Evaluation of Seed Treating Chemicals against Seed Borne Fungi on Germinating Wheat Seed

A Thesis

By

Marziha Mannan Mim
Student Id: MS 190808

A Thesis (Course No. PLP- 6104) Submitted for Partial Fulfillment of the Degree
Master of Science in Plant pathology
of
Khulna University

Agrotechnology Discipline
School of Life Science
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March, 2022

DEDICATED TO

MY

BELOVED PARENTS

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ABSTRACT

Seed borne pathogens of wheat plays vital role in causing plant diseases and constrain wheat productivity in Bangladesh. An experiment was conducted to evaluate four seed treating chemicals namely Knowin 50 WP, Sunvit 50 WP, Neuben 72 WP and Tilt 250 EC against wheat seed borne fungi in plant protection laboratory, Agrotechnology Discipline, Khulna University Khulna, Bangladesh during January, 2021 to October 2021.The experiment was carried out to detect the associated seed borne fungi on two wheat varieties and find out the efficacy of fungicides to control seed borne fungi of wheat. Wheat seed samples (Farmer's seed) of two (*Triticum aestivum* L.) varieties (BARI Gom-25 and BARI Gom-26) were collected from Regional Agricultural Research Station, Jashore. The experiment was conducted following completely randomized design (CRD) with four replications. Different seed borne fungi *Alternaria* spp., *Bipolaris* spp., *Curvularia* spp. and *Aspergillus* spp. were identified as associated fungi on these two varieties of seed. The prevalence of different seed borne fungi was varying independently and individually in wheat varieties. In case of germination between two wheat varieties, the highest germination percentage was recorded in BARI Gom-26. All the tested fungicides increased germination percentage as compared to untreated seed. Knowin 50 WP showed superior influence on germination of wheat variety over untreated seed in Blotter method. Among the tested fungicides Tilt 250 EC showed potential performance in controlling all the associated fungi on wheat seed.

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CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the top growing cereal crops belonging to the family Gramineae (FAO, 2016) has served as a staple food source for mankind since times immemorial (Satish et al., 2010). It is counted as the most important cereal crops, with over 600 million tons being harvested annually worldwide (Shewry et al., 2015).This has a prominent position among the cereals that supplements nearly one third of the world population's diet by providing half of the dietary protein and more than half of calories (Dhanda et al., 2004). In 2020 over 763 million tons wheat being harvested annually worldwide (FAO, 2020). According to BBS, it is considered as the second most staple food crop next to rice in Bangladesh. Besides human nutrition, wheat is also used as animal feed (Islam et al., 2015).

Bangladesh Bureau of Statistics (BBS, 2020) data also shows that both acreage and production of wheat have been declining in the last 22 years. According to the BBS data the acreage of wheat fell to 10.29 lakh tonnes in FY2019-20 in a land of 21, 79,640 acres from 19.08 lakh tonnes in FY1998-99. The data showed the downward trend as 18.40 lakh tonnes in FY1999-2000, 16.73 lakh tonnes in FY2000-01, 16.06 lakh tonnes in FY2001-02, 15.07 lakh tonnes in FY2002-03, 12.53 lakh tonnes in FY2003-04, 9.76 lakh tonnes in FY2004-05 and 7.35 lakh tonnes in FY2005-06 (BBS, 2020). In Bangladesh the production of wheat and yield is too low in comparison to the world wheat production (BBS, 2020).

In Bangladesh the lower productivity is due to diseases and poor management. Here out of 16% annual crop losses due to plant diseases, at least 10 percent incurred due to seed borne disease (Fakir, 1983). Wheat is affected by as many as 120 different diseases, of which 42 are seed borne (Wiese, 1987). Seed plays vital role in the transmission of plant pathogens causing plant diseases. The pathogen may be externally or internally seed borne or associated with seed as contaminant. The seed borne pathogens may cause seed abortion, seed rot, seed necrosis or reduction in germination as well as seedling damage by systemic or local infection resulting in development of disease at later stages of plant growth (Neergaard, 1977).

Healthy seed plays an important role for increasing successful cultivation and yield of crops. Seed borne pathogens of wheat responsible for causing variation in plant morphology and reducing yield (15% - 90%). The yield loss in wheat due to different disease have been reported to be 20 % in Wheat var. Sonlika, whereas 14% and 8% in Akbar and kanchan respectively (Razzaque and Hossain, 1991). It is also reported that in the farmers field in case of severe attack it may result even 100% yield loss (Hossain and Azad, 1994). The Department of Agricultural Extension (DAE) report reveals that wheat fields were burned by wheat blast pathogen, which contributes to 15% decrease in wheat production of the eight infected districts (Islam et al., 2016 and Malaker et al., 2016).

As many plant pathogens are seed borne and seed is the most important input for crop production. Diseases, especially caused by seed borne fungal agents, are the main factors reducing yield and quality of wheat. Seed borne pathogens reduce viability, vigor, and germination capability of the seeds resulting in lower yield (Majumder et al., 2013). The fungi which are exclusively seed-borne in nature can be controlled only through the treatment of seeds. Almost all methods are aimed for protecting plant becoming disease rather than curing them after they become diseased (Singh et al., 1995).

The chemicals are used to treat seeds to prevent their decay after planting by controlling pathogens carried on, inside or existing in the seed and soil (Singh et al., 1995). Fungicidal seed treatment may kill or inhibit seed borne pathogen and may form a protective zone around seeds that can reduce seed decay and seedling blight caused by soil borne pathogen, and resulting vigorous seedlings (Nene and Thapliyal, 1979).

Seed treatment by chemicals is the best economical way to keep good seed health condition and environment safe because in this management mostly very low dose of chemicals are used as compared to foliar application. It was reported seed treating fungicides viz. vitavex-200 @ 0.3% of seed weight eliminated all the seed borne pathogen and increased germination 25.70% over control. Many scientist observed the effect of fungicide, biological agents and host resistance in managing and inhibition of pathogen growth in *in vitro* by using fungicide like as Carbendazim, Hexaconazle, Tebuconazle and Sulphur (Bhuiyan et al., 2013 and Musyimi et al., 2012).

For the control of seed-borne fungi, fungicides viz. Mancozeb, Carbendazim and Mancozeb plus Carbendazim mixture were tested and the results indicated that different concentrations of fungicides were found significantly effective in percent growth inhibition of *A. alternata, R. stolonifer, Mucor spp., F. moniliforme* and *A. flavus* (Ahmad and Zaidi, 2018). Seed

Naznine et al. (2016) were tested wheat seed samples of 11 varieties viz. Akbar, Kanchan, Barkat, Sonalika , Paban, Prodip, Satapdi, BARI gom-25, BARI gom-26, BARI gom-27 and BARI gom-28 from 7 different Upazillas of Bogra and 5 different Upazillas of Naogaon Health test by Blotter method resulted eight different seed borne fungi belonging to six genera viz. *Bipolaris sorokiniana* (0.5 to 30.5%), *Alternaria tenuis* (0.5 to 25%), *Fusarium moniliforme* (0.0 to 33.5%), *Fusarium oxysporum* (2.7 to 53%), *Curvularia lunata* (0.0 to 5.5%), *Aspergillus niger* (0.0 to 18.5%) and *Penicillium spp*. (0.0 to 1.5%).

Islam et al. (2015) conducted a study to detected fungi by the methods as recommended by the ISTA. The experiment was conducted at the Seed Pathology Centre, Bangladesh Agricultural University, Mymensingh during January, 2006 to February, 2007 to record and identify the seed borne fungi associated with wheat seeds as well as their control with some chemicals. The seeds were collected from ten villages of each of two different locations of Dinajpur district (Dinajpur, Sadarupazila and Birgonjupazila). Different seed borne fungi such as *Bipolaris sorokiniana*, *Ahernaria tenuis*, *Curvularia lunata*, *Fusurium* spp., *A. niger* and *A. flavus* were predominant with the wheat seeds of two locations of Dinajpur district.

