milky white spherical droplets are seen hanging at the tip of long thin stalks. These stalks are primary conidiophores, which arise laterally from the hyphae in the aerial mycelium. Microconidia are hyaline, 1-2 septate oval, ellipsoidal to sub- cylindrical and measure 5-20 x 2.8-7 µm. Macroconidia are hyaline, stout, measured 22-75 x 35- 7 µm, subcylindrical (or) slightly curved, with short blunt and rounded apical cells and indistinctly pedicillate basal cells. They are glubose to subglubose, smooth (or) rough- walled and 6-11 µm in diameter (Kraft, 1969, Ram Nath *et al.*, 1970; Booth, 1971).

Plate 2: *Fusarium sp*

Rhizopus sp.

Colonies are greyish white in colour. Mycellium are filamentous, branching. Three types of hyphae are found stolons, rhizoids and usually unbranching Sporangiophores. Sporangiophores are rounded and produce numerous nonmotile multinucleate spores for asexual reproduction. Sporangia are black in colour.

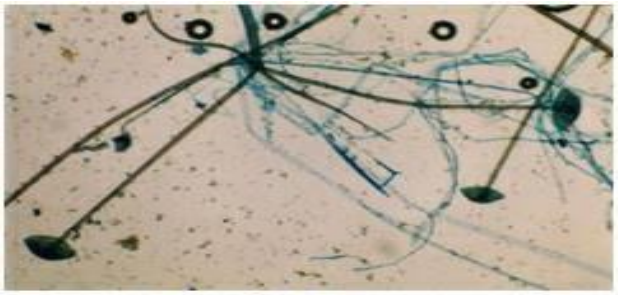


Plate 3: *Rhizopus sp*

Alternaria sp.

Colonies are usually gray, dark blackish brown and black. Mycelium all are immersed or partly superficial, hyphae colourless, olivaceous brown or brown. The conidiophores of the pathogen are pale brown, cylindrical, erect and not rigid and arise singly with a size of 34- 54 x 4-7 µm. Conidiophores produces conidia at the apex which are in chains of one or two. The conidia are straight or slightly curved, obclavate, yellowish brown to dark brown in colour and measures 30- 120- x 4-7 µm. The conidia have 4- 12 transverse septa and 0- 6 longitudinal septa. The conidia have long beak which may be simple or branched and about 24 to 220 x 2-4 µm in size (Ellis and Holiday, 1970).

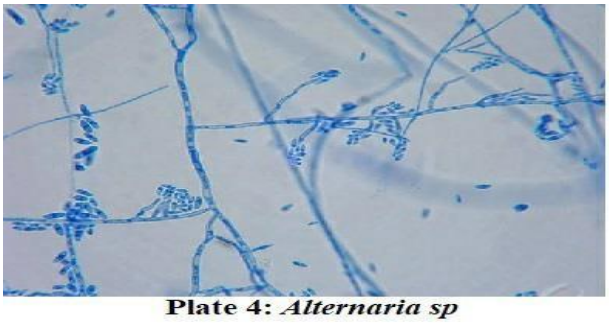


Plate 4: *Alternaria sp*

Rhizoctonia sp.

The pathogen is not currently known to produce any asexual spores. Colonies are distinguishing right angles. Conidia are hyaline basidiospores are borne. Conidiophores forms club shaped basidia with four apical sterigmata on which oval.

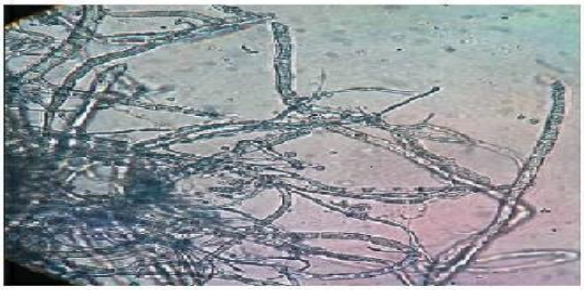


Plate 5: *Rhizoctonia sp*

Cochleobolus sp.

These fungi are able of showing different interaction with their host and different life style depending on their interaction. Shape various from small circular to oval. Colonies are oblong, chocolate coloured sports. Conidia are reddish brown to tan lesions and over time more greyish.

