SEED PATHOLOGY OF RAPESEED-MUSTARD

BY

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Dissertation

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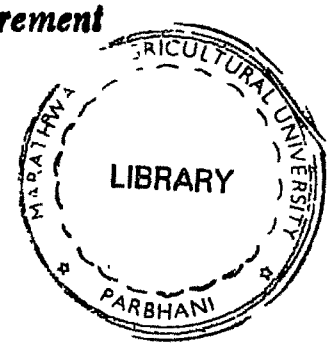

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DEDICATED TO

LATE. GRAND FATHER

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AND

BELOVED PARENTS

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I, hereby declare that,the dissertation or part thereof has not been previously submitted by me for the degree of any University.

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Shri.Patil Shashikant Manoharrao has satisfactorily prosecuted his work of research for a period of not less than four semisters and that the dissertation entitled **" Seed Pathology of Rapeseed Mustard"**, submitted by him is the result of original work and is of sufficient high standard to warrant its presentation to the examination.

I also certify that the dissertation or part thereof has not been previously submitted by him for a degree of any University.

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CERTIFICATE - II

This is to certify that the dissertation entitled **" Seed Pathology of Rapeseed-Mustard"**, submitted by Shri.Shashikant Manoharrao Patil to the Marathwada Agricultural University, Parbhani in partial fulfilment of the requirement for the degree of **MASTER OF SCIENCE (Agriculture)** in the subject of Plant Pathology has been approved by the students advisory committee after oral examination in collaboration with the external examiner.

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Introduction

CHAPTER I

INTRODUCTION

The first report of seed-borne pathogen came in 1755 when Tillet, a French Botanist, established that the casual fungus of the hill-bunt of Wheat Tilletia caries is seed borne. Since then more than 1,000, fungi, 100 bacteria, 126 viruses and about 14 nematodes have been found associated with seed (Richardson,1979).

Plant protection testing technique will depend invariably on the importance of the pathogen carried in the seed and the disease potential assigned to this pathogen, in a given situation. Seed is multiferous and therefore, it is highly essential that the seed-borne fungi should be thoroughly studied with respect to their biology and control. Seed treatment not only kills or inhibits the pathogen associated with seeds, but also protects the seed from seed-borne pathogen in initial stage of growth by forming a zone of protection arround the seeds.

Seed treatment helps in establishment of sound healthy plant by improving germination stand and vigour. It is also cheapest and safest method of direct plant disease control. Relatively small quantities of plant materials are handled and in principle most of the seeds are treated. Fungicidal seed treatment therefore, is highly essential and probably the major method of direct plant disease control (Nene and Thapliyal,1979).Storege of treated seed some time is benefial by increasing the

germicidal effect or it may be detrimental by increasing phytotxicity (Paul Neegard,1977).

Among oil seed crops of India, rapeseed (*Brassica campestris* Linn.) and mustard [*Brassica juncea* Linn (Zern and Coss)], together refered as rapeseed mustard (RM) are an important group of oil seed crops grown under wide range of agro-climatic conditions. They belong to family; Cruciferae and also termed as oil *Brassicas*. In India, the Indian mustard (*Brassica juncae*), brown sarson (*Brassica campestris* Var. yellow sarson), and toria (*Brassica campestris* Var.toria) are widely grown for oil purposes. The other *Brassicas* such as gobi sarson (*B. napus*) and wild mustard (Jungli rai) (*B. tournefortii*) are grown on very small scale. The improtant varieties of rapeseed mustard released for cultivation in India during the past few years are given below.

1)	Indian mustard (*B. juncea*)	: Prakash,RLM-514,Seeta,Varuna,Kranti, Pusa-bold,Saurabh,Vaibhav,Vardan, Bhagirathi, Sanjucta,Pusa-Basant, Pusa-Bahar.
2)	Toria (rapeseed) (*B. campestris* Var toria)	: DKL-1,Sangam,Agrani,TL-15,TH-68, Bharani,PT-30,TH-63,Panchali,RAUT-17.
3)	Brown sarson (Rapeseed) (*B. campestris* Var sarson)	: Pusa-Kalyani, Suphala, KOS-1.
4)	Yellow sarson (Rapeseed) (*B. campestris* Var. sarson)	: K-88, PYS-6, Benoy, 66-197-3,Subinoy, YSK-1, T-151, B-9.

Rapeseed and mustard are economically important crops in local and international trade as they yield edible oil ranging from 30-40 per cent which is used as the main cooking medium in Northern India. The seed and oil are used as condiments in preparation of pickles and flavouring curries and vegetables. The leaves of young plants are used as green vegetable.Rapeseed mustard oil contains erucic acid (38-57%), linilenic acid(4.5 to13), oleic(27%)and linolic acids which are of high nutritive value for human consumption.

In India winter season cultivation increased production and productivity of rapeseed mustard resulting into increased edible oil supplies. Since 1987-88, the countries vegetable oil scenario showed a remarkable transformation from its decade oil stagnation to one of self reliance. The dream of countrys potential and capabilities to become self sufficient in oilseed production become true by the record out put of 18 million tonnes in 1988-89 and current production level of 21.5 million tonnes from 26.8 million hactares. The spectacular progress of 70 per cent increase in 8 years is considered worth recording as " Yellow Revolution" (Sovenier, ISPR, DOR, Hyderabad, Aug.2-5, 1993).

The production which was having around 3 million tonnes until 1986-87,has increased to a level of 6 million tonnes. The productivity has increased from 417 kg/ha in 1949-50 to 903 kg/ha in 1991-92 (Kumar,1993).

Rapeseed mustard group of crops accounts for 25.4 per cent of total acerage and contribute 31.1 per cent of the total production of oilseed in the country. The crop is traditionally grown in states like Rajasthan, Uttar Pradesh, Madhya Pradesh, Gujarat, West Bengal, Assam, Orissa, Bihar, Haryana and Punjab.Now its cultivation is spreading to non-traditional states such as Maharashtra, Andra Pradesh, Tamil Nadu and Karnataka. In Maharashtra the crop is now fairly popular and even in vertisols of Marathwada region, the farmers have obtained yields to the tune of 12-15 Qu./ha.during 1992-93 and 1993-94 in post-rainy season. The area under rapeseed-mustard is increasing in Maharashtra and the crop occupies around 13,000 ha. in the state with production of 5,000 metric tonnes (Oil Seeds Development Board, Govt.of India, 1995). However, the productivity of 380 kg/ha in Maharashtra is far below the national average of 980 kg/ha.

Like any other crop, rapeseed-mustard are subjected to attack by number of fungal, bacterial and viral diseases as well as other biotic anbd abiotic factors. Blight, [Alternaria brassicae (Berk) Sacc.],dark leaf spot, [A.brassicicola) Schw.) Wiltshire.] white rust, [Albugo candida (Lev.) kuntze) downey mildew,[Pernospora parasitica (Pers.)] and powdery mildew,(Erysiphe cruciferarum Opiz.) are considered major diseases while number of root rots, damping off, wilt, leaf spots, mosaic,phyllodyand grey stem etc.are considered minor

diseases (Shaha and Singh,1998). The pathogens are known to cause losses in qualitative and quantitative yield (Vasudeva,1958; Chohan,1978; Chahal and Kang,1979; Kolte,1985). These fungi also reduce the germination and storability of the seed. Further they also cause seed rot, seedling mortality and leaf spot disease to the crops. Previous research work was mostly related with crops and limited work is attempted on seed. Study regarding seed mycoflora and effect of seed treatment with fungicide are not attempted. Present study involved experiments on one of the important oilseed crops viz.,rapeseed mustard cv. Pusa-Bold, Seeta, TN-17, MCN-60 with following objectives

1) To isolate and identify fungi associated with rapeseed mustard by different methods and study of pathogenicity.

2) To study the sensitivity of fungi to different funicides.

3) To study the effect of fungicidal seed treatment on seed mycoflora and storability in important varieties.

4) To study the effect of seed treatment on seed and seedling health, vigour and seedling emergence.

5) To study the persistance of seed dressing fungicides.

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

GENERAL :

Seed borne nature of plant pathogen was recognised more than 200 years ago, when Tillet (1755) reported *Tilletia caries*, the cause of wheat bunt, to be carried by seeds. Since, then a number of reports have been made on various crops. Orton (1931) published a list of seed-borne parasites which subsequently was updated by Noble *et al.*,(1958) and Richardson (1979) for pathogens.

The role of seed-borne fungi in causing diseases of plant has been known for a long time and yet this important field of seed pathology has almost been neglected in all these years. Raychaudary (1973) stated that seed-borne pathogens affect adversly the stand of the crop, build up of inoculum in the field cause disease during the cropping season and market value of the produce is reduced. The work on seed-borne pathogens has increased currently and number of institutes are devoting full time to seed pathology studies like " The Danish Government Institute of Seed Pathology for developing countries, Copenhagen, Denmark".

SEED MYCOFLORA :

Petrie,(1978) examined commercial seed lots of rape and turnip rape in Western Canada.He reported *Alternaria brassicae*,*A. raphani*,*Fusarium roseum*,*Botrytis cinerea*, *Sclerotinia sclerotiarum* and *Albugo candida*.

Winter and Huber, (1978) reported Alternaria brassicae, Fusarium avenaceum and Botrysis cinera from winter rapeseed. Chahal, (1981) reported seed-borne infection of Alternaria brassicae in Indian mustard. Infection gradually eliminated during storage. Discoloured seeds having 84-92 % infection in April were completely free from it when tested in September after storage at room temperature.Sudarmadi and Wallace, (1982) reported the major disease i.e. stem canker of Brassica napus and B. campestris.Gaur and Ahmed, (1983) reported seed mycoflora of four varieties. They listed 19 fungal species. Seed of Brassica juneca (Indian mustard) cv. RL-18, Durgamani, Pant Raj-15 and Varuna-8 were found infected with Aspergillus spp.Humpherson and O'brien,(1984) obtained Alternaria brassicae and A. brassicola in oilseed rape and cabbage.

Tripati and Kaushik, (1984) reported the intensity of Alternaria brassicae with seed from three crops of rapeseed (Brassica campestris) and Mustard (B. junica). He also reported that the population of the pathogen decreased with increasing temperature and storage time.

Brokenshire and Prasanna, (1984) recorded all the major diseases of winter oilseed rape in SE Scotland in 1982-83, Peronospora parasitica was the most common pathogen and occurred in more than 50% of crop Purenopeziza brassicae attained significant level in the spring and continued to develop on top levels and pods because of the conductive cool wet spring. Leaf spotting

caused by Botrytis was also common. Alternaria was recorded at trace level through the vegetative phase of the crop .

Gurmile Singh and Negi, (1984) reported seed mycoflora of some Brassicae varieties and their impact on seed quality. They also reported that Alterneria alternata was the most common among 20 fungi isolated from varieties of Brassica spp. They also observed that due to infection of seed mycoflora there was reduction in germination percentage and oil content.

Vishnuavat et al.,(1985) reported that Alternaria brassicae was more frequently isolated from rapeseed (Cv.DYS-1) with gray to dry gray discolouration. The affected seeds were reduced in seed size and seed weight, viability and seed germination.

White et al.,(1984) tested chemical compound on seed treatments for brassica seedling establishment,seed vigour, phythium interaction with various brassicas, brassica seed treatments against Alternaria and Phoma.

Daebelez et al.,(1986) reported that leaf spot of rape is caused mainly by Alternaria brassicicola and also 20 % losses occured due to this pathogen in the Northern areas of Germany.Asharaf and Chaudhary, (1986) reported effect of Fusarium oxysporum,F. moniliforme (Gibberella fujitiuroi)
and F. Semitectus (F. pallidoroserum) on rapeseed. All 3 spp. reduced the oil content and altered its colour. The oil had a mouldy odour and its refractive index was

increased. The free fatty acid content and saponification value increased and the iodine value decreased. Kolte et al.,(1987) reported that infection by Alternaria brassicae and A. brassicicola reduced 1000 seed weight and seed yield, causing losses of 46.57 % in rape and 35.38 % in mustard.

Satyabrata Maiti and Raoof, (1988) discussed diseases of rapeseed mustard (including Indian mustard) (Brassica Juneca) B. campestris Var. Brown sarson, yellow sarson and toria and taramira (Eruca sativa (E. Vesican) of viral, mycoplasmal, bacterial and fungal origin and gave brief diagonstic symptoms and control measures.

White and Ho, (1988) measured the emergence from Brassica seedlots designated by seed merchants as being of high vigour in sterilized soil and in sterilized soil re-incubated with Pythium sylvaticum. The mean emergence level of 93.9 % in the former compared favourably with the known mean total viability of the seedlots and 97.7%, However, P. sylvaticum reduced emergence to the tune of 16 in untreated seed lots. The majority of seed lots gave more than 75 % emergence but in 3 cases emergence was reduced by 32.5 to 40.0 %. The effect were not related directly to time taken to emergence, nor were the seedlots most affected those with high level of infection. A compound seed treatment containing metalaxyl, Fenpropimorph and Gamma HCH restored high emergence level.

Ansari et al.,(1988) identified A.brassica as the caus al agent of a blight of rape and Indian mustard in Uttar Pradesh, India. The pathogen caused damping off of seedlings as well as blight of the leaves, stems and pods on maturity plants in the field. Lower leaves were attacked first and then the remaining leaves, stems and pods. The pathogen grew and sporulaced well on a wide range of media. Variation in colony characteristics were observed on different solid media. Potato dextrose agar was the best for pathogen growth and sporulation. Morphological characteristics of the pathogen were similar to those previously described for the epecies.