Pathak et al. (2013) conducted experiment with three different variety of Seeds (WH896, PBW-373 and HD264) of wheat (*Triticum aestivum*) were collected from Quarsi Agriculture Farm Aligarh. These three seed samples of wheat showing different forms of discoloration and abnormalities were screened for associated fungi. Seed-borne fungi, *viz. Fusarium moniliforme, Rhizopus* spp., *Mucor* spp., *Alternaria alternata, Aspergillus niger, Aspergillus flavus, Curvularia lunata, Drechslera* spp, *Alternaria* spp. and *Penicillium* spp. were isolated from the wheat variety HD264. Blotter method was found to be the best media for the isolation of mycoflora whether borne externally or internally.

Hajihasani et al. (2012) conducted experiment with three different variety of wheat. A total of 15 fungal species including *Tilletia laevis, Tilletia tritici, Ustilago tritici, Fusarium graminearum, Fusarium culmorum, Microdochium nivale, Bipolaris sorokiniana, Alternaria alternata, Curvularia* spp., *Aspergillus niger, Aspergillus candidus, Aspergillus flavus, Penicillium* spp., *Mucor* spp. *and Rhizopus* spp. were identified in three wheat cultivars.

Rajput (2005) tested one hundred twenty wheat seed samples collected from Sindh wheat growing areas for fungal seed-borne pathogens by using the standard blotter method. Five seed borne fungi viz., *Alternaria tenuis, Aspergillus niger, Fusarium moniliforme, Curvuluria* *lunata and Stemphylium herhurum* were isolated from 12 wheat varieties viz., Mehran, T.J-83, Soghat, Sarsabz, Anmol, Johar, C-591, Sindh-81, Pak-70, Mexipak-65, H-68 and Faisalabad-85 respectively. *Alternaria tenuis* was predominance with an infection range from 22.5-47.5%. Maximum seed germination was observed in Anmol and minimum in Pak-70. Maximum root and shoot length of seedlings was recorded in Anmol and Sarsabz followed by H-68 and minimum in Pak-70, Mehran-89, Soghat and Johar.

Siddique et al. (2002) assessed the reaction of five wheat varieties to *Bipolaris sorokiniana* and *Fusarium moniliforme* in terms of prevalence of the pathogen on the seeds, symptom severity on the growing plants and effects of the pathogens on the yields. Prevalence of *B. sorokiniana* was 4-4.25% in Kanchan and Akbar and of *Fusarium monilifome* was 2.25-2.75% in Gourab and Kanchan. Disease severity was the highest in Barkat both in laboratory and field conditions. However, *Bipolaris sorokiniana* infection was more severe than that of *Fusarium moniliforme*. Percent diseased grains were high in Barkat, while grain yield was low in Barkat and Sonalika due to inoculation of *B. sorokiniana* and *F. moniliforme*. Thus, there was a significant variation in reaction among the wheat varieties to the pathogens.

Hyder-Ali et al. (1992) collected seeds of seven selected cultivars of wheat in two seasons from five agricultural research stations at Gazipur, Ishurdi, Jamalpur, Jashore and Mymensingh of Bangladesh and detected Sixteen fungi, representing nine genera. The most predominant fungi, in order of prevalence, were *Alternaria tenuis, Bipolaris sorokiniana, Aspergillus* spp., *Curvularia lunata and A. flavus*. The fungi varied in prevalence with respect to location, cultivar and duration of seed stored. *A. tenuis, B. sorokiniana, Fusarium graminearum, F. oxysporum and F. semitectum* were associated with diseased seedlings. *A. flavus* more frequently associated with ungerminated seeds was found to be the causal agent of germination failure. *Sordaria funicola* was found as newly recorded seed-borne fungi of wheat in Bangladesh.

2.3 Efficacy of seed treating chemicals

Antonkiewicz et al. (2021) found that Carboxin and Thiram fungicide reduce microorganisms involved in the pathogenesis of pre-emergence damping-off and limit the presence of antagonistic fungi. The effectiveness of the chemical fungicide is 44.2% and 25.9%. Under the influence of *P. oligandrum*, there was a five-fold increase in the population of antagonists. Also with increase in humus in the soil reduces the percentage of diseased broad bean seedlings.

Addrah et al. (2020) conducted a study to efficiently control seed-borne fungi with four broad spectrum fungicides (Carbendazim, Triadimefon, CaprioF-500, and Flusilazole) were screened against *V. dahliae,* which was isolated from a diseased sunflower plant. Flusilazole was selected based on its low half-maximal effective concentration value (EC_{50}), 78.7μg/mL. Seeds of diseased plants obtained from two different locations treated with formulated Flusilazole fungicide at optimum parameters showed a significant increase in seed germination and a decrease in contamination rate from 98% to less than 10%. The results affirmed that confectionery cultivars are much more susceptible to fungal contamination than oilseeds, and also that seed pretreatment is a suitable way to prevent the spread of soil and seed-borne fungi in sunflower production.

Ahmed et al. (2018) evaluated the efficacy of three seed treating chemicals viz. Provax 200 WP, Brine solution, Rovral 50 WP and one Biofungicide viz. BAU-Biofungicide were evaluated against *Alternaria* blight of mustard caused by *Alternaria brassicae* and *Alternaria brassicicola*. Three varieties viz. var. BARI-6, Tori-7 and SAU-Shorisha-1 were used in this study. Among the seed treating chemicals, Rovral 50WP showed the best performance in reducing disease incidence and severity as well as increasing seed yield.

Naher et al. (2016) conducted a study with three seed treating fungicides viz. Vitavax 200 (0.25%), Thiovit (0.25%) and Cupravit (0.25%). Collected seed samples were tested by dry inspection and blotter method. Seed health test by blotter method revealed seed borne fungi belonging to six genera viz. *Bipolaris oryzae* (2.5 to 8.53%), *Alternaria padwickii* (5.3 to 13.35%), *Fusarium moniliforme* (11.66 to 21.67%), *Fusarium oxysporum* (1.25 to 4.35%), *Curvularia lunata* (1.95 to 7.5%) and *Aspergillus* spp. (1.75 to 6.54%) were encountered. But after seed treatment remarkable improvement viz. *Bipolaris oryzae* (0.15 to 3.75%), *Alternaria padwickii* (0.0 to 3.0%), *Fusarium moniliforme* (2.16 to 5.83%), *Fusarium oxysporum* (0.0 to 3.0%), *Curvularia lunata* (0.0 to 2.56%) and *Aspergillus* spp. (0.0 to 1.5%) were encountered. Vitavax 200 was found most effective against the seed borne pathogens of rice.

Naznine et al. (2016) were treated wheat seeds of variety Kanchan with chemical fungicides (Bavistin, Tilt and Provax), plant extracts and BAU Biofungicide while the highest (11%)

incidence of *Bipolaris sorokiniana* was recorded in untreated control and the lowest (0.0%) seed treatment with Provax and Tilt after Biofungicide. Seed germination ranged from 56.0 to 98.0% where the highest (98.0%) and lowest (56.0%) germination was recorded in case of seed treatment with Biofungicide and Tilt.

Shehzadi et al. (2016) tested seed sample of wheat varieties (Inqalab-91, Fareed 2006 and Sehar 2006) were collected from Faisalabad, Sargodha, Khanpur and Sanglahil areas of Punjab, Pakistan; and studied to detect the mycoflora associated with them. Keeping in view the importance of *Drechslera sorokiniana* the efficacy of different fungicides Topsin-M (Thiophanate Methyl 70 WP), Score (Difenoconazole) and Precure (Thiophanate-methyl + Diethofencarb 65WP) were evaluated at three different levels (20, 50 and 100 ppm). The results show that 17 different fungi species were detected from the wheat seed with varying frequency levels. *Alternaria alternata* was the most abundant fungi detected from tested wheat varieties at all locations with an incidence of 40 to 83 percent. Score at a concentration of 50 ppm was proved to be the most effective and reduced the growth of *Drechslera sorokiniana* up to 71 percent, while this reduction in case of Topsin-M and Precure was only 60 and 37 percent, respectively.