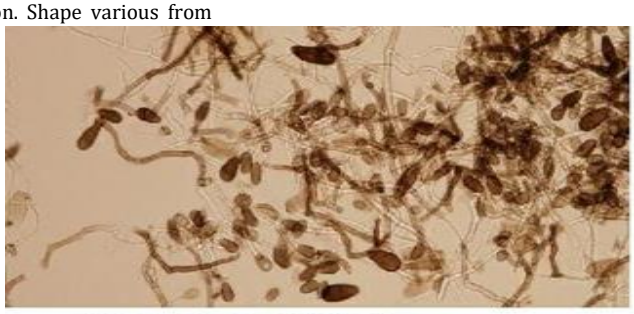


Plate 6: *Cochliobolus sp*

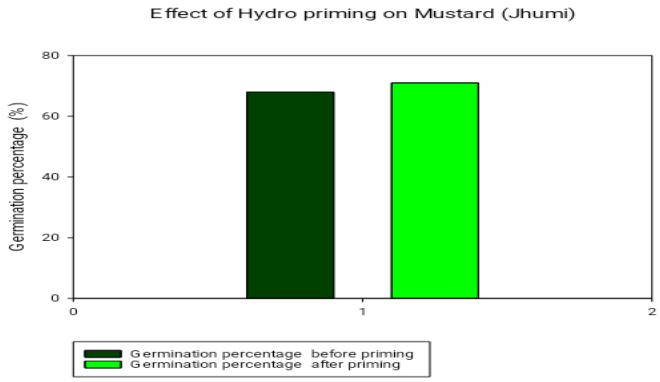


Fig No.-4 Evaluation of Hydro priming on the germination of Oil seed

Seed priming plays an important role on the germination percentage of seeds. In Hydro priming it was shown that the germination percentage of mustard increased from 68% to 71% (Table no: 4)

Evaluation of Halo priming on the germination percentage of oil seed:

Table no:-4 Effect of Halo priming on Mustard (Jhumi)

Sl No.	Name of Priming Salt	Germination percentage before Priming.	Germination percentage after Priming.
		Mustard (Jhumi)	Mustard (Jhumi)
1.	NaCl	69	73
2.	KNO3	65	68
3.	$CaCl_2$	68	73
4.	$CaSO_4$	63	68
S.D		2.387	1.924
Mean		65.130	72.130

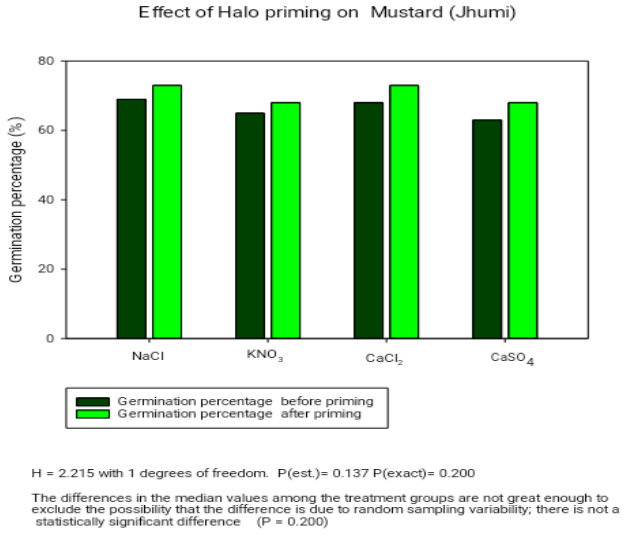


Fig No.-5 Evaluation of Halo priming on the germination percentage of oil seed

In Halo priming it was shown that Nacl increased the germination percentage of mustard from 69% to 73%. KNO_3 increased the germination percentage of mustard from 65% to 68%. $Cacl_2$ increased the germination percentage of mustard from 68% to 73% and $caso_4$ increased the germination percentage of mustard from 63% to 68 %.(Table no: 5).

Evaluation of Osmo priming on the germination percentage of oil seed:

Table no:-5 Effect of Osmo priming on Rice (Khas) and Mustard (Jhumi)

SL.	Name of Priming agent	Germination percentage before Priming.	Germination percentage after Priming.
1.	Sugar	68	71
2.	PEG (Poly ethelene glycol)	66	70
3.	Glycerol	65	69
4.	Sorbitol	69	73
5.	Manitol	63	68
	S.D	2.387	1.924
	Mean	66.200	70.200