Ashwanikumar et al.,(1989) they isolated 65 fungal spp. from 30 seed samples of mustard. The most of prominent species were Aspergillus flavus, A. fumigatus, A. nelulans, A. niger, A. sydonii, A. tewens and A. Brassicolor. A. flavous isolates screnned for aflatoxin production 61.9% were positive and all seed samples were found to be contaminated with aflatoxinogenic strains.

Shivpuri et al.,(1990) tested eighty—two Indian mustard seed samples from nine agro-climatic zones of Rajasthan, India and 16 fungal species were isolated and effect was studied of these fungi on the quantity and quality of Indian mustard oil. Fusarium oxysporum, Phomalingam (Leplospheria maculans) and P. nebulosa reduced. All the fungi caused an unpleasent odour in oil and changed the oil colour significantly.

Churasia, (1992) isolated 44 species from the seed of three varieties of Indian mustard. Out of them the 12 species occurred frequently on all cultivars (extrenaly borne) and 10 pathogenic species were internally borne. Alterneria padvickii and Periconia saraswati purensis was isolated for the first time from Indian mustard seed.

Rakeshkumar et al.,(1993) isolated 16 fungal and 3 bacteria species on leaf extract agar medium. They also observed that Alternaria spp. were the most abundant, occuring on 50% of the seed.

Karona Vishnuvat and Kolte,(1993) studied oospores of Perenospora parasitica that occurred on the seed surface and in the hypodermis of seed coat tissue in sarson, toria and Indian mustard. P. parasitica was seed transmitted in the non-systematic manner in sarson and toria at rate of 0.9 and 0.4 % respectively but not in Indian mustard. Infected seedling showed downy growth of the fungus on the lower surface of the cotyledon,leaves and upto 2-3 sussessive true leaves. Further growth of such seedlings appreared normal,no hypertrophy of the inflorescence and seed collected from such a plants showed no infection.

SEED TREATMENT

Winter and Huber, (1978) reported that seed treatment with 0.2%, benlate at 50 °C for 25 min. was effective against. Leptospharia maculans, Alternaria brassicae, Fusarium avenaceum, Botrytis cinerea.

Chahal,(1981) reported that Alternaria brassicae in mustard need to be controlled with fungicide sprays on the field to obtain better quality, healtheir seed.

Rana and Tripathi,(1983) tested 19 fungicides,out of them carbendazim, carboxin, thiophanate- methyl, captafol, Mancozeb and Agrasan-GN were effective at all concentrations (0.05, 0.1, 0.15 and 0.2 % a.i.) against Rhizoctonia bataticola (Macrophormina phaseolina) on Brassica juncea. Seed treatment with 11 selected fungicides showed that carbendazim,carboxin and panoram checked the disease completely under glass house conditions. Soil drenches using these fungicides and benomyl were also effective, but panoram was phytotoxic at the higher rate.

Dueck et al.,(1983) reported that a single application of the benomyl and vinclozolin controlled sclerotial stem rot on rapeseed when applied at 25% bloom. The effective control by foliar application of fungicides indicated that most infections were caused by airborn ascospores.

Ogilvy, (1984) reported that Alternaria brassicae in winter oil seed rape (Brassica napus) was reduced due to the seed treatment or sprays of fungicides.

Rawlinson et al.,(1984) tested five fungicides and applied at different times, a single autumn spray of benomyl or prochloroz at 0.5 kg a.i./ha consistently decreased incidence and severity of the principal

disease, light leaf spot (Pyrenopeziza brassicae) and some times decreased stem canker (Leptosphaeria maculans). In Cv. Primor under severe disease conditions as autumn fungicide spray was more effective than a spring one. A decrease in P. brassica was detectable upto 8 months after application. The autumn spray maintained plant population density, increased leaf area index dry matter, crop growth rate, earliness of flowering and yield by upto 0.6 qu/ha an autumn + spring spray increased yield by upto 0.83 t/ha. Effects on growth and yield were due to disease control and not due to chemical stimulation. Fungicide effects were more clearly revealed by crop growth and population measurement than disease assessment based solely and randomly selected plants. Triaclinefon sprayed on rape stubble at high rate (1 kg a.i/ha) decrease incidence and severety of P. brassica through out the growing season of a subsequent rape crop, improving plant population on density, growth, flowering and yield. Electrostatically charged rotary atomizer and conventional hydraulic spray applications were equally effective. A reduction to 1/4 in dose rate of prochloraz and 100 fold reduction in the amount of water carrier (125 g ai in 4.3 lit/ha) when applied electrostatically in autumn or autumn + spring had similar effect on disease crop growth and yield to those obtained with a conventional sprayer delivering 500 g a.i. Prochloraz in 410 lit/ha fungicide, spraying time and methods of

applications are discused in relation to epidemiology of P. brassicae and L. maculans and the economics of disease control.

Randhawa and Aulakh, (1984) reported that seed treatment (of Indian mustard) at 50°C for 20 minutes was highly effective in controlling seed borne fungi including Alternaria brassicae, the major pathogen of cruciferous crops, without any pronounced effect on germinability of the seed.

Gupta et al.,(1985) reported that in field test with 6 fungicides against Alternaria brassicae, Difolaton (captafol) (0.2 %) was highly effective when 4 sprays were given at 10 days interval.

White et al.,(1984) reported studies of testing compounds seed treatments for brassicae seedling establishment, seed vigour-Pythium interaction with various brassicas, brassica seed treatment against Septoria apicola, infection tests on rapeseed to detect Alternaria brassicae and Leptosphaeria maculans, pea seed treatment to control Ascochyta pisi and thiram soaking to control Phoma (Pleospora) betae on red beet.

Mirdha and Safa,(1985) isolated seed-borne fungus Alternaria brassicae from 97.6 % of 84 mustard (Brassica campestris) seed samples and it was associated with 9.5% of the total seed studied, 6.41% of infected seed failed to germinate and 73.4% of seedlings died after germination. Association of A. brassicae with surface sterilized intact seeds and seed coats and its

complete absence from dehusked grains indicated that most infections were confined within seed coats and fungus is seed-borne. A. brassicae associated with mustard seed was controlled by Vitavax-200 (Carboxin), Granosam M and Benlate (benomyl).

Chahal, (1986) estimated yield losses due to Alternaria brassicae as 43.62% in Brassica compestris and 38.36% in Indian mustard, four sprays of Bacor, Difolatan,Blitox, Dacoril or Dithane M-45 controlled the disease and resulted in significant yield increase. The best cost/benifit ratio was obtained with copper oxychloride applied four times starting when the crop was 75 days old.

Kumudkumar and Singh, (1986) tested 9 fungicides against Alternaria brassicae infection in mustard and rapeseed. They concluded that Bavistin (Carbendazim), Difolatan (Captafol) (each at 1.5g/kg seed) and Dithane M-45 (Mancozeb) (2g) eradicated the pathogen. Atleast 24 hours storage after treatment was needed for effective control with Thiram. Soaking was more efficient then slurry treatment.

Saha,(1986) tested 10 fungicides against Rhizoctonia solani infection in RM.He found Bavistin (carbendazim),Brassicol (quintozene),Vitavax (carboxin), Ziram and Caresan wet (Methoxyethyl mercury chloride) performed best followed by Dithane Z-78 (zineb) and Dithane M-45 (mancozeb).

Kumudkumar and Singh,(1986) tested 10 fungicides for seed treatment on seed germination, shoot and root length development and seedling and seed rot. All were effective than control,but Difolatan (captafol),Vitavax (carboxin),Dithane M-45 (mancozeb) and Bavistin (carbendazim) performed best. Difolatan completely controlled seed rot. They also reported species of Fusarium, Rhizoctonia and Alternaria (including A. brassicae and A. brassicicola).

Tripathi et al.,(1987) found that Alterneria brassicae of rape and mustard can be controlled with 4 sprays of captafol followed by mancozeb 30 days after sowing at 15 days interval was the best combination for maintaining a disease free crop.

Satyabarta Maiti and Raoof,(1988) reported disease of rapeseed mustard (including Indian mustard) (Brassicae juneca), B. compestris var. Brown sarson, yellow sarson and toria and taramira [Eruca sativa (E. vasican) of viral, mycoplasmal, bacterial and fungal origin, including brief diagnostic symptoms and control measures.

White and Ho, (1988) calculated emergence from Brassica seed in sterilized soil and in sterilized soil reincubated with Phythium sylvatium. The mean emergence level of 93.9 % in the former compared favourably with the known mean total viability of the seed lots 97.7 %.

Saha,(1989) reported in vitro growth of Alterneria brassicae and A. brasicicola isolated from the rape and

Indian mustard which was reduced by each of 10 fungicides tested. Ziram and Ceresan wet (Phenylmercury acetate) completely inhibited growth. The second greatest growth reduction occurred when Alterneria brassicae was treated with Dithine M-45 (Mancozeb) or Difolatan (Captafol) and when A.brassicicola was treated with Dithine Z-78 (Zineb) or Mancozeb. In all cases increasing the concentration of fungicide increased growth inhibition.

Kamat and Rajendra Prasad, (1989) described the major fungal and bacterial diseases affecting rapeseed and Indian mustard in India and gave details of their incidences life cycles and currently available control measures.

Ansari and Khan, (1990) evaluated in vitro 18 fungicides for their effect on the growth of Alterneria brassicae and then for their efficacy as seed treatment and foliar applications in the management of damping off of seedling and blight of rape. In vitro Dithane M-45, Dithane Z-78, Ziram, Difolatan-80, Blitox-50 and Benlate completely inhibited growth of the pathogen and were fungicidal in action. Thiram and Brestan-60, which also caused total growth inhibition, were however, fungistatic, Benlate (0.1%) followed by Dithane M-45 was the best seed dressing for controlling damping off of seedling. Dithane M-45 (0.2%) followed by Dithane Z-78 as a foliar spray was most effective for controlling blight and increasing the yield in field trials.

Kharbanda, (1992) evaluated several fungicides in the laboratory growth chamber and field as seed treatment and foliar sprays for their protective and curative action against black leg for rape (Brassica napus and Brassica campestris) caused by Leptosphaeria maculans, seed treatment with Benomyl, Carboxin + Thiram, Iprodine with Benomyl, Thiabendazole and Tolclofos-Methyl) significantly suppressed seed-borne L. maculans in agar plates. In the growth chamber Iprodine and Prochloraz seed treatment effectively protected seedling from infection upto 21 days after seedlings. In the field test however, non of this seed treatment prevented infection in seedling artifically inoculated with the pathogen 15 days after seedlings Iprodine or Prochloraz, sprayed once either four days before foliage inoculations or four days after stem inoculations with L. maculans in a growth chamber test, controlled the diseases significantly.

Rakesh Shah and Jain, (1993) isolated 16 fungi from samples of the Indian mustard seed, using agar plate and bottle test, the former giving the better results. Reduction in germination was greatest in seed inoculated with Aspergillus flavus while Petnlla setifela caused the most emergence rot. Of 12 fungicides tested as seed dressing on inoculated seed, Carboxin gave the best control of all the seed-borne fungi and highest germination while Zineb was best for checking post emergence rot.

Materials and Methods

CHAPTER III

MATERIALS AND METHOD

3.1 Isolation :

The seed samples of rapeseed (Brassica campestris Linn.) and mustard (Brassica juncea Linn.) Cv. Pusa-Bold, Seeta, TN-17 and MCN-60 were collected from Department of Agronomy, M.A.U.,Parbhani.

An attempt was made to pick up those seeds from the mixture which appeared unhealthy. International rules for seed testing (ISTA)* were followed throughout the studies. Four methods viz. Agar plate (Musket,1948), Blotter paper (De.Tempe, 1955),Moist sand (Suryanarayana and Bhombe, 1961) and Rolled towel (Deshkar and Khare, 1975) were followed for isolation of seed-borne fungi.

To isolate external and internal seed mycoflora associated with the samples of rapeseed mustard, four hundred seed of each variety were selected at random and tested for mycoflora as given below.

3.1.1 Agar Plate Method :

For isolation of internal seed-borne fungi two hundred seeds of each variety viz. Pusa-Bold, Seeta, TN-17 and MCN-60 were selected and surface sterilized by dipping in 0.1 per cent mercuric chloride solution for 1 to 2 minutes, then they were washed in three changes of sterile water and then seeds were placed in petridish containing potato dextrose agar (PDA). Similarly for

isolation of external seed-borne fungi two hundred seeds of each variety i.e. Pusa-Bold, Seeta, TN-17 and MCN-60 was directly placed in petridishes containing sterile potato dextrose agar media.All petridises were incubated at 28 °C ± 1 °C. Petridishes were exposed for 12 hours to ultraviolate light and 12 hours to dark. Observation were recorded for the growth of different mycoflora with the help of stereobinocular microscope after 7 days, and mycelial fragments were transfered to potato dextrose agar slants for further studies.