Islam et al. (2015) conducted a study with three chemical fungicides such as Vitavax 200, Bavistin and Captan. Among the used chemicals Vitavax 200 showed potential effect compared to others. However, if considerable effect of Vitavax 200 fungicide to the environment and it performance in field trail were observed Vitavax 200 could be used to control wheat seed born fungi.

Hoque et al. (2014) conducted an experiment to test fungicides like as Rovral (0.2%), Secure 600WG (0.2%), Bavistin 70WP (0.2%), Captan 50WP (0.2%) in BARI Masur-1. Around 57% healthy seed was recorded from the secure 600WG sprayed plot. Germination was 21.63% in case of Secure 600WG against 18.94% in Control. Four fungal pathogens namely *Aspergillus flavus, Aspergillus niger, Penicillium* spp. *and Fusarium* spp. were associated with lentil seeds. Among the treatments, Secure 600WG (0.2%) was found to be the most effective fungicide in controlling seed borne fungi.

Hajano et al. (2012) tested five fungicides viz. Thiophanate-methyl, Carbendazim, Fosetyle-aluminium, Mancozeb and Copper oxychloride against *Magnaporthe oryzae*. Out of these fungicides, only mancozeb appeared as the highly effective fungicide that completely inhibited the mycelia growth of the fungus. All other fungicides proved inferior as they showed little inhibitory effect even at their higher concentrations.

Khanzada et al. (2002) conducted a study by using ISTA technique seed samples of 12 wheat (*Triticum aestivum* L.) varieties were tested, seed borne fungi isolated viz., *Alternaria tenuis, Aspergillus niger, Stemphylium herbarum, Fusarium moniliforme and Curvularia lunata.* Efficacy of different fungicides was evaluated for the control of seed borne fungi associated with wheat of which Baytan, Vitavax, Benlate and Captan were found highly effective. All fungicides significantly increased the seedling emergence, number of grains per spike, 1000 grain weight, grain yield per plot and per hectare over control.

Mortuza et al. (2002) observed the effect of four fungicides as seed dress, namely Vitavex-200, Captan, Bavistin and Baytan on fungal infection, germination and nodulation against *fusarium* wilt of chickpea. The highest germination in blotter and and the field was observed in the seed treated with Vitavex-200 and Baytan. *Aspegillus niger* and *A, flavus* were completely controlled by Captan and Bavistin. Bavistin exhibited appreciable protection against *Fusarium oxysporum* in blotter as well as in the field.

Mazur et al. (2002) evaluated both *In vitro* and *In vivo* efficacy of 6 chemical fungicides Namely Amistar (Azoxystrobin 250 g/L), Topsin (70 % thiophanate-methyl), Chitosol (B-1, 4-D glucosamine polymer), Biosept (33% grapfrute extract) and Plyversum (containing 1x106/g *Pythium oligandrum* oospore) to control pathogenic fungi of chickpea (*Fusarium oxysporum, F avenaceum, F culmorum, Alternariatenuissima, A. alternate and Botrytis cinera*). Amistar was found as the most effective fungicide for tested fungi both in *in vitro* and *in vivo.*

Aveling et al. (1993) tested Ailazine, Benomyl, Carbendazim (Bavistin), Flusilazole mixture, Procymidone, Tebuconzle and Thiram for their efficacy in reducing pathogens *Alternaria porri* and *Stemphylium vescarium* of onion both on seed and in culture. An untreated control, hot water soaked and sodium hypochlorite treatment also included for comparison. Treated seeds were rated for trays in the green house. None of the treatments eradicated *Alternatia porri* and *stemphylium vescarium* from onion seed.

Khan et al. (1992) conducted an experiment to evaluate the efficacy of plant products and Benlate on mycoflora of chickpea seeds and he found the fungicide at 0.2% concentration was effective in minimizing the frequency of micoflora and also increased germination.

Gupta and Srivastava (1988) studied eight fungicides namely Copper oxychloride, Mancozeb, Capafol, Thiram, Carbendazim, Ziram, Captan and Caboxin for the control of *Stemphylium vesicarium in* onion prevented the disease.

Mortuza and Bhuya (1988) reported Pre-sowing seed treatment with Baytan10 DS and Vitavex-200 at 0.25% w/w of dry seed reduced disease in cadence 39.8% and 74% respectively.

2.3.1 Effect of fungicide against the growth of *Alternaria*

Madadi et al. (2021) conducted a study to identify the causal agent, and to test the efficacy of several fungicides with different modes of actions against *A. alternata*. Five different chemical fungicides including; Copper oxychloride (Sufer Copper Oxychloride® 50% WP), Carbendazim (Carbendazim Aria® 60% WP), Penconazole (Penconazole® 20% WE), Mancozeb (QadriMancozeb® 80% WP) and Flutriafol 6.94% + Tebuconazole 20.8% (Topgaurd® 30% SC) were tested at four different concentrations of; 20, 100, 300 and 500 mg\ l. Among the tested fungicides, the highest *in vitro* inhibition (%) of mycelial growth of the pathogen was recorded on using Flutriafol 6.94% + Tebuconazole 20.8% causing complete inhibition (100 %) at 100, 300 and 500 mg\ l, followed by Penconazole causing 100 % inhibition at 300 and 500 mg\ l; moreover, it caused strong inhibition of 90.17 % at 100 mg\ l. Both fungicides proved to be the most effective expressing strong inhibition potency, Mancozeb was the third effective fungicide causing strong mycelial inhibition of (41.91, 75.24 and 84.21 %) at 100, 300 and 500 mg\ l, respectively. The least mycelial growth inhibition was observed with Copper oxychloride (8.77 %) followed by Carbendazim (21.05 %) at maximum concentration of 500 mg\ l.

Pun et al. (2020) conducted *in vitro* experiment to evaluate the efficacy of six chemical fungicides at five different concentrations i.e. 50ppm, 100ppm, 250ppm, 500ppm and 1000ppm six botanical extracts at three concentrations i.e. 5%, 10% and 15%, and *Trichoderma harzianum* against *Alternaria brassicicola*. The study was carried out in Completely Randomized Design (CRD). Hexaconazole proved to be the most effective chemical fungicide recording 100% growth inhibition at all the tested concentrations which was at par with 1000ppm Metalaxyl + Mancozeb (Kriloxyl Gold), 500 and 1000 ppm Mancozeb and 1000 ppm Carbendazim + Mancozeb (SAAF) whereas, 50 ppm Carbendazim was least effective in reducing fungal growth (7.16%). *T. harzianum* showed 65.02% inhibition of test fungus. The study indicated better performance of some chemical fungicides even at lower concentrations i.e. 100ppm and 250ppm. So, such effective fungicides could be used to minimize hazardous effect.

Kakraliya et al. (2018) conducted an experiment under *in vitro* and field conditions to observe the effect of fungicides, botanical and bio-agents against *Alternaria triticina*. Eight treatments were taken up with three replications and data collected was analyzed using CRD. Maximum inhibition per cent mycelia growth was observed in Propiconazole (89.72%), Hexaconazole (88.44%), Vitavax (87.70%), followed by *Trichoderma harzianum* (85.50%), *Trichoderma viride* (83.30%), *Pseudomonas fluorescens* (80.73%) and neem leaf extract (73.57%) as compared to control (0).

2.3.2 Effect of fungicide on the growth of *Bipolaris*

Gupta et al. (2013) worked on the effect of seven fungicides Propiconazole, Hexaconazle, Tricylazole, Carbendazim, Triadimefon, Mancozeb and Azoxystrobin at 50, 100, 150, 200 and 250 ppm fungicide on *Bipolaris oryzae* in laboratory and conducted that Propiconazole was most effective with maximum inhibition of 97% at 250 ppm concentration. In field condition application of Propiconazle significantly reduced the disease severity (69, 73 and 70%) and increased the grain yield.