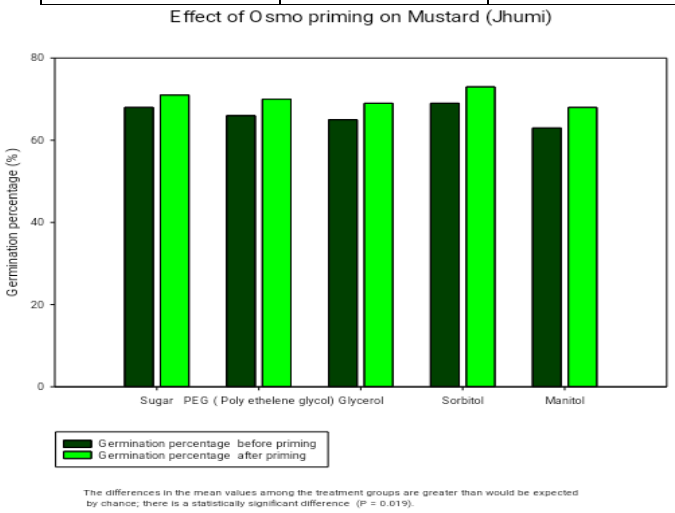


Fig No.-6 Evaluation of Osmo priming on the germination percentage of oil seed

In case of Osmo priming it was shown that Sugar increased the germination percentage of mustard from 68% to 71%. PEG increased the germination percentage of mustard from 66% to 70%. Glycerol increased the germination percentage of mustard from 65% to 69%. Sorbitol increased the germination percentage of mustard from 69% to 73%. NaCl increased the germination percentage of rice mustard from 63% to 68%. (table no:6)

Evaluation of Hormonal priming on the germination percentage of oil seeds:

Table no :-6 Effect of Hormonal priming on Mustard.

SL. No.	Name of Priming agent	Germination percentage before Priming.	Germination percentage after Priming.
		Mustard (Jhumi)	Mustard (Jhumi)
1	Salicylic acid	64	73
2	Ascorbic acid	66	75
3	Kinetin	68	76

S.D	2.000	1.528
Mean	66.000	74.667

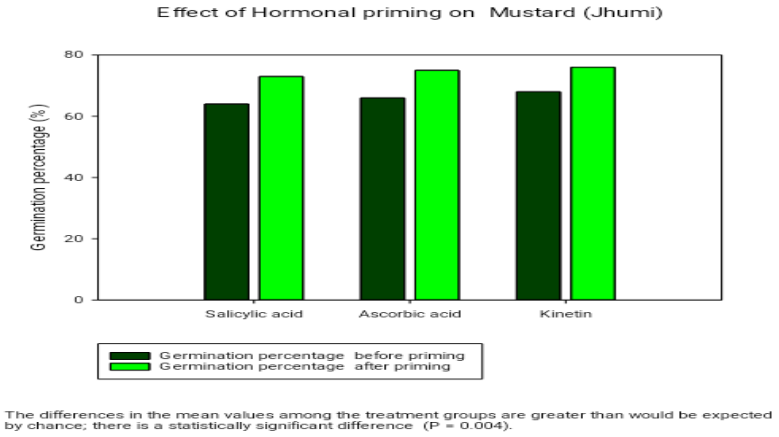


Fig No.-7 Evaluation of Hormonal priming on the germination percentage of oil seeds

In Hormonal priming Salicylic acid increased the germination percentage of mustard from 64% to 73%. Ascorbic acid increased the germination percentage of mustard from 66% to 75%. Kinetin increased the germination percentage of mustard from 68% to 76%.

Evaluation of Bio-agents on the basis of germination percentage of oil seeds:

Three bio- agents i.e., *Trichoderma viride* and *Pseudomonas fluorescens* and *Bacillus subtilis* were used as seed treating agents to assess their efficacy on seed germination. Soaking method which was described under materials methods was followed for the experiment. Data pertaining to the various parameters are presented in Table no (7) The results revealed the positive effects of most of the bio- agents against the infection of seed mycoflora which reduced the infection percentage considerably and enhanced the germination percentage of different seeds of oilseed crops. All the treatments differ significantly over control. *Trichoderma viride* showed good for all the seeds of oilseeds with the germination percent increasing 68%(before treatment) to 79%(after treatment) Followed by *Pseudomonas fluorescens 65%(*before treatment) to *70%* (after treatment)and *Bacillus subtilis* 65% (before treatment) to 69% (after treatment).

Table No:7 Evaluation of Bio-agent (*Trichoderma viridae, Pseudomonas fluorescens, Bacillus subtilis)* on oil seed (Jhumi).