3.2.2 Blotter Paper Method :

Three layers of blotter paper, size equivalent of petridish were soaked in sterile water and kept in the petridish. Two hundred seeds of each variety i.e. Pusa-Bold, Seeta, TN-17 and MCN-60 were selected for isolation of internal seed-borne fungi. They were surface sterilized by dipping in 0.1 per cent mercuric chloride solution for 1 to 2 minutes and washed in three changes in sterile water. Then twentyfive seeds were placed equidistantly on three layers of moist blotter paper in petridish. External seed-borne fungi were isolated by placing two hundred seeds of each variety directly on the three layers of moist blotter paper in petridish. All petridishes were incubated at 28 °C ± 1 °C and exposed to ultraviolet light and dark as described in earlier method. Then seeds and seedlings were

examined after seven days with stereobinocular microscope, the percentage of individual seed mycoflora was recorded and mycelial fragments were transfered to potato dextrose agar slants for further studies as and when required, sterilied distilled water was added to moisten the blotter paper.

3.2.3 Rolled Towel Method :

A lot of two hundred seeds of each variety was surface sterilized by dipping in 0.1 per cent mercuric chloride solution for 1 to 2 minutes then they were washed in three changes of sterile water. One hundred seeds were placed on moist towel paper and covered with polythene paper and rolled carefully avoiding disturbance of the seeds from their place. A second lot of two hundred seeds of each variety was directly placed on moist towel paper and covered with polythene paper and rolled carefully. The rolled towel paper were kept in slanting position along the wall on laboratory tables and incubated at 28 °C ± 1 °C. After 7 days, the seeds and seedlings were examined with stereobinocular microscope, the percentage of individual seed mycoflora was recorded and mycelial fragments were transfered to PDA slants for further studies.

3.2.4 Moist Sand Method :

For the isolation of internal seed-borne fungi two hundred seeds of each variety were surface sterilized by

dipping in 0.1 per cent mercuric chloride solution for 1 to 2 minute and washed in three changes of sterile water. Then one hundred seeds were placed in big glass petriplate containing sterilized moist sand. Similarly for the isolation of external seed-borne fungi two hundred seeds of each variety were directly placed in big petriplate containing sterilized moist sand. Then these petriplates were incubated at 28 ^{o}C ± 1 ^{o}C temperature in a chamber specially designed with arrangement of 12/12 hour light and darkness cycle. Four hundred seeds were examined and observation were recorded after 7 days for the development of fungi on seeds. As and when required, sterilized distilled water was added to moisten sand. The result of which are given in Table No.1 to 4.

3.3 Pathogenicity :

All cultures were brought into pure form following hypal tip method and grown on PDA slants.One hundred apparently healthy seeds of four varieties viz.Pusa-Bold, Seeta, TN-17 and MCN-60 were surface sterilized in 0.1 per cent mercuric chloride solution for 1.5 to 2 minutes and then washed in three changes of sterile water. All seeds were rolled on actively sporulating cultures and were placed on moist blotter paper.The material was incubated at 28 ^{o}C ± 1 ^{o}C and exposed to 12 hours dark and 12 hours light. Observations were recorded after 7 days for normal and abnormal seedling and were continued upto

15 days. The culture of the fungus was identical with original culture. Reisolation was made and same cultures were used throughtout the course of experimentation.

3.4 Evaluation of efficacy of different fungicides :

For evaluation of efficacy of six fungicides viz.Thiram,Carbendazim (Bavistin),Thiram + Carbendazim, Mancozeb (Dithane M-45),Captan, Captafol were used. Five hundred grams healthy seeds of each variety i.e. Pusa-Bold, Seeta, TN-17 and MCN-60 was selected and divided into seven lots. Six lots were treated by dry seed treatment with Thiram, Carbendazim, Thiram + Carbendazim, Mancozeb, Captan, Captafol. Seventh lot was kept as untreated control. Then these seed samples were stored in cloth bags at room temperature (24 ^{o}C to 35 ^{o}C) in the laboratory.These seed samples were tested further for seed mycoflora, seed germination, seedling vigour index* keeping an interval of 30 days by following blotter paper method, moist sand method and rolled towel method upto 180 days.

! * Vigour Index = Germination % x (Root + Shoot length in cm)!

3.5 Sensitivity of <u>Fusarium moniliforme</u> :

Six fungicides viz. Thiram, Carbendazim, Thiram + Carbendazim, Mancozeb, Captan, Captafol at the rate of 3 gm/lit of water were used for this study. The fungicides were placed in conical flasks containing warm PDA, shaked throughly and poured in sterilized petridishes

(20 ml per petridish). The seeds of each variety was rolled on actively growing culture of Fusarium moniliforme and placed in petridishes containing PDA with fungicides. The effect of each fungicide was observed. Colony diameter was measured after inoculation of plates for nearly 48 hours at 28 °C ± 1 °C in the dark. Inhibition percentage was calculated by following formula

$$\text{Inhibition } \% = \frac{\text{Diameter of colony of test pathogen in control} - \text{Diameter of test pathogen in seed treatment}}{\text{Diameter of colony of test pathogen in control}} \times 100$$

Table 3 A : Details of fungicides used in the study.

Common name	Trade product used	Chemical name	Manufactured by
Thiram	Hexathir	Tetramethylthiuram disulfide or bis (dimethyl-thio carbamoyl) disulphide.	Bharat Pulvarsi Mills Pvt.Ltd. Bombay.
Carbendazim	Bavistin	Methyl 1H-benzimidazole - 2 - y 1 carbamate	BASF India Ltd. Bombay.
Mancozeb	Dithane M-45	Manganese ethylen - ebisdithiocarbamate plus Zinc	Indofil Chemica Company,Bombay.
Captan	Orthocide	N-(trichloromethyl thio)- 4- Cyclohexene -1,2- dicarboximide	Rallis India Lt Bombay.
Captafol	Difolatan	Cis-N-(1,1,2,2-tetra chloroethylthio)-4- cyclohexene-1,2-dicarboximide	Rallis India Lt Bombay.

Table 3 B : The fungicidal seed treatment rapeseed mustard seed.

Crop	Fungicides/ treatments	Rate of application to seeds
		Method of application - Dry
Rapeseed Mustard	Thiram	3 gm/kg seed
	Carbendazim	3 gm/kg seed
	Thiram + Carbendazim	3 gm/kg seed
	Mancozeb	3 gm/kg seed
	Captan	3 gm/kg seed
	Captafol	3 gm/kg seed

Results

CHAPTER IV

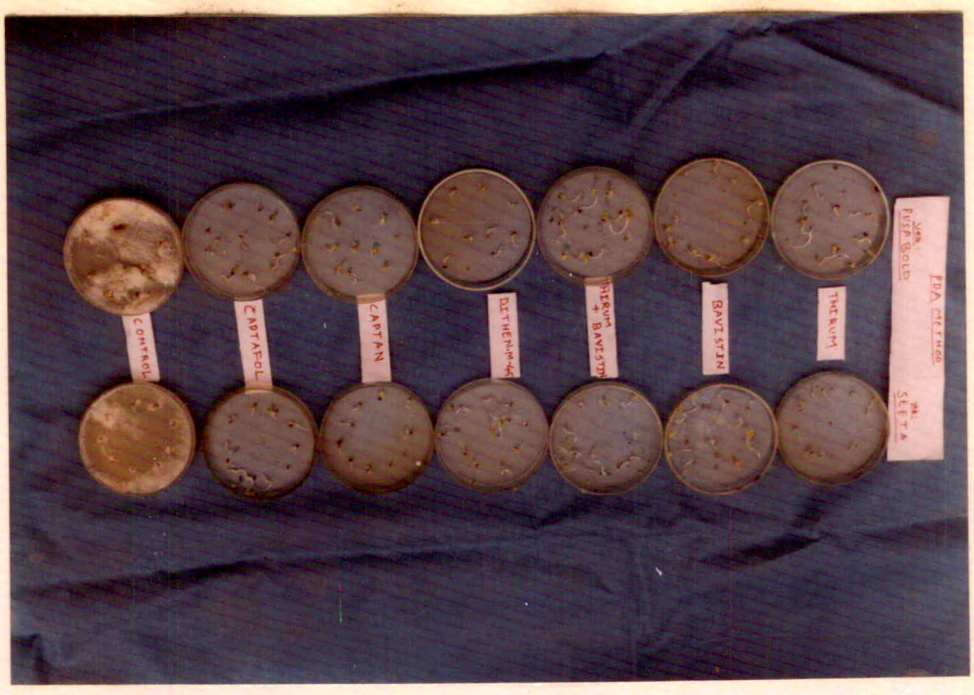
RESULTS

4.1.1 Isolation of mycoflora from rapeseed-mustard seeds (*Brassica juncea* (Zein and Loss):

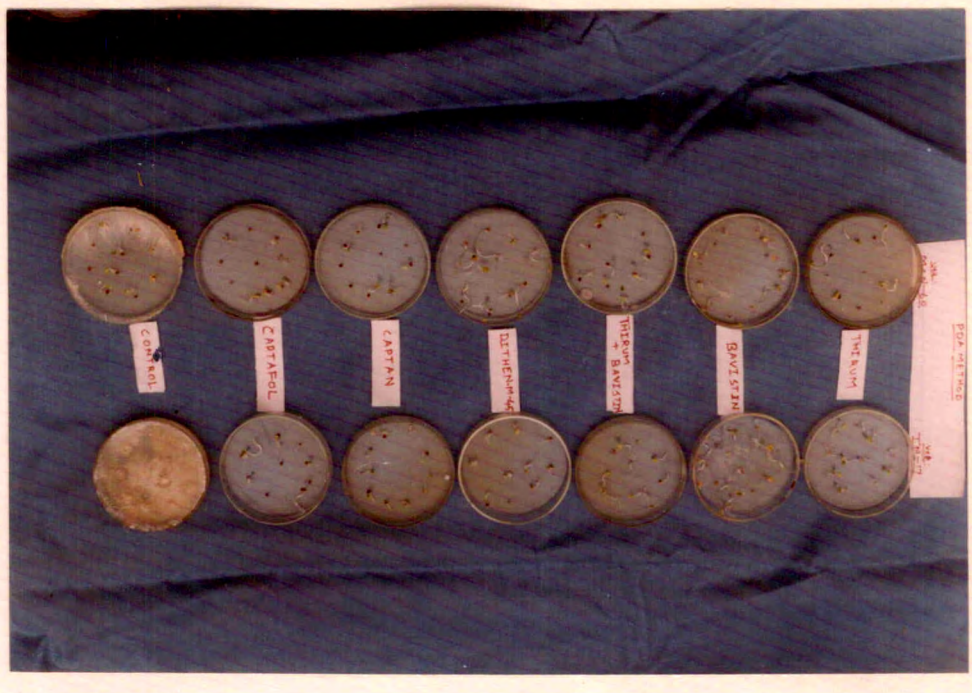
The results given in table 1 to 4 indicate that the fungi isolated by agar plate, blotter paper,rolled towel and moist sand (Fig.1) were *Fusarium moniliforme*, *Aspergillus flavus*, *Macrophomina phaseolina*, *Aspergillus niger*, *Alternaria alternata* and *Curvularia lunata* from rapeseed -mustard Cv.Pusa-Bold, Seeta, TN-17 and MCN-60 seeds.

All the methods yielded more or less the same set of seed mycoflora. The result in Table 1 to 4 indicate that the seed mycoflora per centage was maximum in unsterilized seeds than sterilized seeds in all the cultivars.It was also observed that seed mycoflora per centage was maximum in agar plate method as compared to blotter paper, rolled towel paper and moist sand methods in sterilized and unsterilized seeds in Pusa-Bold, Seeta,TN-17,MCN-60. The total seed mycoflora was found less in all the methods in Pusa-Bold as compared to Seeta, TN-17,MCN-60. The fungi *Fusarium moniliforme*, *Macrophomina phaseolina*, *Aspergillus niger* and *Alternaria alternata* were more prominant than *Aspergillus flavus* and *Curvularia lunata* in all the methods in all the cultivars.

In general it was observed that agar plate method is superior than all other methods in yielding more number of fungi from the rapeseed-mustard.

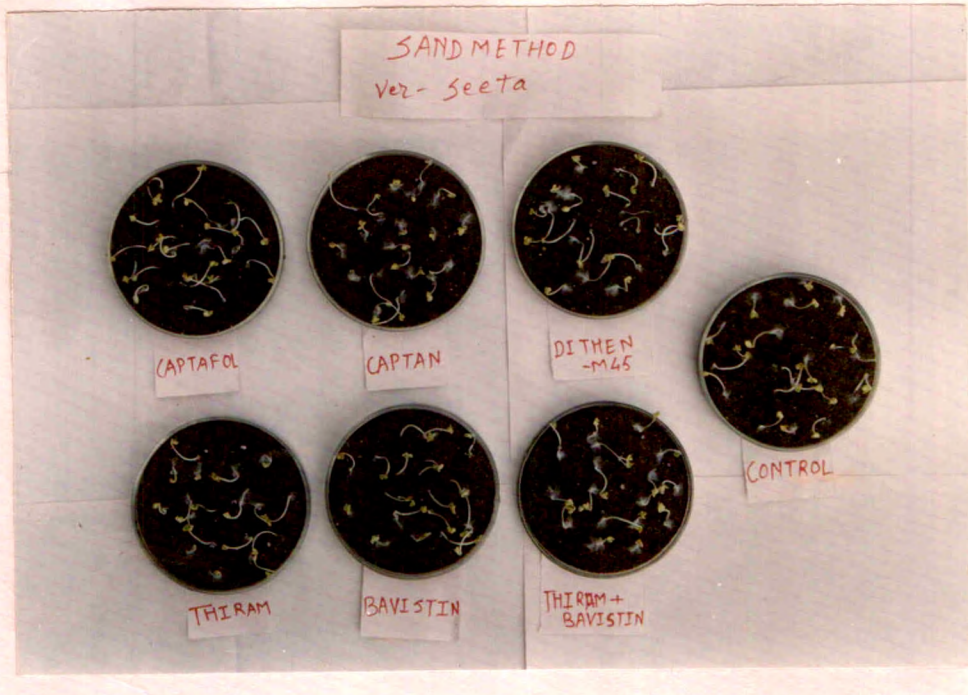


Fungi isolated with rapeseed mustard Cv. Pusa bold and Seeta in agar plate method.