Rahman et al. (2013) evaluated the efficacy of eight fungicides viz. Tilt 250 EC (0.05%), Farzim 50 WP (0.1%), Onthan 80 WP (0.2%), Propiconazle 250 EC (0.05%), Sunconazole 250 EC (0.05%) and Dentol 80 WP (0.2%), Baizeb 80 WP (0.2%) and Shadid 250 EC (0.05%) to control the leaf bight (caused by *Bipolaris sorokiniana*) of wheat were studied under natural field condition at Bangladesh Agricultural research Institute during the year of 2005 to 2008. All the fungicides reduced the disease and increase the yield grain yield of the crop. Tilt 250 EC was found to be the best for controlling leaf blight of wheat followed by the Propiconazle 250 EC and Sunconazle 250 EC. Among the fungicide groups, Propiconazole group were found to be better than Carbendazim and Mancozeb group in terms of controlling leaf blight of wheat and economic point of view.

Gontariu and Enea (2012) found two treatments are necessary for maximum production of grain. They concluded the highest yield was obtained by following seed treatment with Amiral (Nupid) product followed by treatment with Chlorothalonil (Bravo 500 EC) in the vegetation.

Hasan et al. (2012) studied the effect of five fungicides namely Hexaconazole, Carbendazim, Mancozeb, Difenoconazle + Propiconazole and Propiconazole on *Bipolaris sorokiniana* at different concentration (100ppm, 200ppm, 300ppm, 400ppm and 500ppm) on the mycellial growth and dryweight. The hightest control was found in case of fungicides in Mancozeb (1.86g) at 400ppm concentration where lowest was found in case of fungicides Propiconazole, Hexaconazole and Difenoconazle + Propiconazole (0.00g) at all concentrations.

Yamaguchi et al. (2010) observed severity of *Bipolaris sorokiniana* was very much less in media containing Carbendazim at low concentration and mostly suppressed at higher concentration.

Yamashita et al. (2010) successfully controlled the growth of *Bipolaris maydis with* Tetraconazole and Trifloxystrobin + Propiconazole *in vitro* condition.

Ahmed et al. (2002) evaluated the effect of fungicide Bavistine, Hinosun, Tilt 250 EC and Dithane M-45 on against *Bipolaris oryzae* of rice under *in vivo* condition. All the fungicides and their different concentrations significantly inhibited the mycellial growth and effectively at high concentration. Dithane M-45 was the best with 100% reduction of prevalence of the pathogen and inhibited the mycellial growth at 0.3% of the seed weight as seed treatments and 500 ppm as mycellial growth.

2.3.3 Effect of fungicide against the growth of *Curvularia*

Abubakar et al. (2021) carried out an experiment to determine the effective fungicides and rate of application for control of fungal pathogen (*Curvalaria lunata*). Five fungicides; Mancozeb, Carbendazim, Mancozeb + Carbendazim, Benomyl and Imidacloprid + Metalxylm + Tebuconazole were evaluated *in vitro* on *Curvularia lunata* at three levels (manufacturers recommended rate, half the recommended rate and one and half the recommended rate) in a CRD in the laboratory. Mancozeb, Mancozeb+ Carbendazim and Imidacloprid + metalaxyl-m + tebuconazole proved to be the best, showing little or no mycelia growth in 2 – 14 days after inoculation. However, Mancozeb + Carbendazim (Fungu force) and Imidacloprid + Metalaxyl-m + Tebuconazole (Dress force) had the greatest inhibitory effect on mycelia growth in all the leaf blight fungal pathogens identified when applied at the manufacturers recommended rate probably being combined systemic and contact fungicides and combined systemic fungicides respectively.

Ramdan et al. (2020) conducted a study to determine the effects of various plant extracts and chemical fungicide on the germination capability, as well as the inhibition of fungal infection in calliandra seeds. Parameters observed in this study were seed germination and fungi infection percentage. Seeds were treated by immersing them in hot water (five minutes) followed by chemical fungicides (i.e., Benomyl, Mancozeb) or plant extracts (i.e., ginger, onion, garlic, turmeric) for one hour. The study design used was a completely randomized design. The results showed that all the treatments could increase seed viability significantly. Chemical treatment with concentrations of 0.5%, 2%, and 3% had a significant effect on the *Fusarium* infection level, which was higher than the control, which was 0.18%, 0.17%, and 0.19%. Meanwhile, for *Curvularia* and *Aspergillus*, physical and chemical treatments did not have a significant effect.

2.3.3 Effect of fungicide against the growth of *Aspergillus*

Nahar and Shamsi (2020) isolated twelve species of fungi from the seeds of three cotton varieties (HC-1, HC-2 and HC-3) following "Tissue planting" and "Blotter" methods Amongst five selected fungicides the complete inhibition of the radial growth of *Aspergillus flavus* was observed with Autostin 50 WDG and Nativo 75 WP at all the treated concentrations. Capvit 50 WP showed complete growth inhibition of the fungus at 300, 400 and 500 ppm concentrations. Out of five fungicides, the complete inhibition of the radial growth of *Aspergillus niger* (Type-I) was observed in Autostin 50 WDG and Nativo 75 WP at all the treated concentrations. At 100 ppm Capvit 50 WP showed the lowest inhibition (48.7%). Thiovit 80 WG showed no inhibitory activity at all.

Suharti et al. (2020) assessed the effects of various plant extracts and chemical fungicide on the germination capability, as well as the inhibition of fungal infection in calliandra seeds. Parameters observed in this study were seed germination and fungi infection percentage. Seeds were treated by immersing them in hot water (five minutes) followed by chemical fungicides (i.e., Benomyl, Mancozeb) or plant extracts (i.e., ginger, onion, garlic, turmeric) for one hour. The study design used was a completely randomized design. The results showed that all the treatments could increase seed viability significantly. The highest reduction of fungal infection by *Aspergillus* spp. on calliandra seeds were found by Mancozeb.

Masiello et al. (2019) evaluated the sensitivity of *F. graminearum, F. proliferatum, F. verticillioides,* and *A. flavus* to eleven fungicides, selected based on their different modes of action in both *in vitro* assays and, after selection, in the field. *In vitro*, Demethylation inhibitors (DMI) showed excellent performances, followed by Thiophanate-methyl and Folpet. both Succinate dehydrogenase inhibitors (SDHI), and Fludioxonil were more active against *A. flavus* than *Fusarium* species. In field trials, Prothioconazole and Boscalid could reduce *A. flavus* contamination at values of 75% and 56%, respectively.

Khan et al. (2017) treated onion (*Allium cepa* L.) seeds separately with five fungicides namely Bavistin 50 WP (Carbendazim) and Dithane M-45 (Mancozeb) @ 2.2 g/kg, Homai (Thiophanate) and Rovral (Iprodione) @ 2.0 g/kg and Provax-200 (Carboxin) @ 3.0 g/kg of seeds before storage.under laboratory condition. S Provax-200, Rovral, Dithane M-45 and Bavistin were found effective in reducing seed-borne infection of *Aspergillus* spp. (*A. niger* and *A. flavus*) of onion seeds, and improved germination and vigor index were also recorded for these four fungicides. Therefore, seed treatments with Provax200, Rovral, Bavistin or Dithane M-45 are suggested as effective fungicides to control seed borne fungi and for better germination and vigor of onion seed.

CHAPTER III

METHODOLOGY

3.1 Location of the study

The experiment was conducted at the Plant Protection Laboratory, Agrotechnology Discipline, Khulna University, Khulna, Bangladesh during January, 2021 to October 2021. Four different fungicides were used to evaluate the efficiency against seed borne fungi on germinating wheat seed.

3.2 Collection of Sample

The experiment was carried out to detect the seed borne fungi on wheat seed and find out the efficacy of fungicides to control seed-borne fungi of wheat. Wheat seed samples (Farmer's seed) of two (*Triticum aestivum* L.) varieties (BARI Gom-25 and BARI Gom-26) were collected from regional agricultural research station, Jashore.

3.3 Seed treating chemicals used in this experiment

Using fungicides and their recommended doses (0.25%) against fungal pathogens in seed were selected based on the Islam et al. (2015) protocol.