SL No.	**Bio-Agent**	**Germination percentage before treatment**	**Germination percentage after treatment**
1.	***Trichoderma viridae***	68	79
2.	***Pseudomonas fluorescens***	65	70
3.	***Bacillus subtilis***	65	69
SD		1.732	5.508
Mean		66.000	72.667

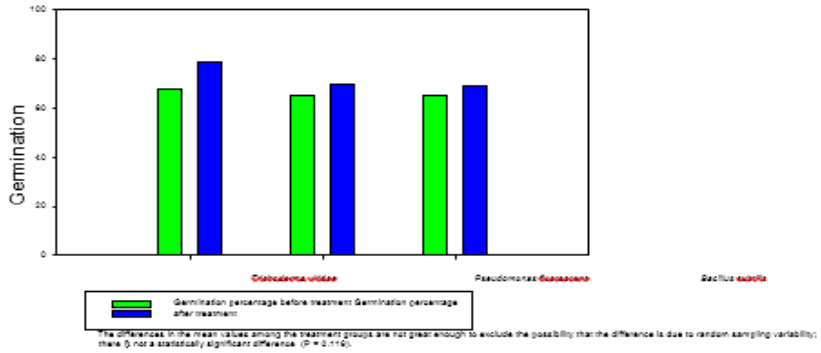


Fig no:8 Evaluation of Bio-agent (*Trichoderma viridae, Pseudomonas fluorescens, Bacillus subtilis)* on oil seed (Jhumi).

Evaluation of plant extracts on the basis of germination percentage of oil seed (Standard Blotter Method and Agar Plate method):

Four plants extracts viz. leaf extract of Ban Tulsi, Neem, Datura and Sajne were used as seed treating agents to assess their effect on seed germination. Soaking method which was described under materials methods was followed for the experiment. Data pertaining to the various parameters are presented in Table no (9) and Fig. no (9). All the treatments differ significantly over control. However, the extract of Tulsi showed 72% (500ppm), and 76% (1000ppm) germination percentage against the seed mycoflora. The Datura leaf extract showed maximum germination on Jhumi 96% (1000ppm) followed by Sajne 84% (1000ppm)and Neem 80% (1000ppm).

Table No –8 Evaluation of plant extracts on the basis of germination percentage of different oil seeds under in-vitro condition (Standard Blotter method

Sl. No.	Oil Seeds	Plant Extracts								Control	
		TULSI		**NEEM**		**SAJNE**		**DATURA**			
		500 ppm	**1000 ppm**	**500 ppm**	**1000 ppm**	**500 ppm**	**1000 ppm**	**500 ppm**	**1000 ppm**		
1.	Jhumi (large)	72.00	76.00	72.00	80.00	80.00	84.00	92.00	96.00	52.00	54.00
	SD	11.588	12.362	11.588	12.362	11.588	12.362	11.588	12.362	11.588	12.362
	Mean	73.600	78.000	73.600	78.000	73.600	78.000	73.600	78.000	73.600	78.000

Evaluation of plant extracts on the basis of germination percentage of different oil seeds under in-vitro condition (Standard Blotter method)

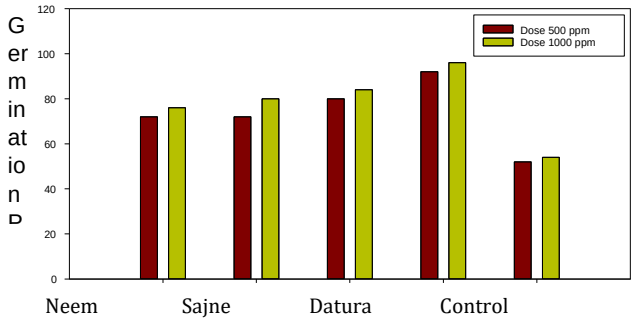


FIG NO:9 Evolution of plant extracts on the basis of germination percentage of different oil seeds under in-vitro condition (standard Blotter method)

Table No – 9 Evaluation of plant extracts on the basis of germination percentage of Oil seed under invitro condition (Agar pate method)

Sl. No.	Oil Seeds	Plant Extracts								Control	
		TULSI		NEEM		SAJNE		DATURA			
		500 ppm	1000 ppm	500 ppm	1000 ppm	500 ppm	1000 ppm	500 ppm	1000 ppm		
1.	Jhumi (large)	40.00	48.00	36.00	40.00	40.00	44.00	52.00	56.00	20.00	22.00
SD		11.524	12.198	11.524	12.198	11.524	12.198	11.524	12.198	11.524	12.198
Mean		37.600	40.400	37.600	40.400	37.600	40.400	37.600	40.400	37.600	40.400