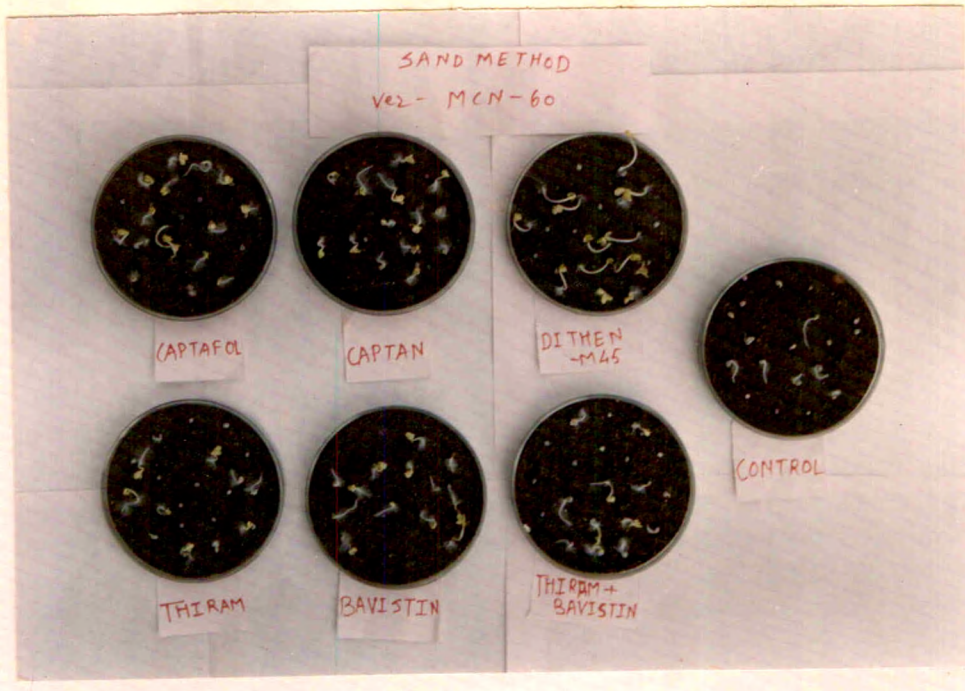


Fungi isolated with rapeseed mustard Cv. MCN-60 and TN-17 in agar plate method.

Fungi isolated with rapeseed mustard Cv. Pusa bold in sand method.



Fungi isolated with rapeseed mustard Cv. Seeta in sand method.



Fungi isolated with rapeseed mustard Cv. MCN-60 in sand method.

Fungi isolated with rapeseed mustard Cv. TN-17 bold in sand method.

Table 1 : Percentage of mycoflora isolated from sterilized and unsteriled rapeseed mustard seeds Cv. Pusa-Bold by various methods.

Sr. No.	Fungi isolated	Agar Plate		Blotter Paper		Rolled Towel		Moist Sand	
		S S	U S S	S S	U S S	S S	U S S	S S	U S S
1.	Fusarium moniliforme	5.33	7.67	4.67	6.33	4.00	6.00	2.33	5.00
2.	Asperigillus flavus	4.67	6.00	3.33	5.00	5.00	7.33	3.00	5.00
3.	Macrophomina phaseolina	5.00	7.67	4.00	6.00	5.00	6.67	4.00	6.33
4.	Asperigillus niger	5.33	6.67	4.67	6.00	3.67	6.33	3.00	5.33
5.	Alterneria alternata	6.67	7.67	5.67	7.67	4.00	6.00	4.00	4.67
6.	Curvularia lunata	4.00	6.00	3.00	5.67	2.33	3.33	3.00	4.67
	S.E.±	0.46	0.46	0.59	0.62	0.48	0.65	0.54	0.47
	C.D.± (P = 0.05)	1.45	1.44	1.87	1.96	1.52	2.04	1.71	1.49

Note : S S - Sterilized seed, U S S - Unsterilized seed

Table 2 : Percentage of mycoflora isolated from sterilized and unsteriled rapeseed mustard seeds Cv.Seeta by various methods.

Sr. No.	Fungi isolated	Agar Plate		Blotter Paper		Rolled Towel		Moist Sand	
		S S	U S S	S S	U S S	S S	U S S	S S	U S S
1.	Fusarium moniliforme	7.33	8.67	5.00	7.00	5.67	7.64	6.00	7.67
2.	Asperigillus flavus	6.00	8.00	4.33	6.67	5.00	6.67	5.00	6.67
3.	Macrophomina phaseolina	7.33	9.33	5.00	6.67	5.33	7.00	4.00	5.67
4.	Asperigillus niger	6.00	7.33	5.67	7.00	5.00	7.00	4.33	6.33
5.	Alterneria alternata	7.00	9.00	6.67	7.67	5.33	7.00	5.67	7.67
6.	Curvularia lunata	5.67	7.67	4.00	6.67	5.00	6.67	3.33	5.67
	S.E.±	0.55	0.68	0.33	0.54	0.35	0.57	0.50	0.57
	C.D.± (P = 0.05)	1.75	2.02	1.03	1.75	1.74	1.79	1.58	1.80

Note : S S - Sterilized seed, U S S - Unsterilized seed

Table 3 : Percentage of mycoflora isolated from sterilized and unsteriled rapeseed mustard seeds Cv. MCN-60 by various methods.

Sr. No.	Fungi isolated	Agar Plate		Blotter Paper		Rolled Towel		Moist Sand	
		S S	U S S	S S	U S S	S S	U S S	S S	U S S
1.	Fusarium moniliforme	7.00	8.33	6.33	7.33	6.00	7.67	5.33	7.00
2.	Asperigillus flavus	6.00	7.67	6.00	7.67	5.00	7.00	4.00	6.00
3.	Macrophomina phaseolina	5.67	7.00	5.00	7.00	5.67	7.33	5.33	6.67
4.	Asperigillus niger	6.33	7.33	5.00	6.67	4.00	6.00	4.67	6.67
5.	Alterneria alternata	7.00	8.33	4.00	6.00	5.33	7.00	3.67	5.67
6.	Curvularia lunata	6.67	7.67	3.66	6.00	5.00	7.00	4.33	6.33
	S.E.±	0.61	0.42	0.59	0.64	0.66	0.49	0.69	0.59
	C.D.± (P = 0.05)	1.93	1.31	1.85	2.09	2.07	1.56	2.19	1.87

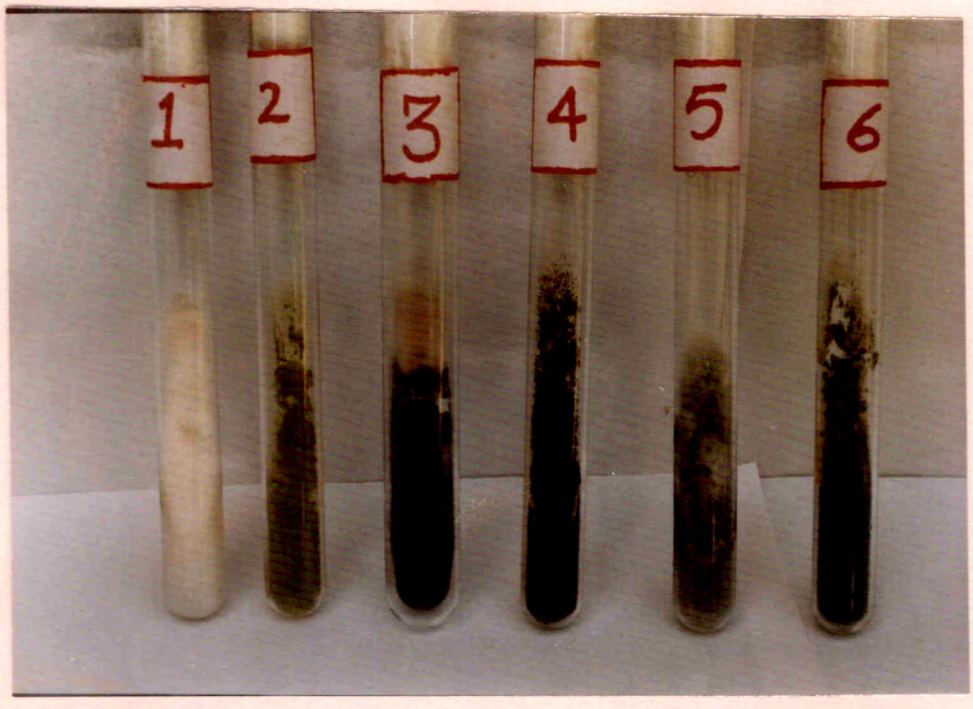
Note : S S - Sterilized seed, U S S - Unsterilized seed

Table 4 : Percentage of mycoflora isolated from sterilized and unsteriled rapeseed mustard seeds Cv.TN-17 by various methods.

Sr. No.	Fungi isolated	Agar Plate		Blotter Paper		Rolled Towel		Moist Sand	
		S S	U S S	S S	U S S	S S	U S S	S S	U S S
1.	Fusarium moniliforme	6.33	7.67	5.33	7.33	4.00	5.67	2.33	5.00
2.	Asperigillus flavus	5.33	7.33	4.33	6.00	5.00	7.00	3.33	5.00
3.	Macrophomina phaseolina	5.33	7.00	5.00	6.67	5.00	5.67	4.00	6.00
4.	Asperigillus niger	6.00	7.33	5.67	7.67	3.67	5.33	3.00	5.33
5.	Alterneria alternata	6.33	8.00	6.33	7.33	4.00	6.00	3.00	5.67
6.	Curvularia lunata	4.33	5.67	3.33	5.67	2.33	5.00	4.00	6.00
	S.E.±	0.54	0.38	0.58	0.44	0.53	0.47	0.55	0.71
	C.D.± (P = 0.05)	1.72	1.98	1.82	1.40	1.66	1.55	1.75	2.23

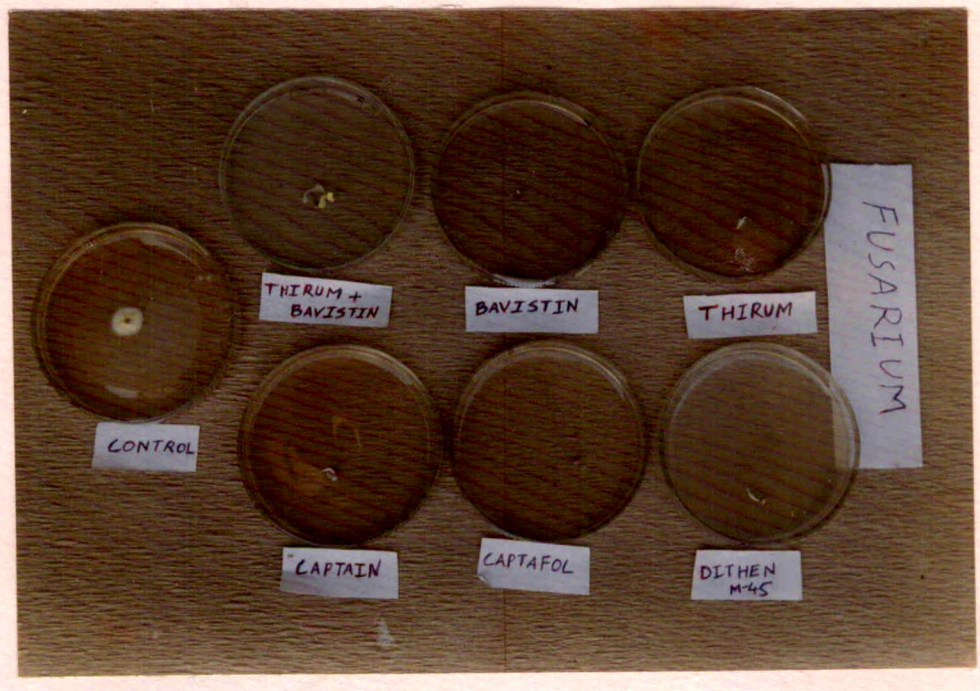
Note : S S - Sterilized seed, U S S - Unsterilized seed

Major fungi isolated from rapeseed mustard Cv. Pusa bold, Seeta, MCN-60 and TN-17.



1. *Fusarium moniliforme* Sheldon (Fig.1)
2. *Aspergillus flavus* (Link) ex.Fries (Fig.2)
3. *Macrophomina phaseolina* (Tassi) Goid (Fig.3)
4. *Aspergillus niger* Van Tiegh (Fig.4)
5. *Alternaria alternata* (Fr.) Keissler (Fig.5)
6. *Curvularia lunata* (Fig.6)

Response of rapeseed mustard Cv. Pusa bold Seeta, MCN-60 and TN-17 with *Fusarium moniliforme* Sheldon, inoculation to seed for germination and mortality.