Table1. List of seed treating chemicals that were used in this experiment

Trade name	Chemical name	Mode of action	Concentration/ Dose
Knowin 50 Wp	Carbendazim	Systemic	250ppm
Sunvit 50 WP	Cupper oxychloride	contact	250ppm
Neuben 72 Wp	64% Mancozeb +8 % Metalaxil	contact	250ppm
Tilt 250 EC	Propiconazle	Systemic	250ppm

3.3 Seed treating Method

3.3.1 Blotter method

According to International Seed Testing Association (2001) the percentage seed germination and fungal association were determined. From each sample 400 seeds were taken randomly for the test. Seeds were placed on three-layered blotting paper moistened with sterilized water in glass petridish (9cm) (120°C for 12 hours in oven) by using sterilized forceps. Each petrideish contained 25 seeds. Then petridishes were kept at room temperature under 12/12 hours light and dark for seven days.

3.4 Preparation of fungicidal solutions for seed treatment

In this method, four different types of fungicides viz Knowin 50WP (0.25%), Sunvit 50 WP (0.25%), Neuben 72 WP (0.25%) and Tilt 250 EC (0.25%) were taken and poured in four different conical flask. According to Islam et al. (2015)100ml distilled water was poured in each conical flask and shaken continuously to make stock solution. Then 250ppm working sample for each fungicide like as Knowin 50 WP, Sunvit 50 WP, Neuben 72 WP and Tilt 250 EC solutions were made and used for seed treatment.

3.5 Seed treatment with chemicals

In this method, four hundred seeds were randomly taken from each sample. The four fungicides namely, Knowin 50 WP, Sunvit 50 WP, Neuben 72 WP and Tilt 250 EC were tested at 250 ppm. Requisite amount of each fungicide and seeds from each sample were taken in a 250 ml Erlenmeyer flask and were shaken mechanically for 10 minutes for proper coating of fungicides. Then excess chemical drained off and moistened treated seeds were air dried to remove excess moisture from the seed surface. Then seeds were ready for plating. Seed without any treatment served as control (Islam et al. 2015). The efficacy of the 4 test fungicide, were evaluated by comparing with an untreated control using the Standard Blotter Method (ISTA, 2001).

3.6 Plating the treated seeds on petridish

The petridish were first cleaned with distilled water, dried and then strelized in an oven. Petri dishes were taken and put on the accession number, date was written on the petrideish. A set of three sterilized filter paper were dipped in sterilized water to provide sufficient moisture for the germination of seed. The seeds were placed on water soaked three layered Whatman No. 1 filter paper at the bottom of 9 cm diameter petridish. In each petridish, 25 seeds were plated at equal distance using a sterilized forceps. The petridishes containing treated seeds were then incubated at 25±2°C under 12 hours alternate cycle of light and darkness. After 7 days of incubation, seeds were observed under stereomicroscope and preparing temporary slide for confirmation of identified fungi.

3.7 Germination test of treated seed

Four hundred seeds per treatment were used for germination test. Seeds were treated as described above. Untreated seeds were also subjected to similar germination test to serve as control. Germinated seeds were counted manually after 7 days of germination according to ISTA, (2007) protocol.

3.8 Examination of incubated seeds and recording of infectious fungi

Numbers of germinated seeds were recorded along with the seed-borne fungi after seven days of incubation. Count of each fungus from each dish was entered in working recording sheets immediately after examination of the dish (Mathur and Kongsdal, 1994; ISTA, 2007) the results were expressed in percentage.

3.9 Morphological Identification of fungi in seed

After 7 days of incubation, each incubated seed was observed under stereomicroscope. Reproduction characters of each fungus were observed for identification. Where identification was difficult or doubtful under the stereomicroscope, temporary slide was prepared and examined under the compound microscope and identified with the help of keys (Chidambarain et al. 1973). Image of conidia ofungi was taken by using Carl Zeiss Software.

3.10 Calculation of germination percentage in blotter method

Four hundred seeds were used in each treatment and within the four hundred seeds germination percentage was calculated using the following formula-

$$\text{Germination (\%)} = \frac{\textit{Number of seeds germinated}}{\textit{Total number of seeds placed in experiment}} \times 100$$

3.11 Calculation of reduction percentage of fungal association

Reduction percentage of fungal association was calculated based on the Islam et al. (2015) protocol using following formula-

% Reduction of fungal association = 100 - Total No. of % seed borne fungi

3.12 Experimental design and statistical analysis

The experiments were conducted following the Completely Randomized Design (CRD). Analysis of variance and the mean differences were done to determine the efficacy of the treatments by using Statistical Tool for Agricultural Research program (STAR, IRRI, Philippines) software. The noticeable and significant differences among the results were compared by Least Significant Difference (LSD) Test.

CHAPTER IV

RESULTS AND DISCUSSION

In the present study, efficacy of four different fungicides has been governed on the germinating wheat seed at 250ppm concentration against seed borne fungi of wheat. The collected data on different parameter of the present study were analyzed statistically and the results have been presented and discussed in this chapter under following headings.

4.1 Identification of seed borne fungi

In this present study two different varieties of seeds BARI Gom-25 and BARI Gom-26 were investigated to identify the seed borne fungi. Seeds of these varieties incubated for seven days on wet blotter paper. After seven days of incubation the yielded fungi were detected and then identified (Table 2). The fungi yielded were *Alternaria*spp. *Bipolaris* spp. *Curvularia* spp. and*Aspergillus* spp.In this experiment, only these major ones were taken into account.

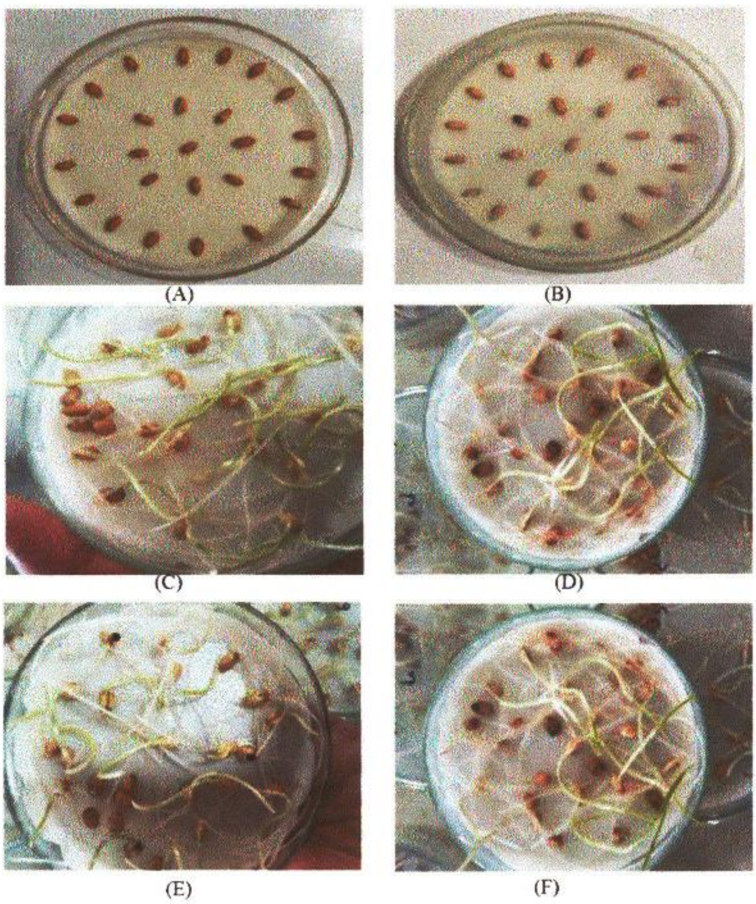
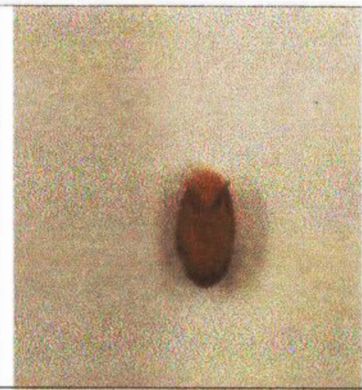
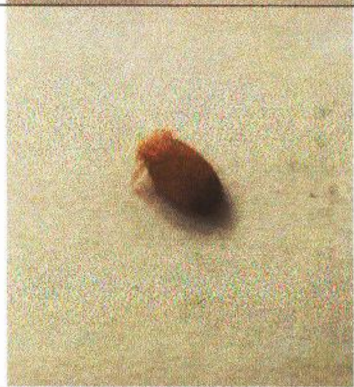
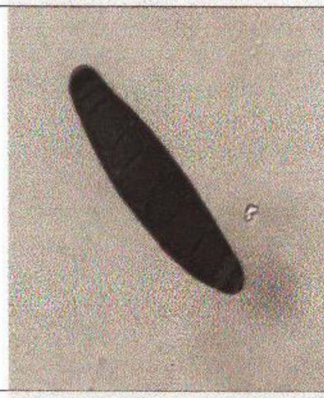
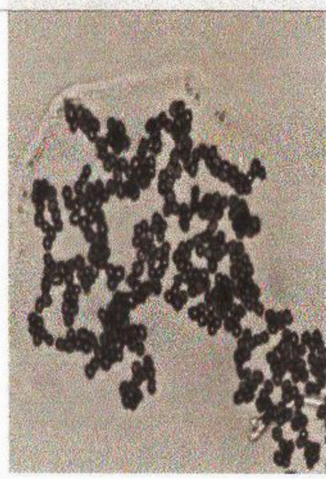