Table No – 9 Evaluation of plant extracts on the basis of germination percentage of Oil seed under invitro condition (Agar pate method)

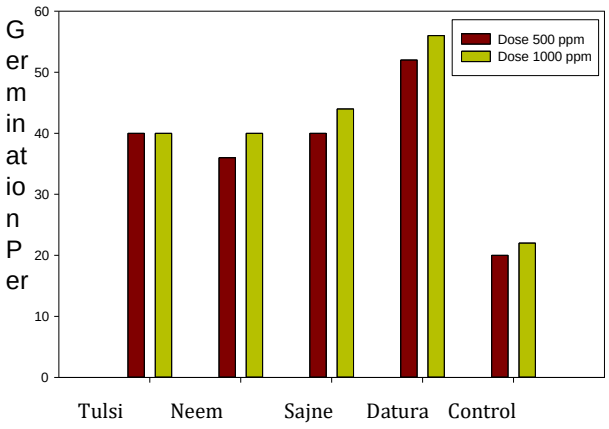


FIG NO:10 -Evolution of plant extracts on the basis of germination percentage of different oil seeds under invitro condition (Agar plate method)

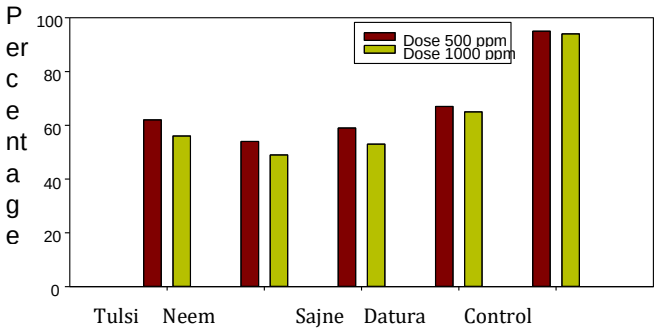
Evaluation of plant extracts on the basis of inhibition of fungal growth of different oilseeds under invitro condition: (Standard Blotter method and Agar plate method:

Four plant extracts viz. leaf extract of Ban Tulsi, Neem, Datura, Sajne were used as seed treating agents to assess their effect on seed germination. Soaking method which was described under materials methods was followed for the experiment. Data pertaining to the various parameters are presented in Table no. (11) and Fig no. (11). All the treatments differ significantly over control. The Datura leaf extract showed maximum inhibition of seed mycoflora on Jhumi 67% (500ppm) followed by Bon Tulsi 62% (500ppm) and Sajne 59% (500ppm).

Table No. 10 – Evaluation of plant extracts on the basis of fungal growth inhibition of Oil seed under invitro condition (Agar plate method)

Sl. No.	Oil Seeds	Plant Extracts								Control	
		BONTULSI (*Croton bonplandianum*)		NEEM (*Azadirachta indica*)		SAJNE (*Moringa oleifera*)		DATURA (*Datura Stramonium*)			
		500 ppm	1000 ppm	500 ppm	1000 ppm	500 ppm	1000 ppm	500 ppm	1000 ppm		
1.	Jhumi (large)	62.00	56.00	54.00	49.00	59.00	53.00	67.00	65.00	95.00	94.00
	SD	16.134	18.091	16.134	18.091	16.134	18.091	16.134	18.091	16.134	18.091
	Mean	67.400	63.400	67.400	63.400	67.400	63.400	67.400	63.400	67.400	63.400

Evaluation of plant extracts on the basis of fungal growth inhibition of Oil seed under invitro condition (Agar plate method)



The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.722).