Growth inhebition of Fusarium moniliforme Sheldon, inoculation to rapeseed mustard Cv. Pusa bold in media containing 3 gm of fungicide per litera.

Table 5 : Response of rapeseed mustard to *Fusarium moniliforme* inoculation and seed treatment with fungicides.

Sr. No.	Fungicide	Variety	Germination % * (15 DAP) **	Mortality % (15 DAP)	
				Pre	Post
1.	Thiram (2.5 g/kg)	Pusa-Bold	80	14	6
		Seeta	78	17	5
		MCN-60	78	16	6
		TN-17	77	18	5
2.	Carbandazim (2.5 g/kg)	Pusa-Bold	71	20	9
		Seeta	70	20	10
		MCN-60	69	22	9
		TN-17	69	20	11
3.	Thiram + Carbandazim (2.5 g/kg)	Pusa-Bold	81	12	7
		Seeta	80	14	6
		MCN-60	78	18	4
		TN-17	79	20	9
4.	Dithane M-45 (2.5 g/kg)	Pusa-Bold	77	14	9
		Seeta	76	19	5
		MCN-60	75	18	7
		TN-17	73	17	10
5.	Captan (2.5 g/kg)	Pusa-Bold	76	14	10
		Seeta	75	16	9
		MCN-60	73	14	13
		TN-17	71	19	10
6.	Captafol (2.5 g/kg)	Pusa-Bold	74	20	6
		Seeta	73	18	9
		MCN-60	72	17	11
		TN-17	71	22	7
7.	Control (Untreated)	Pusa-Bold	64	24	12
		Seeta	62	23	15
		MCN-60	60	25	15
		TN-17	60	26	14

* Based on four hundred seeds in each treatment.

** DAP = Days after planting.

The identification of fungi isolated from rapeseed-mustard cv.Pusa-Bold,Seeta,TN-17,MCN-60 seeds were done on the basis of cultural and morphological characters which was also confirmed by senior Mycologist,I.A.R.I., New Dehli - 110012. The list of fungi is given below:

1. Fusarium moniliforme Sheldon (Fig.1)
2. Aspergillus flavus (Link) ex.Fries (Fig.2)
3. Macrophomina phaseolina (Tassi) Goid (Fig.3)
4. Aspergillus niger Van Tiegh (Fig.4)
5. Alternaria alternata (Fr.) Keissler (Fig.5)
6. Curvularia lunata (Fig.6)

4.2.1 Interactions between fungicides and cultivars under Fusarium moniliforme inoculation condition in rapeseed-mustard.

The seeds of rapeseed mustard cv.Pusa-Bold, Seeta, TN-17,MCN-60 were treated with seed dressing fungicides (Thiram,Carbendazim, Thiram + Carbendazim, Mancozeb,Captan,Captafol) keeping untreated control. Then these treated and untreated seeds were rolled on actively growing culture of Fusarium moniliforme and sown on sterilized agar media plate. The result given in Table 5 indicated that all chemical improved the germination and reduced the mortality in rapeseed mustard cv.Pusa-Bold, Seeta, TN-17 and MCN-60. Thiram and Thiram + Carbendazim improved maximum germination percentage than other fungicides and less pre and post emergence mortality.

4.3.1 Effect of fungicidal seed treatment during the storage period on germination, vigour index and seed mycoflora in rapeseed mustard.

The seeds of rapeseed mustard Cv. Pusa-Bold, Seeta, TN-17 and MCN-60 were used for study. The first lot of seed of each cultivar was treated with six seed dressing fungicides viz. Thiram, Carbendazim, Thiram + Carbendazim, Mancozeb, Captan, Captafol at the rate of 3.0 g/kg seed as a dry seed treatment. The second lot of the seeds of each cultivar was kept as untreated control. These lots were stored under ambient conditions for 180 days in cloth bags. The moisture percentage was 9 at the time of storage. The seed samples of each cultivar were tested for germination, vigour index and seed mycoflora at monthly intervals by blotter paper method, rolled towel method and moist sand method.

The data in Table No. 6 to 8 indicate that there was significant reduction in germination percentage, vigour index and increase in seed mycoflora after 6 months of storage in Pusa-Bold in blotter paper, rolled towel and moist sand methods.

In blotter paper method the germination percentage was decreased from 88.2 to 79.5, vigour index was also decreased from 821 to 729 and seed mycoflora was increased from 3.82 to 5.05.

In rolled towel method the germination and vigour index were decreased from 90.5 to 78.9 and 850 to 828

respectively and seed mycoflora was increased from 3.65 to 5.10.

In moist sand method germination and vigour index were decreased from 91.2 to 80.4 and 1026 to 904 respectively and seed mycoflora was increased from 3.55 to 4.97 in 6 months storage period.

It maintained certifications standards of germination (75%) upto 180 days in all the methods.

It was concluded that the germination percentage and vigour index was maximum and less seed mycoflora in moist sand method as compaired to blotter and rolled towel method in Pusa-Bold cultivar.

4.3.2 Effect of individual fungicide on germination, vigour index and seed mycoflora.

The seed germination percentage and vigour index were significantly superior in Thiram + Carbendazim and Thiram than other fungicidal seed treatment and untreated control in blotter paper, rolled towel and moist sand method and also seed mycoflora was significantly less in the same fungicidal seed treatment in all the methods. All the fungicidal seed treatments increase the germination percentage, vigour index and decreased seed mycoflora than untreated control in all the methods (Table 6 to 8).

Table 6 : Effect of fungicidal seed treatment during the storage period on Germination, Vigour Index and Seed Mycoflora in mustard Cv. Pusa-Bold by Blotter paper method.

Sr. No.	Period in days (P)	Germination %	Vigour Index	Seed Mycoflora
F1.	0	88.2	821	3.82
	30	88.0	808	3.94
	60	86.4	792	4.22
	90	84.7	777	4.46
	120	82.8	759	4.70
	150	81.1	744	4.87
	180	79.5	729	5.05
	S.E.±	0.55	2.25	0.01
	C.D.±	1.54	6.25	0.04
F2.	Fungicide (F)			
	Thiram	89.3	819	3.87
	Carbandazim	78.8	723	5.14
	Thiram + Carbendazim	89.8	835	3.61
	Dithane M-45	86.9	796	4.20
	Captan	85.4	783	4.42
	Captafol	83.9	770	4.60
	Control	76.9	705	4.07
	S.E.±	0.55	2.25	0.01
	C.D.±	1.54	6.25	0.04
	Intraction			
F1 x F2	P x F			
	S.E.±	1.47	5.96	0.04
	C.D.± (P=0.05)	4.07 (NS)	16.53 (NS)	0.1 (NS)

Note : P = Period, F= Fungicide,

Table 7 : Effect of fungicidal seed treatment during the storage period on Germination, Vigour Index and Seed Mycoflora in mustard Cv. Pusa-Bold by Rolled Towel method.

Sr. No.	Period in days (P)	Germination %	Vigour Index	Seed Mycoflora
F1.	0	90.5	950	3.65
	30	89.1	936	3.88
	60	87.6	920	4.05
	90	85.7	900	4.35
	120	83.6	881	4.62
	150	82.0	861	4.78
	180	78.9	820	5.10
	S.E.±	0.38	4.76	0.04
	C.D.±	1.06	13.19	0.11
F2.	Fungicide (F)			
	Thiram	89.8	944	3.76
	Carbandazim	80.8	849	4.92
	Thiram + Carbendazim	91.1	957	3.59
	Dithane M-45	87.8	922	4.09
	Captan	86.8	907	4.23
	Captafol	84.8	891	4.46
	Control	76.4	802	5.35
	S.E.±	0.38	4.76	0.04
	C.D.±	1.06	13.19	0.11
	Intraction			
F1 x F2	P x F S.E.±	1.01	12.59	0.10
	C.D.± (P=0.05)	2.80 (NS)	34.89 (NS)	0.28

Note : P = Period, F= Fungicide,

Table 8 : Effect of fungicidal seed treatment during the storage period on Germination, Vigour Index and Seed Mycoflora in mustard Cv. Pusa-Bold by Moist Sand method.

Sr. No.	Period in days (P)	Germination %	Vigour Index	Seed Mycoflora
F1.	0	91.2	1026	3.55
	30	89.7	1010	3.78
	60	88.9	995	4.00
	90	86.3	971	4.27
	120	84.3	949	4.53
	150	82.3	928	4.77
	180	80.4	904	4.97
	S.E.±	0.21	2.53	0.03
	C.D.±	0.59	7.02	0.08
F2.	Fungicide (F)			
	Thiram	90.3	1016	3.72
	Carbandazim	82.1	927	4.79
	Thiram + Carb@ndazim	91.6	1033	3.51
	Dithane M-45	88.1	992	4.04
	Captan	87.0	979	4.20
	Captafol	85.3	961	4.21
	Control	78.0	878	5.20
	S.E.±	0.21	2.53	0.03
	C.D.±	0.59	7.02	0.08
	Intraction			
F1 x F2	P x F S.E.±	0.57	6.69	0.07
	C.D.± (P=0.05)	1.57 (NS)	18.56 (NS)	0.20

Note : P = Period, F= Fungicide,

4.3.3 Seed mycoflora as influenced by period into fungicides in Pusa-Bold in rolled towel method and sand method.

In storage period stuides for 6 months in Pusa-Bold germination and vigour index was non-significant in the interaction between period into fungicides in rolled towel and moist sand method. But seed mycoflora was significantly superior in the interaction between period into fungicides in rolled towel and moist sand (Table 9 and 10). The seed mycoflora was less in Thiram + Carbandazim and Thiram than other fungicidal seed treatment in rolled towel and sand method.

Table 9 : Seed mycoflora influenced by period into fungicides in Cv. Pusa-Bold by Rolled Towel method.

Treatment --> / Period !	Thiram	Carbandazim	Thiram + Carbandazim	Dithane M-45	Captan	Captafol	Control
0	3.0	4.45	2.80	3.30	3.40	3.80	4.80
30	3.30	4.60	3.05	3.55	3.75	3.95	4.80
60	3.40	4.85	3.15	3.70	3.95	4.25	5.10
90	3.80	4.95	3.55	4.20	4.20	4.50	5.30
120	4.05	5.15	3.95	4.45	4.55	4.65	5.55
150	4.20	5.15	4.15	4.60	4.80	4.95	5.65
180	4.60	5.35	4.50	4.85	5.0	5.15	6.30

S.E. $\pm$ - 0.10

C.D. $\pm$ - 0.28
(P = 0.05)

Table 10 : Seed mycoflora influenced by period into fungicides in Cv. Pusa-Bold by Moist Sand method.

Treatment --> / Period !	Thiram	Carbandazim	Thiram + Carbandazim	Dithane M-45	Captan	Captafol	Control
0	3.05	4.25	2.65	3.15	3.30	3.65	4.80
30	3.10	4.40	2.95	3.55	3.70	3.95	4.85
60	3.50	4.65	3.10	3.70	3.90	4.20	4.95
90	3.60	4.80	3.55	4.05	4.30	4.50	5.15
120	4.02	5.00	3.85	4.40	4.45	4.70	5.30
150	4.35	5.15	4.15	4.60	4.75	4.90	5.55
180	4.45	5.30	4.35	4.85	4.95	5.05	5.85

S.E. $\pm$ - 0.07

C.D. $\pm$ - 0.20
(P = 0.05)

4.3.4 Effect of fungicidal seed treatment during the storage period on germination, vigour index and seed mycoflora in rapeseed mustard Cv. Seeta.

The data in Table No.11 to 13 indicated that there was significant reduction in germination percentage from 88.6 to 77.3 in blotter paper, 89.4 to 79.1 in rolled towle paper and 90.4 to 79.8 in moist sand. It also indicated that the vigour index was decreased from 826 to 727 in blotter paper, 908 to 803 in rolled towel paper and 1028 to 907 in moist sand. The seed mycoflora was significantly increased from 3.94 to 5.08 in blotter paper, 3.82 to 5.10 in rolled towel and 3.6 to 5.02 in moist sand method in Seeta after the 6 months storage period. It maintained certification standards of germination (75%) upto 6 months in all the methods.

It was also concluded that the germination and vigour index were maximum with less seed mycoflora in moist sand method as compared to blotter paper and rolled towel method in Seeta cultivar.

4.3.5 Effect of individual fungicides on germination, vigour index and seed mycoflora.

The germination percentage and vigour index were significantly higher in Thiram + Carbendazim followed by Thiram than other fungicidal seed treatment and untreated control in blotter paper, rolled towel and moist sand method. It was also observed that the seed mycoflora was also less in the same fungicidal seed treatment in all the methods (Table 11 to 13).

Table 11 : Effect of fungicidal seed treatment during the storage period on Germination, Vigour Index and Seed Mycoflora in mustard Cv. Seeta by Blotter paper method.