Figure 1. Incubated Seed of BARI Gom-25 (A) and BARI Gom-26 (B) treated with Tilt 250 EC (C) Sunvit 50 WP (D) Neuben 72 WP (E) Knowin 50 WP (F)

Table 2. List of associated fungal genera with their identifying characteristics

Sl. No.	Identified Fungal Genera	Infected Seed	Fruiting Body of Identified Fungi	Identification Key Book
1	*Alternaria* spp.			Illustrated Genera Of Imperfect Fungi (Barnett, 1972); Basic Plant Pathology Method (Dingra, and Sinclair, 1986).
2	*Bipolaris* spp.			Illustrated Genera Of Imperfect Fungi (Barnett, 1972); Basic Plant Pathology Method (Dingra, and Sinclair, 1986).
3	*Curvularia* spp.			Illustrated Genera Of Imperfect Fungi (Barnett, 1972); Basic Plant Pathology Method (Dingra, and Sinclair, 1986).
4	*Aspergillus* spp.			Illustrated Genera Of Imperfect Fungi (Barnett, 1972); Basic Plant Pathology Method (Dingra, and Sinclair, 1986).

4.2 Effect of seed treating chemicals against associated fungi on BARI Gom-25

Four fungicides namely Knowin 50 WP, Sunvit 50 WP, Neuben 72 WP and Tilt 250 EC were used to treat seed in this experiment. The effect of different fungicides on germination percentages and fungal association on BARI Gom-25 was presented in figure 2 and table 3.

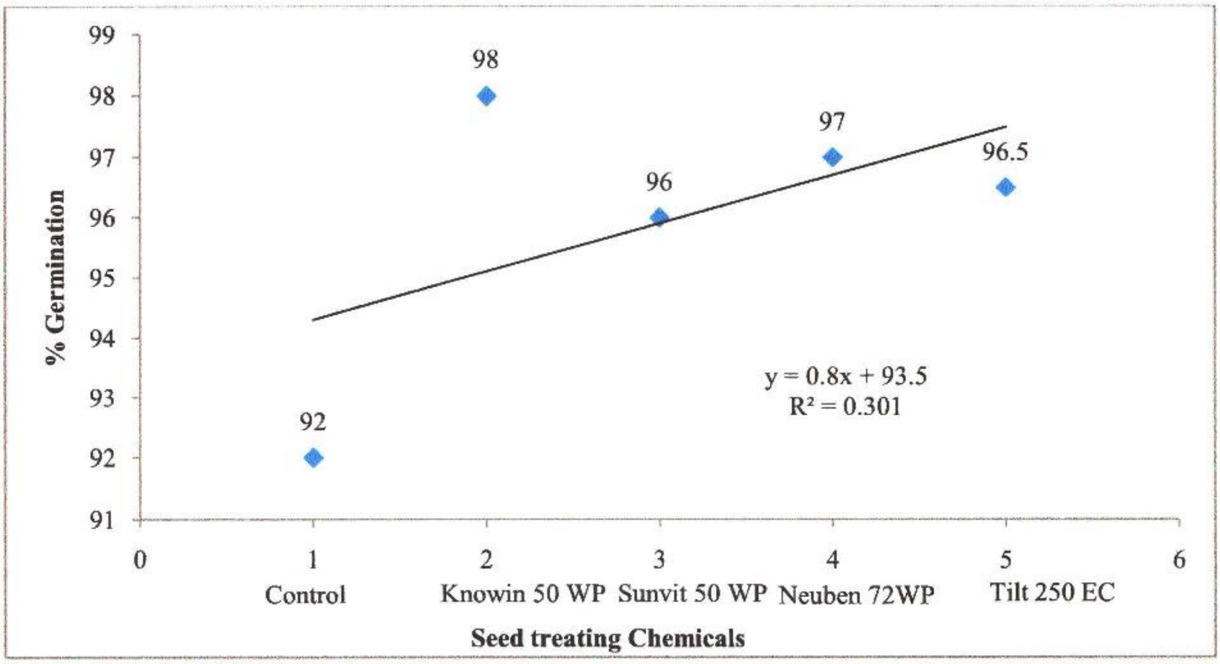


Figure 2. Effect of seed treating chemicals on germination percentage of BARI Gom-25

It was observed that the treated seeds showed significantly higher rate of germination than untreated seed (control) (Figure 2). Germination ranged from 92% to 98%. The highest 98% germination was recorded in Knowin 50 WP treated seed and the lowest was 92% found in control treatment. But according to the gazette published by Seed Wing, Ministry of Agriculture, Government of the Peoples Republic of Bangladesh (2010) the minimum percent germination for seed standardize 96%. So the germination percentage should be increased by treating seeds. The percent of germination was positively linearly correlated with the seed treating chemicals. Their relationship could be explained by regression equation Y=93.5+0.8X, where Y= estimated germination and X= regression coefficient. The correlation coefficient was r=0.301(Fig.2).

Table 3. Effect of seed treating chemicals against fungi associated with BARI Gom-25 at 250ppm concentration

Treatments	Seed Borne Fungi			
	Alternaria spp.	*Bipolaris* spp.	*Curvularia* spp.	*Aspergillus* spp.
Control (no treatment)	15.50a	12.50a	7.25a	13.25a
Knowin 50 WP	7.00b	4.00b	2.50b	5.00b
Sunvit 50 WP	5.75c	2.75c	2.25b	5.00b
Neuben 72 WP	2.50d	2.25c	1.00c	2.00c
Tilt 250 EC	2.00d	1.00d	0.00d	2.00c
Level of significance	**	**	**	**
LSD	0.64	0.64	0.61	0.33

** =1% level of significance

The effects of different fungicidal treatments on associated seed borne fungi are presented in Table 3. All the treatments for controlling these four fungi were significant at 1% level of significance.

In case of *Alternaria* spp. (15.50) maximum association was observed in control treatment and minimum association was found by using Tilt 250 EC (2.00) and Neuben 72 WP (2.50) both of which were statistically similar (Table 3).

The lowest association of *Bipolaris* spp. (1.0) was observed when seeds were treated with Tilt 250EC followed by Neuben 72 WP (2.25) which was statistically similar with Sunvit 50 WP (2.75). The highest association of *Bipolaris* spp. (12.50) was found in control treatment.