Fig No:11- Evaluation of plant extracts on the basis of fungal growth inhibition of Oil seed under in-vitro condition (Agar plate method)

Table No.-11 Evaluation of plant extracts on the basis of fungal growth inhibition of Oil seed under invitro condition (Standard Blotter method)

Sl. No.	Oil Seeds	Plant Extracts								Control	
		BONTULSI (*Croton bonplandianum*)		NEEM (*Azadirachta indica*)		SAJNE (*Moringa oleifera*)		DATURA (*Datura Stramonium*)			
		500 ppm	1000 ppm	500 ppm	1000 ppm	500 ppm	1000 ppm	500 ppm	1000 ppm		
1.	Jhumi (large)	85.00	79.00	83.00	79.00	87.00	81.00	95.00	88.00	110.00	100.00
	SD	11.045	08.961	11.045	08.961	11.045	08.961	11.045	08.961	11.045	08.961
	Mean	92.000	85.400	92.000	85.400	92.000	85.400	92.000	85.400	92.000	85.400

Evaluation of plant extracts on the basis of fungal growth inhibition of Oil seed under in-vitro condition (Standard Blotter method)

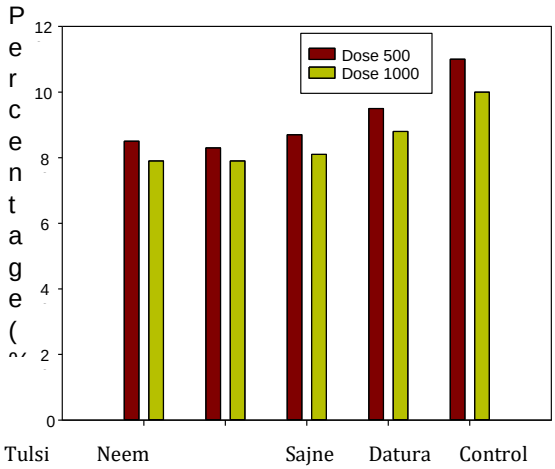


Fig No:12 – Evaluation of plant extracts on the basis of fungal growth inhibition of Oil seed under in-vitro condition (Standard Blotter method)

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.330).

Detection of level of effectiveness of plant extract on seed bone mycoflora by SEM study:

The seed borne fungi, *Rhizopus sp., Aspergullis sp., Fusarium sp., Cochliobolus sp., Alternaria sp.* and *Rhizoctonia sp.* growing over the seed of Rice and Mustard were observed under Light microscope. Then the microscopically identified samples of each plate was marked, thereafter treatment via plant extracts it supplied for SEM identification The Scanning Electron Microscopy showed different rapture stages of these fungi.. The result of each plate is below-

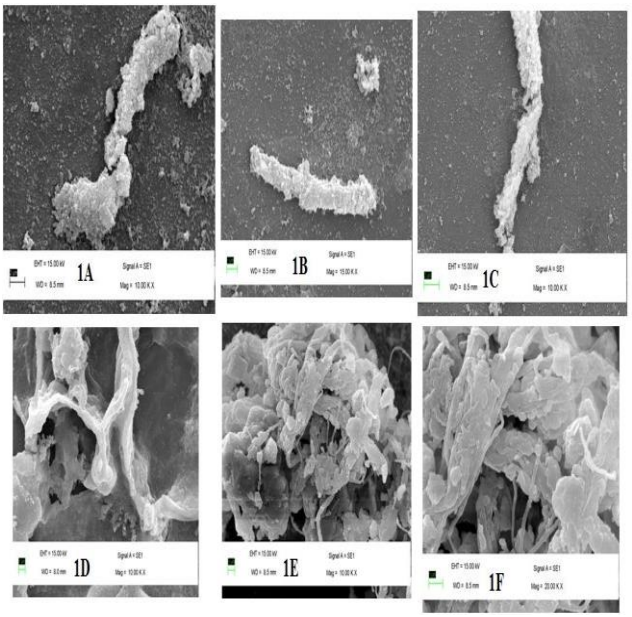


Figure-13 : Effect of Ethyl acetate extract of *Moringa oleifera* and the raptureness occurred in those seed borne fungi (*Rhizopus sp.* {1A}, *Aspergullis sp.* {1B}, *Fusarium sp.* {1C}, *Cochliobolus sp.* {1D}, *Alternaria sp.* {1E} and *Rhizoctonia sp.* {1F}) respectively.