Sr. No.	Period in days (P)	Germination %	Vigour Index	Seed Mycoflora
F1.	0	88.6	826	3.94
	30	87.0	818	4.19
	60	85.1	796	4.42
	90	83.3	779	4.66
	120	81.1	758	4.87
	150	79.1	744	5.08
	180	77.3	727	5.24
	S.E.±	0.13	3.47	0.03
	C.D.±	0.35	9.62	0.08
F2.	Fungicide (F)			
	Thiram	87.5	823	4.12
	Carbendazim	79.0	744	5.10
	Thiram + Carbendazim	89.6	842	3.83
	Dithane M-45	84.7	791	4.49
	Captan	83.3	777	4.65
	Captafol	81.8	764	4.80
	Control	75.7	707	5.42
	S.E.±	0.13	3.47	0.03
	C.D.±	0.03	9.62	0.08
	Intraction			
F1 x F2	P x F S.E.±	0.34	9.19	0.07
	C.D.± (P=0.05)	0.93	25.47	0.21

Note : P = Period, F= Fungicide,

Table 12 : Effect of fungicidal seed treatment during the storage period on Germination, Vigour Index and Seed Mycoflora in mustard Cv. Seeta by Rolled Towel method.

Sr. No.	Period in days (P)	Germination %	Vigour Index	Seed Mycoflora
F1.	0	89.4	908	3.82
	30	88.1	894	4.01
	60	86.6	879	4.29
	90	84.4	857	4.52
	120	81.9	832	4.82
	150	80.7	820	4.95
	180	79.1	803	5.10
	S.E.±	0.07	0.69	0.03
	C.D.±	0.18	1.92	0.09
F2.	Fungicide (F)			
	Thiram	88.6	900	3.98
	Carbendazim	80.1	814	5.02
	Thiram + Carbendazim	90.1	915	3.74
	Dithane M-45	86.0	873	4.35
	Captan	85.3	867	4.40
	Captafol	83.8	851	4.58
	Control	76.0	772	5.44
	S.E.±	0.07	0.69	0.03
	C.D.±	0.18	1.92	0.09
	Intraction			
F1 x F2	P x F			
	S.E.±	0.18	1.84	0.09
	C.D.± (P=0.05)	0.47	5.09	0.25

Note : P = Period, F= Fungicide,

Table 13 : Effect of fungicidal seed treatment during the storage period on Germination, Vigour Index and Seed Mycoflora in mustard Cv. Seeta by Moist Sand method.

Sr. No.	Period in days (P)	Germination %	Vigour Index	Seed Mycoflora
F1.	0	90.4	1028	3.60
	30	89.3	1008	3.86
	60	87.4	987	4.09
	90	84.0	963	4.39
	120	83.6	944	4.62
	150	81.4	913	4.85
	180	79.8	907	5.02
	S.E.±	0.49	5.86	0.02
	C.D.±	1.36	16.25	0.06
F2.	Fungicide (F)			
	Thiram	90.2	1013	3.73
	Carbendazim	81.1	922	4.92
	Thiram + Carbendazim	91.8	1038	3.47
	Dithane M-45	87.6	989	4.12
	Captan	86.0	971	4.33
	Captafol	83.1	953	4.52
	Control	76.1	864	5.39
	S.E.±	0.49	5.86	0.02
	C.D.±	1.36	16.25	0.06
	Intraction			
F1 x F2	P x F			
	S.E.±	1.29	15.51	0.06
	C.D.± (P=0.05)	3.59 (NS)	42.99 (NS)	0.15

Note : P = Period, F= Fungicide,

4.3.6 Germination, vigour index and seed mycoflora as influenced by period into fungicides in rapeseed mustard Cv. Seeta in blotter paper and rolled towel paper method.

In storage period of 6 months in Seeta (Table 14 to 19) indicated that germination, vigour index and seed mycoflora were significantly superior in the interaction between period into fungicides in blotter paper and rolled towel paper. The germination percentage and vigour index was higher in Thiram + Carbendazim followed by Thiram than other fungicidal treatments. It was also observed that the seed mycoflora was less in Thiram + Carbendazim and Thiram seed treatment in blotter paper and rolled towel paper also.

4.3.7 Seed mycoflora as influenced by period into fungicides in Seeta in moist sand method.

In storage period of 6 months in Seeta germination and vigour index was non-significant in the interaction between period into fungicide. The seed mycoflora was significantly reduced in Thiram + Carbendazim and Thiram than other fungicidal seed treatment and untreated control (Table 20).

Table 14 : Germination influenced by period into fungicides in Cv. Seeta by Blotter paper method.

Treatment --> / Period :	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	92.0	84.5	94.5	90.0	90.5	88.5	80.0
30	91.5	83.0	93.5	88.5	87.5	86.0	79.0
60	89.5	88.5	91.5	87.5	88.5	84.5	78.5
90	87.5	79.0	89.5	88.5	84.5	82.5	75.5
120	85.0	77.0	88.5	82.5	81.0	80.0	74.0
150	84.0	75.5	85.0	81.0	79.0	76.5	72.5
180	83.0	74.0	84.5	78.5	75.5	75.0	71.0

S.E. ± - 0.34

C.D. ± - 0.93
(P = 0.05)

Table 15 : Vigour index influenced by period into fungicides in Cv. Seeta by Blotter paper method.

Treatment --> / Period :	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	860	798	883	837	841	827	748
30	855	810	874	827	818	804	739
60	839	753	855	818	794	785	729
90	817	838	836	894	798	771	705
120	794	719	827	771	757	748	691
150	785	705	829	762	738	715	678
180	810	691	798	729	705	701	664

S.E. ± - 9.19

C.D. ± - 25.47
(P = 0.05)

Table 16 : Seed mycoflora influenced by period into fungicides in Cv. Seeta by Blotter paper method.

Treatment --> / Period !	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	3.40	4.60	3.00	3.80	3.75	4.05	5.00
30	3.50	4.80	3.25	4.05	4.20	4.40	5.15
60	3.90	5.00	3.50	4.20	4.55	4.65	5.15
90	4.20	5.15	3.90	4.55	4.60	4.85	5.40
120	4.45	5.30	4.05	4.80	4.95	5.00	5.55
150	4.65	5.35	4.55	4.90	5.15	5.25	5.75
180	4.86	5.55	4.60	5.15	5.35	5.45	5.95

S.E. ± - 0.07

C.D. ± - 0.21
(P = 0.05)

Table 17 : Germination influenced by period into fungicides in Cv. Seeta by Rolled Towel method.

Treatment --> / Period !	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	93.0	85.0	95.0	91.0	91.5	89.5	81.0
30	92.0	83.0	95.0	89.0	90.0	87.0	80.0
60	91.0	82.0	93.0	88.0	88.0	86.0	78.0
90	88.0	81.0	90.0	86.0	85.0	84.5	76.0
120	86.5	79.0	87.0	84.0	82.0	81.0	74.0
150	86.0	77.0	86.0	83.0	81.0	80.0	72.0
180	84.0	74.0	85.0	81.0	80.0	78.5	71.0

S.E. ± - 0.18

C.D. ± - 0.47
(P = 0.05)

Table 18 : Vigour index influenced by period into fungicides in Cv. Seeta by Rolled Towel method.

Treatment --> / Period !	Thiram	Carb@ndazim	Thiram + Carb@ndazim	Dithane M-45	Captan	Captafol	Control
0	945	863	965	924	929	909	823
30	935	843	965	904	914	889	813
60	924	833	945	894	894	873	792
90	894	823	914	873	863	858	772
120	879	802	884	853	833	822	752
150	874	782	874	843	822	813	731
180	853	752	863	823	813	797	721

S.E. ± - 1.84

C.D. ± - 5.09
(P = 0.05) .

Table 19 : Seed mycoflora influenced by period into fungicides in Cv. Seeta by Rolled Towel method.

Treatment --> / Period !	Thiram	Carb@ndazim	Thiram + Carb@ndazim	Dithane M-45	Captan	Captafol	Control
0	3.30	4.55	2.95	3.65	3.50	3.85	4.95
30	3.40	4.80	2.95	3.95	3.80	4.20	5.00
60	3.65	4.85	3.30	4.15	4.15	4.40	5.55
90	4.15	4.95	3.80	4.35	4.55	4.60	5.30
120	4.35	5.15	4.25	4.65	4.85	4.95	5.55
150	4.40	5.30	4.40	4.80	4.95	5.00	5.80
180	4.65	5.55	4.55	4.95	5.00	5.10	5.90

S.E. ± - 0.09

C.D. ± - 0.25
(P = 0.05)

Table 20 : Seed mycoflora influenced by period into fungicides in Cv. Seeta by Moist sand method.

Treatment --> / Period !	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	3.10	4.40	2.80	3.30	3.40	3.80	4.85
30	3.10	4.65	2.90	3.65	3.80	3.95	5.00
60	3.30	4.80	2.95	3.80	4.25	4.40	5.15
90	3.80	4.85	3.30	4.25	4.55	4.55	5.45
120	4.05	5.15	3.80	4.40	4.60	4.80	5.55
150	4.25	5.30	4.20	4.65	4.80	5.00	5.80
180	4.55	5.30	4.40	4.85	4.95	5.15	5.95

S.E. $\pm$ - 0.06

C.D. $\pm$ - 0.15
(P = 0.05)

4.3.8 Effect of fungicidal seed treatment during the storage period on germination vigour index and seed mycoflora in rapeseed mustard Cv. MCN-60.

The data in Table 21 to 23 indicated that there was significant reduction in germination percentage from 88.2 to 76.1 in blotter paper 89 to 76.8 in rolled towel and 90 to 78.4 in moist sand. Vigour index was also decreased from 821 to 709 in blotter paper, 1007 to 875 in rolled towel paper and 1090 to 950 in moist sand method. The seed mycoflora was increased from 4.01 to 5.35 in blotter paper, 3.85 to 5.29 in rolled towel paper and 3.76 to 5.20 in moist sand method in MCN-60 after the 6 months storage period.

It maintained certification standards for germination upto 6 months in all the methods.

It was concluded that the germination and vigour index were maximum with less seed mycoflora in moist sand as compaired to blotter paper and rolled towel paper method.

4.3.9 Effect of individual fungicide on germination, vigour index and seed mycoflora in MCN-60.

The germination percentage and vigour index were significantly higher in Thiram + Carb@ndazim followed by Thiram than other fungicidal seed treatment and untreated control in blotter paper, rolled towel and moist sand method. Seed mycoflora was also less in the same fungicidal seed treatment in all the methods (Table 21 to 23).

Table 21 : Effect of fungicidal seed treatment during the storage period on Germination, Vigour Index and Seed Mycoflora in mustard Cv. MCN-60 by Blotter paper method.

Sr. No.	Period in days (P)	Germination %	Vigour Index	Seed Mycoflora
F1.	0	88.2	821	4.01
	30	86.8	808	4.20
	60	84.2	786	4.49
	90	82.6	769	4.71
	120	80.4	749	4.97
	150	77.8	724	5.22
	180	76.1	709	5.35
	S.E.±	0.07	0.87	0.02
	C.D.±	0.81	2.41	0.05
F2.	Fungicide (F)			
	Thiram	86.8	810	4.21
	Carbendazim	78.8	734	5.12
	Thiram + Carbendazim	89.1	829	3.92
	Dithane M-45	84.0	782	4.53
	Captan	81.6	760	4.81
	Captafol	81.3	758	4.87
	Control	74.8	697	5.50
	S.E.±	0.07	0.87	0.02
	C.D.±	0.18	2.41	0.05
	Intraction			
F1 x F2	P x F S.E.±	0.18	2.30	0.05
	C.D.± (P=0.05)	0.49	6.37	0.14

Note : P = Period, F= Fungicide,

Table 22 : Effect of fungicidal seed treatment during the storage period on Germination, Vigour Index and Seed Mycoflora in mustard Cv. MCN-60 by Rolled Towel method.

Sr. No.	Period in days (P)	Germination %	Vigour Index	Seed Mycoflora
F1.	0	89.0	1007	3.85
	30	87.7	993	4.15
	60	85.4	967	4.39
	90	82.9	937	4.70
	120	81.0	917	4.92
	150	79.3	899	5.09
	180	76.8	875	5.29
	S.E.±	0.19	1.15	0.03
	C.D.±	0.55	3.18	0.09
F2.	Fungicide (F)			
	Thiram	88.3	1001	4.02
	Carbendazim	79.4	899	5.07
	Thiram + Carbendazim	98.7	1005	4.02
	Dithane M-45	85.3	964	4.42
	Captan	83.6	947	4.61
	Captafol	82.1	934	4.73
	Control	74.7	846	5.50
	S.E.±	0.19	1.15	0.03
	C.D.±	0.55	3.18	0.09
	Intraction			
F1 x F2	P x F S.E.± C.D.± (P=0.05)	0.52 1.45	3.04 8.41	0.09 0.26 (NS)

Note : P = Period, F= Fungicide,

Table 23 : Effect of fungicidal seed treatment during the storage period on Germination, Vigour Index and Seed Mycoflora in mustard Cv. MCN-60 by Moist Sand method.