The lowest association of *Curvularia* spp. (0) was observed when seeds were treated with Tilt 250EC. The highest association of *Curvularia* spp. (7.25) was found in control treatment. Whereas, Knowin 50 WP (2.50) and Sunvit 50 WP (2.25) gave the same result by controlling *Curvularia* spp.

In case of *Aspergillus* spp. (13.25) maximum association was observed in control treatment and minimum association was found by applying Tilt 250 EC (2.00) which was statistically similar with Neuben 72 WP (2.00). Performance of knowin 50 WP (5.00) was statistically similar with Sunvit 50 WP (5.00).

4.3 Effect of seed treating chemicals against associated fungi on BARI Gom-26

The effect of different fungicides on germination percentages and fungal association on BARI Gom-26 was presented in figure 3 and table 4 respectively.

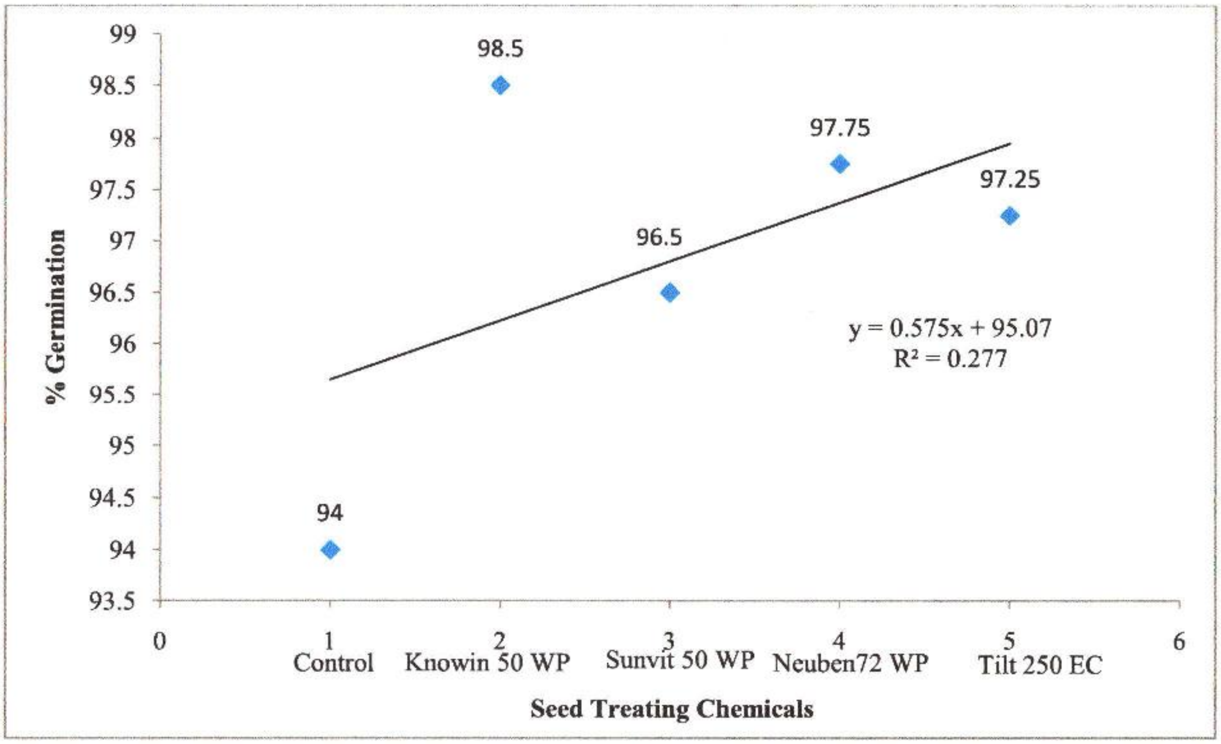


Figure 3. Effect of different seed treatments on germination percentage in BARI Gom-26

The effect of different treatment on germination percentages of BARI Gom-26 ranged from 94% to 98.50%. The highest 98.50% germination was recorded in Knowin 50 WP treated seed followed by Neuben 72 W (97.75%), Tilt (97.25), Sunvit 50 WP (96.50%) and the lowest 94% was found in control treatment. The percent of germination was positively linearly correlated with the seed treating chemicals. Their relationship could be explained by regression equation Y=95.07+0.575X, where Y= estimated germination and X= regression coefficient. The correlation coefficient was r=0.277(Fig.3).

Table 4. Effect of seed treating chemicals against fungi associated with BARI Gom-26 at 250ppm concentration

Treatments	Seed Borne Fungi			
	Alternaria spp.	*Bipolaris* spp.	*Curvularia* spp.	*Aspergillus* spp.
Control (no treatment)	12.75a	11.25a	5.25a	8.25a
Knowin 50 WP	5.00b	3.25b	3.00b	3.75b
Sunvit 50 WP	4.00c	2.75b	2.25c	3.50bc
Neuben 72 WP	3.75c	2.50b	1.00d	3.00c
Tilt 250 EC	2.00d	0.50c	0.00e	1.00d
Level of significance	**	**	**	**
LSD	0.4766	0.80	0.47	0.61

** =1% level of significance

All the treatments for controlling associated fungi were significant at 1% level of significance (Table 4).

The effects of different fungicidal treatments on associated seed borne fungi are presented in Table 4. In case of *Alternaria* spp. the highest association (12.75) was observed in control treatment and minimum association was found by applying Tilt 250 EC (2.00) followed by Neuben 72 WP (3.75) which was statistically similar with Sunvit 50 WP (4.00).

The lowest association of *Bipolaris* spp. (0.50) was observed when seeds were treated with Tilt 250 EC and the highest association of *Bipolaris* spp. (11.25) was found in control treatment. Statistically similar performance was observed by using Neuben 72 WP (2.50), Sunvit 50 WP (2.75) and Knowin 50 WP (3.25) against *Bipolaris* spp.

The lowest association of *Curvularia* spp. (0) was observed when seeds were treated with Tilt 250 EC. The highest association of *Curvularia* spp. (5.25) was found in control treatment. Statistically different performance was observed by using Neuben 72 WP (1.00), Sunvit 50 WP (2.25) and Knowin 50 WP (3.00) against *Curvularia* spp.

In case of *Aspergillus* spp. (8.25) highest association was observed in control treatment and minimum association was found by applying Tilt 250 EC (2.00). Statistically different result was observed by using Neuben 72 WP (3.00), Sunvit 50 WP (3.50) and Knowin 50 WP (3.75) against *Aspergillus* spp.

4.4 Reduction of fungal association on BARI Gom-25

The maximum reduction in fungal association on BARI Gom-25 was found (95%) by using Tilt 250 EC, followed by Neuben (93.25%). The lowest reduction was found in control (51.5 %). Sunvit 50 WP was also effective and reduced at 84.25% fungal infection in seed. Knowin 50 WP showed the lowest reduction of fungal association (81.5%) among the four tested chemicals. It was clear that Tilt 250 EC was significantly effective as a seed treating chemical in reducing fungal associations (Figure 4).