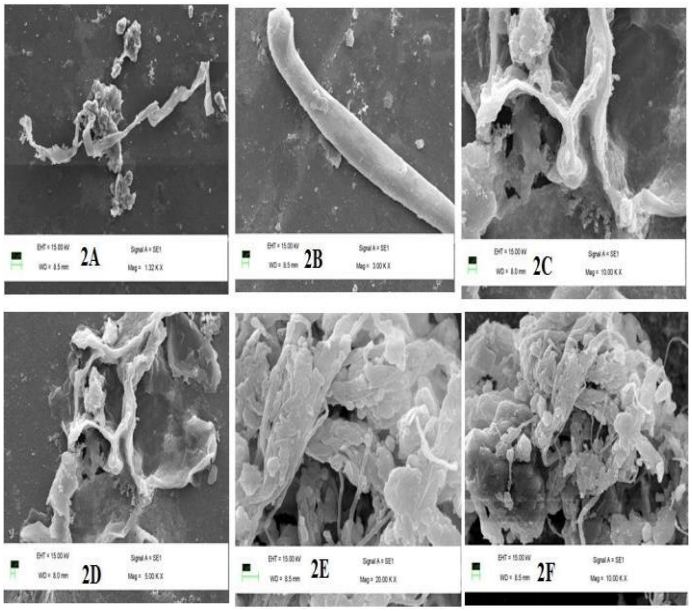


Figure-14 : Effect of Ethyl acetate extract of *Croton bonplandianum* and the raptureness occurred in those seed borne fungi (*Rhizopus sp.* {2A}, *Aspergullis sp.* {2B}, *Fusarium sp.* {2C}, *Cochliobolus sp.* {2D}, *Alternaria sp.* {2E} and *Rhizoctonia sp.* {2F}) respectively.

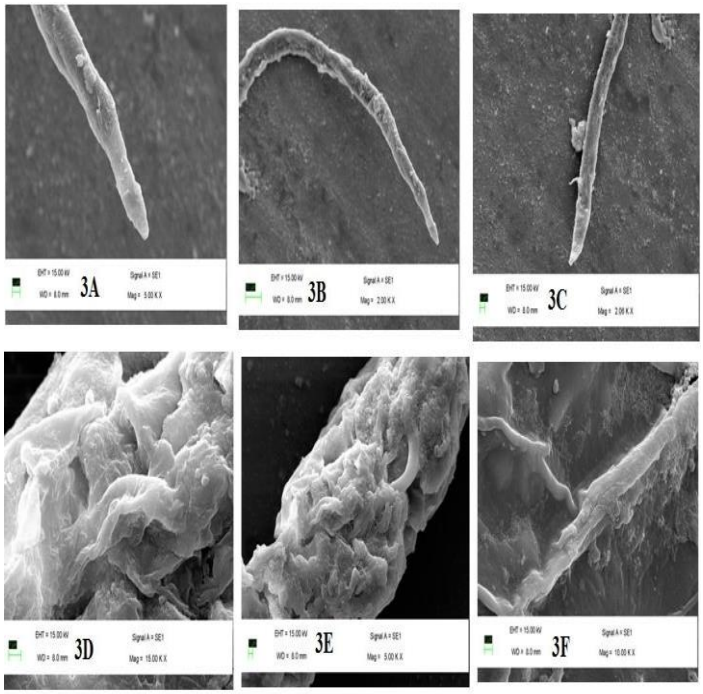
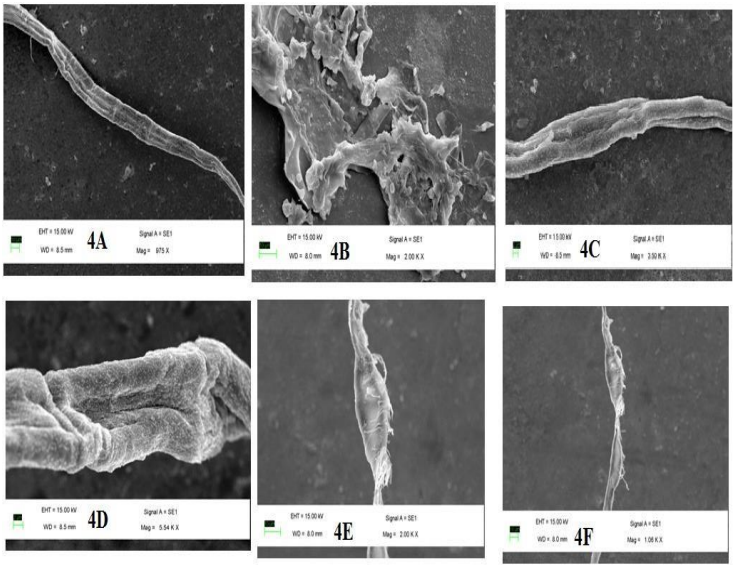


Figure-15 : Effect of Ethyl acetate extract of *Datura stramonium* and the raptureness occurred in those seed borne fungi (*Rhizopus sp.*{3A}, *Aspergullis sp.*{3B}, *Fusarium sp.*{3C}, *Cochliobolus sp.*{3D}, *Alternaria sp.*{3E} and *Rhizoctonia sp.*{3F}) respectively.