Sr. No.	Period in days (P)	Germination %	Vigour Index	Seed Mycoflora
F1.	0	90.0	1090	3.76
	30	89.1	1080	3.85
	60	86.9	1053	4.16
	90	84.3	1022	4.45
	120	82.3	998	4.76
	150	80.6	976	4.90
	180	78.4	950	5.20
	S.E.±	0.11	1.43	0.03
	C.D.±	0.30	3.98	0.09
F2.	Fungicide (F)			
	Thiram	89.7	1085	3.80
	Carbendazim	79.6	964	5.05
	Thiram + Carbendazim	90.5	1101	3.58
	Dithane M-45	86.1	1048	4.27
	Captan	85.0	1032	4.44
	Captafol	84.3	1021	4.58
	Control	76.1	922	5.37
	S.E.±	0.11	1.43	0.03
	C.D.±	0.30	3.98	0.09
	Intraction			
F1 x F2	P x F S.E.±	0.29	3.79	0.08
	C.D.± (P=0.05)	0.80	10.52	0.23

Note : P = Period, F= Fungicide,

4.3.10 Germination, vigour index and seed mycoflora as influenced in rapeseed mustard in blotter paper and sand method.

In storage period of 6 months in MCN-60 (Table 24 to 29) indicated that germination, vigour index and seed mycoflora were significantly superior in the interaction between period into fungicides in blotter paper and moist sand method. The germination percentage and vigour index was higher in Thiram + Carbendazim and Thiram seed treatment than other fungicidal seed treatment and untreated control. It was also found that the seed mycoflora was less in Thiram + Carbendazim and Thiram seed treatment in blotter paper and sand method also.

4.3.11 Germination and vigour index as influenced by period into fungicide in MCN-60 in rolled towel paper method.

In storage period of 6 months in MCN-60 seed mycoflora was non-significant in the interaction between period into fungicides. The germination percentage and vigour index was significantly increased in Thiram + Carbandazim and Thiram seed treatment in rolled towel method (Table 30 and 31).

Table 24 : Germination influenced by period into fungicides in Cv. MCN-60 by blotter paper method.

Treatment --> / Period !	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	92.0	84.5	93.0	90.0	89.0	88.5	80.5
30	91.0	83.0	93.0	89.0	87.0	86.0	78.0
60	89.5	80.0	91.5	87.0	84.0	83.0	76.0
90	88.0	78.0	90.0	84.0	82.0	81.0	75.0
120	85.0	77.0	88.0	82.0	79.0	79.0	73.0
150	82.0	75.0	85.0	79.0	76.0	77.0	71.0
180	80.0	74.0	83.0	77.0	74.0	75.0	70.0

S.E. ± - 0.18

C.D. ± - 0.49
(P = 0.05)

Table 25 : Vigour index influenced by period into fungicides in Cv. MCN-60 by Blotter paper method.

Treatment --> / Period !	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	875	788	865	838	829	825	750
30	846	773	865	829	811	801	732
60	834	745	851	811	783	773	708
90	820	727	848	783	764	755	699
120	792	715	820	764	736	736	680
150	774	699	792	736	708	717	661
180	745	689	773	717	689	699	652

S.E. ± - 2.30

C.D. ± - 6.37
(P = 0.05)

Table 26 : Seed mycoflora influenced by period into fungicides in Cv.MCM-68 by Blotter paper method.

Treatment --> / Period !	Thiram	Carb@ndazim	Thiram + Carb@ndazim	Dithane M-45	Captan	Captafol	Control
0	3.40	4.60	3.30	3.80	3.95	4.05	5.00
30	3.65	4.80	3.30	3.95	4.25	4.40	5.10
60	3.90	5.00	3.55	4.25	4.65	4.80	5.30
90	4.14	5.15	3.80	4.65	4.85	4.95	5.45
120	4.55	5.30	4.15	4.85	5.15	5.15	5.70
150	4.85	5.45	4.55	5.15	5.30	5.35	5.95
180	5.00	5.55	4.80	5.10	5.55	5.45	6.05

S.E. ± - 0.05

C.D. ± - 0.14
(P = 0.05)

Table 27 : Germination influenced by period into fungicides in Cv. MCM-68 by Moist sand method.

Treatment --> / Period !	Thiram	Carb@ndazim	Thiram + Carb@ndazim	Dithane M-45	Captan	Captafol	Control
0	94.0	86.0	95.0	92.0	91.0	90.0	82.0
30	94.0	85.0	95.0	91.0	90.0	89.0	80.0
60	92.0	83.0	94.0	89.0	87.0	86.5	77.0
90	90.0	79.5	91.0	86.0	84.0	84.0	76.0
120	88.5	77.0	89.0	83.5	83.0	81.5	74.0
150	86.0	75.0	87.0	82.0	81.5	80.0	73.0
180	83.5	71.5	85.0	79.0	80.0	79.0	71.0

S.E. ± - 0.29

C.D. ± - 0.80
(P = 0.05)

Table 28 : Vigour index influenced by period into fungicides in Cv. MCN-60 by Moist sand method.

Treatment --> / Period !	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	1139	1042	1151	1115	1102	1091	994
30	1139	1030	1151	1102	1091	1078	969
60	1115	1006	1139	1078	1054	1048	938
90	1090	963	1102	1042	1018	1018	921
120	1073	933	1079	1012	1006	988	897
150	1033	909	1054	994	988	969	885
180	1012	867	1030	957	969	951	860

S.E. ± - 3.79

C.D. ± - 10.52
(P = 0.05)

Table 29 : Seed mycoflora influenced by period into fungicides in Cv.MCN-60 by Moist sand method.

Treatment --> / Period !	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	3.10	4.40	2.95	3.40	3.65	3.80	4.85
30	3.10	4.55	2.95	3.65	3.80	3.95	5.00
60	3.40	4.80	3.10	3.95	4.25	4.35	5.30
90	3.80	5.05	3.30	4.40	4.70	4.65	5.30
120	4.05	5.30	4.00	4.75	4.80	4.90	5.55
150	4.40	5.45	4.25	4.65	4.90	5.00	5.70
180	4.75	5.85	4.55	5.15	5.00	5.15	5.95

S.E. ± - 0.08

C.D. ± - 0.29
(P = 0.05)

Table 30 : Germination influenced by period into fungicides in Cv.MCN-60 by Rolled Towel method.

Treatment --> / Period !	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	93.0	85.0	94.0	91.0	90.0	89.0	81.0
30	93.0	83.0	93.0	90.0	89.0	87.0	79.0
60	91.0	82.0	91.0	87.0	86.0	84.0	77.0
90	90.0	79.0	89.0	85.0	83.0	82.0	74.0
120	85.5	77.0	87.0	83.0	81.5	81.0	72.0
150	85.0	76.0	85.0	81.0	79.0	78.5	71.0
180	83.0	74.0	82.5	80.0	77.0	72.5	69.0

S.E. ± - 0.52

C.D. ± - 1.45
(P = 0.05)

Table 31 : Vigour index influenced by period into fungicides in Cv. MCN-60 by Rolled Towel method.

Treatment --> / Period !	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	1053	963	1065	1030	1019	1004	917
30	1053	940	1053	1019	1008	985	895
60	1031	929	1031	985	974	951	872
90	997	895	1008	949	940	935	838
120	968	872	985	940	923	918	815
150	963	861	963	917	895	889	804
180	940	838	934	906	872	855	781

S.E. ± - 3.04

C.D. ± - 8.41
(P = 0.05)

4.3.12 Effect of fungicidal seed treatment during the storage period on germination, vigour index and seed mycoflora in rapeseed mustard Cv. TN-17.

The data in Table 32 to 34 indicated that there was significant reduction in germination percentage from 86.8 to 75.8 in blotter paper, 88.4 to 75.8 in rolled towel paper and 89.4 to 75.6 in moist sand method. The vigour index was also reduced from 813 to 706 in blotter paper, 1065 to 914 in rolled towel paper and 1210 to 1023 in moist sand method. The seed mycoflora was increased from 4.20 to 5.47 in blotter paper, 3.95 to 5.42 in rolled towel paper and 3.82 to 5.40 in moist sand method in TN-17 after the 6 months storage period.

It maintained certification standards for germination upto 6 months in storage period in all the methods.

It was concluded that the germination and vigour index were higher with less seed mycoflora in moist sand method as compared to blotter paper and rolled towel paper method.

4.3.13 Effect of individual fungicide on germination, vigour index and seed mycoflora in TN-17.

The germination percentage and vigour index were significantly superior in Thiram + Carbendazim and Thiram seed treatment and also less seed mycoflora than other fungicidal seed treatment and untreated control (Table 32 to 34).

Table 32 : Effect of fungicidal seed treatment during the storage period on Germination, Vigour Index and Seed Mycoflora in mustard Cv. TN-17 by Blotter paper method.

Sr. No.	Period in days (P)	Germination %	Vigour Index	Seed Mycoflora
F1.	0	86.8	813	4.20
	30	86.1	813	4.26
	60	84.2	789	4.62
	90	81.3	762	4.85
	120	79.1	741	5.10
	150	77.2	725	5.27
	180	75.8	706	5.47
	S.E.±	0.27	3.49	0.06
	C.D.±	0.75	9.68	0.17
F2.	Fungicide (F)			
	Thiram	86.0	805	4.30
	Carbendazim	77.3	724	5.27
	Thiram + Carbendazim	88.7	831	4.07
	Dithane M-45	83.8	785	4.61
	Captan	81.2	861	4.87
	Captafol	80.3	759	4.93
	Control	73.1	684	5.72
	S.E.±	0.27	3.49	0.06
	C.D.±	0.75	9.68	0.17
	Intraction			
F1 x F2	P x F S.E.±	0.71	9.24	0.16
	C.D.± (P=0.05)	1.98	25.62	0.45 (NS)

Note : P = Period, F= Fungicide,

Table 33 : Effect of fungicidal seed treatment during the storage period on Germination, Vigour Index and Seed Mycoflora in mustard Cv. TN-17 by Rolled Towel method.

Sr. No.	Period in days (P)	Germination %	Vigour Index	Seed Mycoflora
F1.	0	88.4	1065	3.95
	30	87.1	1046	4.14
	60	84.9	1023	4.44
	90	82.5	994	4.72
	120	80.6	971	4.94
	150	78.1	941	5.20
	180	75.8	914	5.42
	S.E.±	0.11	1.82	0.02
	C.D.±	0.31	5.04	0.07
F2.	Fungicide (F)			
	Thiram	87.5	1054	4.18
	Carbendazim	77.9	939	5.22
	Thiram + Carbendazim	89.8	1082	3.78
	Dithane M-45	84.3	1012	4.52
	Captan	82.8	998	4.68
	Captafol	81.9	986	4.79
	Control	73.3	883	5.68
	S.E.±	0.11	1.82	0.02
	C.D.±	0.31	5.04	0.07
	Intraction			
F1 x F2	P x F S.E.±	0.29	4.81	0.07
	C.D.± (P=0.05)	0.80	13.52	0.18

Note : P = Period, F= Fungicide,

Table 34 : Effect of fungicidal seed treatment during the storage period on Germination, Vigour Index and Seed Mycoflora in mustard Cv. TN-17 by Moist Sand method.

Sr. No.	Period in days (P)	Germination %	Vigour Index	Seed Mycoflora
F1.	0	89.4	1210	3.82
	30	88.2	1182	4.00
	60	85.1	1154	4.38
	90	82.8	1120	4.70
	120	80.0	1082	5.01
	150	77.8	1056	5.22
	180	75.6	1023	5.46
	S.E.±	0.11	4.14	0.02
	C.D.±	0.35	11.48	0.06
F2.	Fungicide (F)			
	Thiram	86.7	1173	4.20
	Carbendazim	78.3	1059	5.17
	Thiram + Carbendazim	89.7	1203	3.80
	Dithane M-45	85.7	1159	4.35
	Captan	83.3	1126	4.64
	Captafol	81.6	1108	4.80
	Control	73.8	998	5.67
	S.E.±	0.11	4.14	0.02
	C.D.±	0.35	11.48	0.06
	Intraction			
F1 x F2	P x F S.E.±	0.29	10.96	0.06
	C.D.± (P=0.05)	0.81	30.36	0.16

Note : P = Period, F= Fungicide,

4.3.14 Germination and vigour index as influenced by period into fungicide in TN-17 in blotter paper method.

In storage period of 6 months in TN-17 seed mycoflora was non-significant in the interacton between period into fungicide. The germination percentage and vigour index were significantly increased Thiram + Carb@ndazim and Thiram seed treatment as compaired to other fungicidal seed treatment and untreated control in blotter paper method (Table 35 to 36).

4.3.15 Germination, vigour index and seed mycoflora as influenced in rapeseed mustard Cv. TN-17 in rolled towel paper and moist sand method.

In storage period of 6 months in TN-17 (Table 37 to 42) indicated that germination, vigour index and seed mycoflora were significantly superior in the interaction between period into fungicide in rolled towel paper and moist sand method.

The germination percentage and vigour index was increased in Thiram + Carb@ndazim and Thiram seed treatment and also less seed mycoflora percentage than other fungicidal seed treatment and untreated control in rolled towel and sand method in TN-17.

Table 35: Germination influenced by period into fungicides in Cv.TN-17 by Blotter paper method.

Treatment --> / Period !	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	91.0	83.0	93.0	90.5	83.5	87.5	79.5
30	91.0	81.0	93.0	88.0	87.0	85.0	78.0
60	89.0	79.0	92.0	86.0	85.0	83.5	75.0
90	86.0	76.0	89.0	83.0	82.0	80.0	73.0
120	83.0	75.0	87.0	81.5	79.5	77.0	71.0
150	82.0	74.0	84.0	80.5	77.0	75.5	69.0
180	80.0	73.0	83.0	77.0	74.5	74.0	66.0

S.E. ± - 0.71

C.D. ± - 1.98
(P = 0.05)

Table 36 : Vigour index influenced by period into fungicides in Cv.TN-17 by Blotter paper method.

Treatment --> / Period !	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	852	777	871	848	782	820	745
30	852	759	871	824	815	842	730
60	834	748	868	805	796	782	702
90	805	712	834	777	768	749	684
120	777	702	815	764	745	721	665
150	768	693	787	754	721	707	646
180	749	684	777	721	698	693	618

S.E. ± - 9.24

C.D. ± - 25.62
(P = 0.05)

Table 37 : Germination influenced by period into fungicides in Cv.TN-17 by Rolled Towel method.

Treatment --> / Period ↓	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	92.0	84.0	94.5	91.0	89.5	88.0	80.0
30	91.5	83.0	94.0	89.0	87.5	87.0	78.0
60	89.0	81.0	92.0	86.0	85.0	85.5	76.0
90	87.0	78.0	90.0	84.0	83.5	82.0	73.0
120	86.0	70.0	88.5	83.0	81.0	90.0	71.0
150	84.0	79.5	86.0	79.0	77.5	77.0	69.0
180	82.0	71.0	84.0	70.0	74.0	74.0	66.0

S.E. ± - 0.29

C.D. ± - 0.80
(P = 0.05)

Table 38 : Vigour index influenced by period into fungicides in Cv. TN-17 by Rolled Towel method.

Treatment --> / Period ↓	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	1109	1012	1139	1096	1078	1056	964
30	1103	1000	1133	1050	1054	1040	940
60	1072	976	1109	1036	1024	1030	916
90	1040	940	1004	1012	1006	988	880
120	1036	904	1066	1000	976	964	856
150	1024	886	1036	952	934	920	831
180	988	856	1012	940	960	892	795

S.E. ± - 4.81

C.D. ± - 13.52
(P = 0.05)

Table 39 : Seed mycoflora influenced by period into fungicides in Cv.TN-17 by Rolled Towel method.

Treatment --> / Period !	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	3.40	4.65	3.00	3.65	3.85	4.15	5.00
30	3.55	4.80	3.10	3.95	4.20	4.25	5.15
60	3.95	4.95	3.50	4.40	4.55	4.45	5.30
90	4.25	5.15	3.80	4.65	4.70	4.85	5.70
120	4.40	5.45	4.05	4.80	4.95	5.00	5.95
150	4.55	5.60	4.40	5.10	5.25	5.30	6.20
180	4.85	5.95	4.65	5.15	5.30	5.55	6.50

S.E. ± - 0.07

C.D. ± - 0.18
(P = 0.05)

Table 40 : Germination influenced by period into fungicides in Cv.TN-17 by Moist Sand method.

Treatment --> / Period !	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	93.5	85.0	95.0	92.0	90.5	89.0	81.0
30	93.0	83.0	95.0	91.0	88.5	87.5	79.5
60	90.0	81.0	93.0	88.0	85.0	83.0	76.0
90	87.0	78.0	90.0	86.0	83.0	82.5	73.0
120	83.0	76.0	88.0	83.0	81.0	78.0	71.0
150	81.5	74.0	84.5	81.0	78.0	76.5	67.5
180	79.0	71.0	82.5	79.0	77.0	74.5	66.5

S.E. ± - 0.29

C.D. ± - 0.81
(P = 0.05)

Table 41 : Vigour index influenced by period into fungicides in Cv. TN-17 by Moist Sand method.

Treatment --> / Period !	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	1265	1150	1285	1244	1224	1205	1095
30	1258	1123	1213	1231	1195	1184	1074
60	1217	1096	1258	1190	1150	1136	1028
90	1177	1055	1217	1163	1123	1116	987
120	1123	1023	1190	1123	1196	1055	960
150	1103	1001	1143	1096	1055	1051	940
180	1068	960	1160	1068	1042	1008	899

S.E. ± - 10.96

C.D. ± - 30.36
(P = 0.05)

Table 42 : Seed mycoflora influenced by period into fungicides in Cv. TN-17 by Moist Sand method.

Treatment --> / Period !	Thiram	Carbendazim	Thiram + Carbendazim	Dithane M-45	Captan	Captafol	Control
0	3.25	4.55	2.95	3.40	3.75	3.95	4.95
30	3.30	4.80	2.95	3.65	4.05	4.20	5.10
60	3.80	4.95	3.30	4.15	4.55	4.65	5.30
90	4.25	5.15	3.85	4.40	4.80	4.80	5.70
120	4.80	5.30	4.15	4.80	4.95	5.15	5.97
150	4.90	5.55	4.60	4.95	5.15	5.35	6.10
180	5.15	5.95	4.80	5.15	5.25	5.50	6.45

S.E. ± - 0.06

C.D. ± - 0.16
(P = 0.05)

Discussion

CHAPTER V

DISCUSSION

Seed is multiferous and therefore, it is highly essential that the seed-borne fungi should be thoroughly studied with respect to their biology and control. Fungicidal seed treatment, not only kills or inhibits the pathogen associataed with seed,but also protects the seed from soil-borne pathogen in the initial stages of growth by forming a zone of protection around the seeds. It is also cheapest and safest method of direct plant disease control.

Based on information, the seed mycoflora of rapeseed mustard (Pusa-bold,Seeta,MCN-60 and TN-17) were studied following the methods viz. Agar plate, blotter paper, rolled towel and moist sand method.

The fungi recorded from seeds of Cv.Pusa-bold,Seeta, MCN-60 and TN-17 were*Fusarium moniliforme*, *Aspergillus flavus*, *Macrophomina phaseolina*, *Aspergillus niger*, *Alternariea alternata* and*Rhizopus spp.*

All the methods yield more or less same set of mycoflora. The result indicate that the seed mycoflora percentage was maximum in unsterilized seed as compared to sterilized in all the methods and all the cultivars. It was also indicated that the seed mycoflora percentage was maximum in Agar plate method as compared to blotter paper, rolled towel and moist sand method. The fungi *Fusarium moniliforme*, *Macrophomina phaseolina*,

Aspergillus niger and Alternaria alternata were more prominant than Aspergillus flavous and Rhizopus spp. in all the methods in all the varieties.

Gaur and Ahmed (1983) detected ninteen fungi from four varieties of which eight species were that of Aspergillus and eleven of other.

Fungi detected by blotter technique and agar plate methods were species of Aspergillus,Fusarium,Alternaria, Rhizoctonia, Penicillium,Chaetomium, Rhizopus, Helminthosporium, Curvularia and Macrophomina phaseolina. They also observed that the developement of fungi on the seeds also varied with the varieties.

Gurmail Singh and Negi (1984) recorded twenty fungi of which were Alternaria alternata, Aspergillus flavus, Chaetomium globosum, Cladosporium claclosporides, C. herbarum, Curvularia spicifer, Fusarium oxysporium, Penicillium spp. and Rhizophus nigricans were found to be dominant among the pathogens from five varieties of rapeseed mustard viz.Brassica campestris vari. Varuna and B. nigria (Rai).All the fungi were frequently observed in agar plate method than in blotter method. Seed mycoflora showed variation in frequency of fungal incidence amoung the varieties studied. Similar results have been obtained in present study.

The importance of seed mycoflora of rapeseed mustard has been also recognised by the work of different scientists like Petrie (1978), Chahal(1981), Sudarmadi and Wallage(1982),Humpherson and Brien (1984),

Brokenshix and Rasana (1994), Rasana (1984), Tripati and Kaushik (1984), Ashraf and Chaudhary (1986), Kolte et al (1987), Ansari et al (1988), Satyabrata and Raoof (1988), Kumar et al (1989), Shirpuri et al (1990), Churasia (1992), Karona Vishunavat and Kolte (1993), Rakesh Kumar et al, (1993), Rakesh Shah and Jain (1993). Similar results have been obtained in present studies.

Majority of these fungi are pathogenic and affect in either pre or post emergence of the seedling Vishnuavat et al (1985), observed that due to infection of Alternaria brassicae, the losses were observed in seed size, seed weight, seed germination and seed viability. Chahal (1986) estimated the losses occures due to Alternaria blight as 43.62% in Brassica campestris and 38.36% in Indian mustard.

Effect of fungicidal seed treatment on Fusarium moniliforme of rape seed mustard were studied. One hundred apperently healthy seeds of each cultivars were treated with Thiram, Thiram+Carbendazim, Dithen-M45, Captan and Captafol. Than the treated seeds were rolled on actively growing culture of Fusarium moniliforme and sown on sterilized agar plate. The results indicated that all the fungicides improved the germination percentage and restricted the pre and post emergence mortality. Thiram and Thiram + Carbendazim improved the maximum germination and pre and post montaility as compaired to other fungicides.

Rana and Tripathi(1983)have tested 19 fungicides against collar rot of Indian mustard.Only Carbendazim,carboxin, Thiophanate-Methyl,Captafol,Mancozeb and Agrason were effective at all concentrations against Rhizoctonia bataticola(Macrophomina phaseolina) on Brassicia junicea. Gupta et al(1985) reported that Captafol 0.2 was highly effective against Alternaria brassicae.

Chahal (1986), Kumudkumar and Singh (1986), Saha (1986) have also reported Bavistin (Carbendazim), Difolatan (Captafol),Dithen-M45 (Mancozeb), and Thiram were effective against the control of fungal species of Alternaria, Fusarium, Macrophomina. In storage studies on rape seed mustard (Pusa-Bold, Seeta, MCN-60,TN-17) were conducted with different fungicidal seed treatment (Thiram,Carbendazim,Thiram + Carbendazim,Dithane M-45, Captan and Capatafol for seed germination, vigourindex and seed mycoflora upto 6 months.There was significant reduction in gemination.Vigour index and increase in seed mycoflora in dry fungicidal seed treatment in blotter paper, rolled towel and moist sand method after 6 months storage period. It maintained certification standard for germination upto 6 months storage period in blotter paper, rolled towel and moist sand method. The germination and vigour index was maximum in Thiram + Carbendazim and Thiram than other funicidal seed treatments and untreated control with less seed mycoflora in all the methods.

Rana and Tripathi (1983) reported that out of

19 funicides tested,only Carbendazim,Carboxin,Thiophnate-methyl,Captafol,Mancozeb and Agrasan were effective at all the concentrations aganist seed mycoflora of rapeseed mustard. Gupta et al(1985) reported that Captafol 0.2 was highly effective against Alternaria brassica. Chahal (1986) controled the losses in yield in Brassica compestris of A. brassicae due to seed treatment or sprays of Captafol,Blitox,Dithane M-45. Saha (1986) tested 10 fungicides.Bavistian (Carbendazim), Quintozene,Vitavax (Carboxin),Ziram and Ceresan wet.They performed best followed by Dithane Z-78 (Zinab) and Dithane M-45 (Mancozeb).

Kumudkumar and Singh (1986) reported seed treatment of Bavistin (Carbendazim),Difolatan (Captafol) at (1.5 g/kg seeds) and Dithane M-45 (Mancozeb) (2g) eradicated the Alternaria brassicae.Thiram was also more effective in slurry seed treatment.

Rakesh Shah and Jain (1993) tested 12 fungicides as seed dressing on inoculated seeds, Carboxin gave best control of all the seed borne fungi.

Ansari et al(1990) tested 18 fungicides against seed borne pathogen of rapeseed mustard. Only Dithane M-45,Dithane Z-78,Ziram,Difolatan-80,Blitox-50 and Benlate completely inhibited the growth of the pathogen. They also reported that Thiram and Brestan-60 also caused total growth inhibition of the pathogen. Similar result were also obtained in the present studies.

Summary

CHAPTER VI

SUMMARY

Present investigation was undertaken with a view to isolate and identify fungi and their pathogenicity, to find out effect of fungicidal seed treatment on seed mycoflora, seed health, seed quality and storability in important cultivars of rape seed mustard and to find out effect of seed treatment on seed and seedling health, vigour and seedling mortality.

The fungi isolated by following four methods viz. agar plate, blotter paper, rolled towel and moist sand methods, were Fusarium moniliforme, Aspergillus flavus, Aspergillus niger, Alternaria alternata, Macrophomina phaseolina and Rhizophus spp. from rape seed mustard cultivars Pusa-bold, Seeta, MCN-60 and TN-17 from sterilized and unsterilized seeds. All the methods yielded more or less same set of mycoflora. In general, Agar plate method was superior than all other methods in yielding more number of fungi form the rape seed mustard.

The rape seed mustard Cv. Pusa-bold, Seeta, MCN-60 and TN-17 yieled fungi like Fusarium moniliforme, Aspergillus flavus, Alternaria alternata, Macrophomina phaseolina, Aspergillus niger and Rhizopus spp. Total seed myco-flora was found less in Pusa-bold as compaired to Seeta, MCN-60 and TN-17 in all the methods of isolation.

The interaction studies between fungicides and

cultivars with Fusarium moniliforme inoculation in Pusa bold , Seeta,MCN-60 and TN-17 showed that there was reduction in pre and post emergence mortality in cultivers due to Thiram +Carbendazim and Thiram seed dressing than other fungicides and inoculated control.All the fungicides improved the germination and reduced the mortility in all the cultivars than control.

In storage study there was significant reduction in germination per cent,vigour index and increase in seed mycoflora after 6 months storage period in Pusa-bold, Seeta,MCN-60 and TN-17 in all the methods.The minimum germination standard was maintained up to 6 months in all the cultivars and in all the methods.

The germination per cent and vigour index was significantly higher and seed mycoflora was less in Thiram + Carbendazim and Thiram seed treatment in all the cultivars and in all the methods as compaired to other fungicides and untreated control.

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