Figure-16 : Effect of Ethyl acetate extract of *Azadirachta indica* and the raptureness occurred in those seed borne fungi (*Rhizopus sp.*{4A}, *Aspergullis sp.*{4B}, *Fusarium sp.*{4C}, *Cochliobolus sp.*{4D}, *Alternaria sp.*{4E} and *Rhizoctonia sp.*{4F}) respectively.



DISCUSSION AND CONCLUSION:

The findings pertaining to the investigations carried out on detection and identification of seed mycoflora of mustard isolation of predominant mycoflora *i.e. Aspergillus sp., Rhizopus sp.*, *Fusarium sp.*, *Cochleobolus sp.*, *Rhizoctonia sp.* and *Alternaria sp.*, *in- vitro* evaluation of botanicals, biocontrol agents and chemical on the basis of germination and infection percentage are summarized as below. Occurrence of *Aspergillus sp.*, *Alternaria sp.* was found predominant. The mycofloral species viz. *Rhizopus sp.*, *Fusarium sp.*, *Aspergillus sp.*, *Alternaria sp.* were found associated with all the tested genotypes while species of *Cochleobolus sp., Rhizoctonia sp.* were not detected in some of the genotypes. Among the methods adopted for detection of seed mycoflora, agar plate method proved to be better than the other methods as the number of colonies of the seed mycoflora were more in agar plate method. Four plants extracts viz. leaf extract of Ban Tulsi, Neem, Datura, Sajne were used as seed treating agents to assess their effect on seed germination. Four botanical formulations evaluated against the seed borne mycoflora associated with the oilseed by soaking method. Leaf extract of Tulsi and Neem showed maximum inhibition of mycoflora infestation followed by leaf extract of datura and whereas leaf extract of Sajne showed poor result of inhibition against the seed borne mycoflora. In Agar plate method of Mustard the data revealed the positive effects of most of the plant extracts against the infection of seed mycoflora which reduced the infection percentage considerably and enhanced the germination percentage of different seeds of oilseed crops. However, the extract of Tulsi showed 40% (500ppm), and 48% (1000ppm) germination percentage against the seed mycoflora. The Datura leaf extract showed maximum germination on Jhumi 56% (1000ppm) followed by Tulsi 48% (1000ppm) and Neem 40% (1000ppm). The Datura leaf extract showed maximum inhibition of seed mycoflora on Jhumi 67% (500ppm) followed by Bon Tulsi 62% (500ppm) and Sajne 59% (500ppm). Among the three bio- agents tested for their efficacy against the pathogens by soaking method, maximum percentage of seed germination with less infection was noticed in *Trichoderma viride,* which was found on par with *Pseudomonas fluorescens.* Among the four priming agent viz. Hydro priming, Halopriming, Osmopriming and Hormonal priming, the Hormonal priming showed maximum germination percentage. In Hormonal priming Salicylic acid increased the germination percentage of mustard from 64% to 73%.Ascorbic acid increased the germination percentage of mustard from 66% to 75%. Kinetin increased the germination percentage of mustard from 68% to 76%.

FUTURE ASPECT:

Microbial contamination of seed become in a growing concerned of the farmers as it affect directly and indirectly with germination percentage viability of the seed heath and vigour and also on nutritional property. It is quite obvious that considerable amount of seeds destroyed or rotted every year due to the infection of various mycoflora the quantum of losses is under great threat owing to the fluctuating environmental condition which contributing tremendously towards the multiplication and development of seeds mycoflora is considerable amount of research have been done on the aspect of seed pathology which include identification of seed borne mycoflora, interaction between post pathogens, seed resistance , seed priming use of different indigenous and technological knowhow to Combat seed infection. But till today there is a considerable gap between the proper identification and managements of seed mycoflora under diverse eco-system best on the present research work some emphasis has to be given further on the following aspect-Bio pesticides i.e. botanicals, bio-organism, medicinal plane extract, having an anti microbial property must best utilize in a broad manner. For increase the detection efficacy, alternation in detection methods to be done at regular intervals on all the oilseed crops. For effective detection of bacterial species, special methods need to be developed. Efficacy of various type of indigenous methods to control the seed borne mycoflora associated with the can be practised. Relation with epidemiological factors.

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