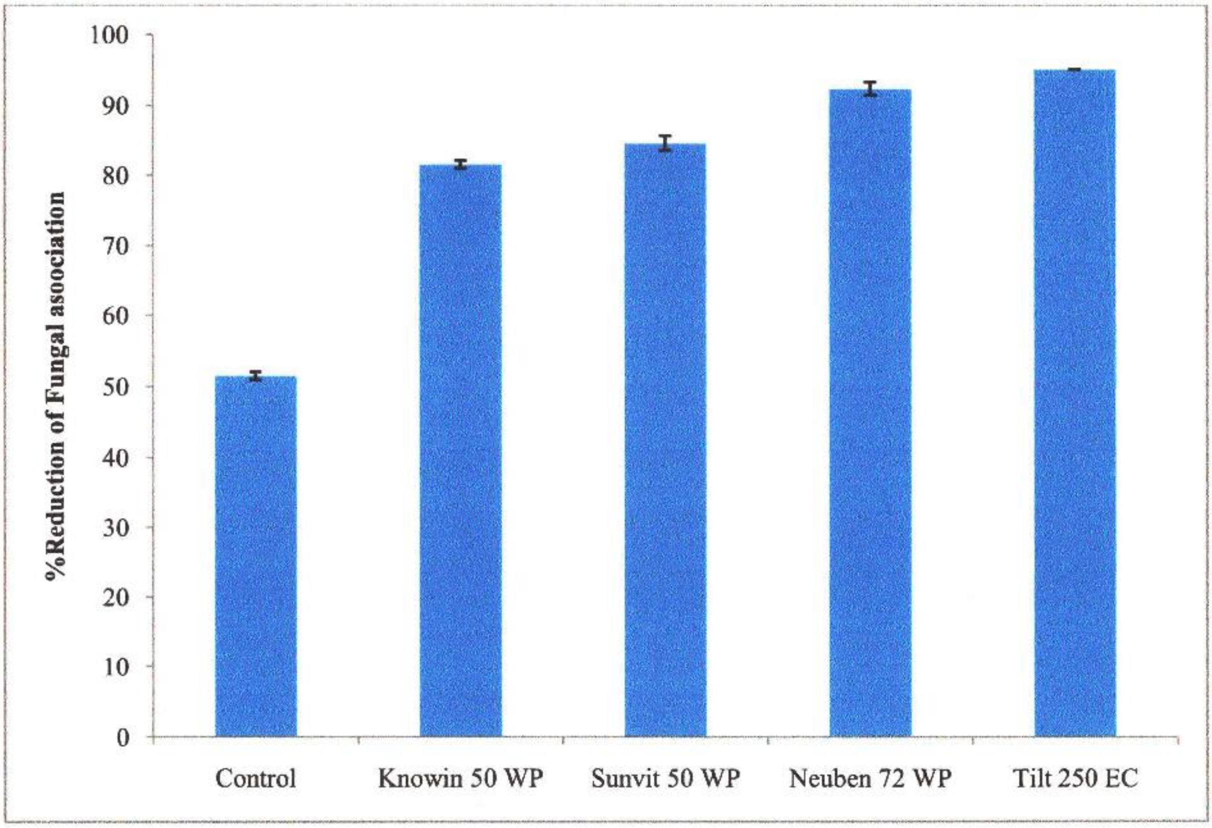


Figure 4. Effect of seed treatment on reduction of fungal association on BARI Gom-25

4.5 Reduction of fungal association on BARI Gom-26

In case of BARI Gom 26, the highest fungal association was 37.5% found in control and the lowest fungal association was 3 % when seed was treated with Tilt 250 EC. The maximum reduction in fungal association in BARI Gom-25 was found 97% by using Tilt 250 EC. Neuben (89.75%) and Sunvit 50 WP was also effective and reduced (87.50%) fungal association. Knowin 50 WP showed the lowest reduction of fungal association among all the tested chemicals and reduction percentage of fungal association was 85% (Fig. 5).

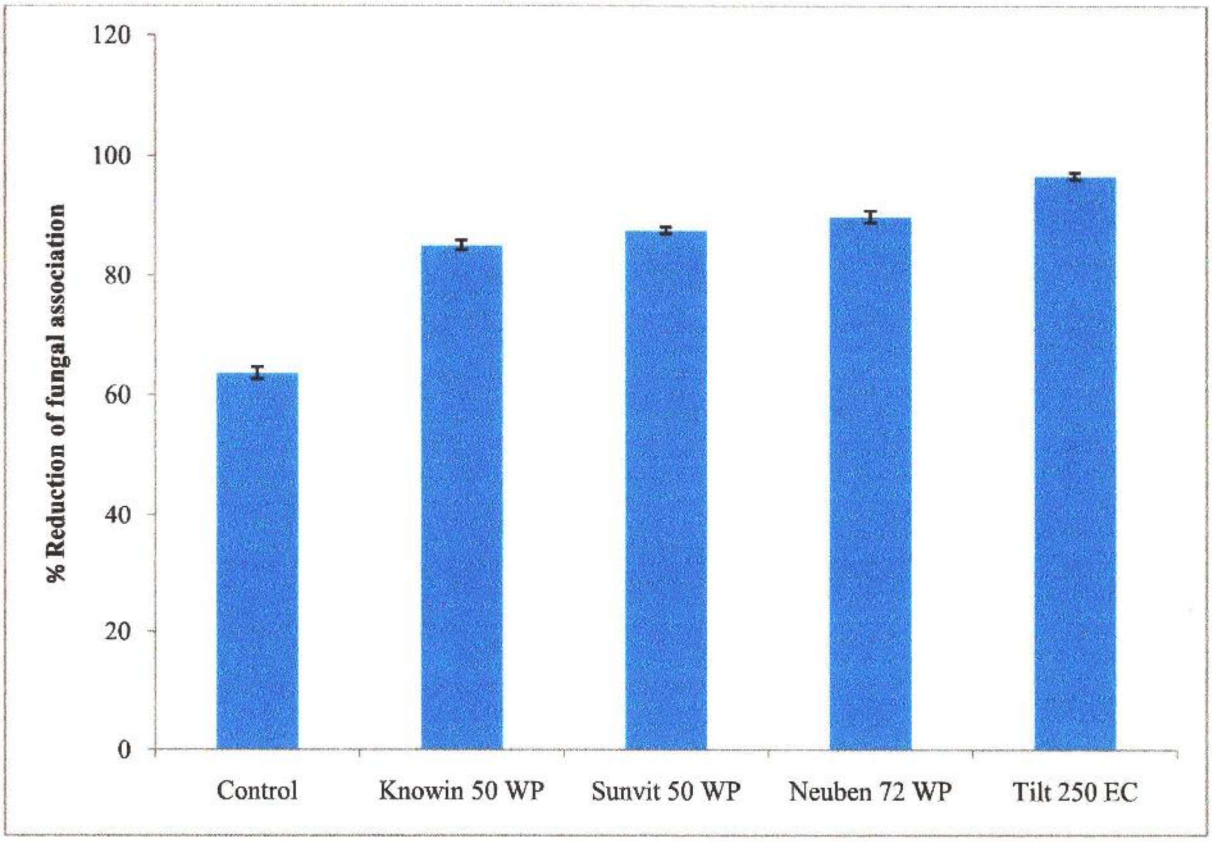


Figure 5. Effect of seed treatment on reduction of fungal association on BARI Gom-26

4.6 Discussion

In the present investigation, four different treatments including a control were tested in blotter method, where their effect on germination and seed borne fungi were evaluated. All the treatments showed superior influence on germination of seeds over control (Figure 2 and 3). Percent seed germination was increased in treated seeds compared to untreated seeds. The seed treated with the Knowin 50 WP showed highest percentage of germination in BARI Gom-25 (98%) and BARI Gom-26 (98.5%) and the other three fungicides were also found to increase the germination percentages over control in both varieties of wheat. They were statistically close with Knowin 50 WP.

In this experiment, the highest germination was recorded in case of seed treatment with Knowin 50 WP and the lowest germination was found in seed treatment with Sunvit 50 WP and Tilt 250 EC. Among the treatments Knowin 50 WP showed superior influence on germination of wheat variety over untreated control in Blotter method. Up to 6% in BARI Gom-25 and 4.5 % in BARI Gom-26 higher germination in Blotter method, respectively over control were achieved by treating seeds with knowin 50 WP. This result supported by Naznine et al. (2016) and Islam et al. (2015) who observed increased germination 4 to 12.5 % over untreated control. The minimum germination % was found in seed treated with Tilt 250EC.

The seed treating chemicals Knowin 50 WP, showed satisfactory effect in increasing germination of wheat seed. This result supported by Bhuiyan et al. (2013) who reported that Bavistin reduced seed-borne infection and increased seed germination over control. But in case of Tilt 250 EC increase of germination percentages of seed was not satisfactory, it reduced seed germination in Blotter. Naznine et al. (2016) also reported reduction of germination percentage with Tilt 250 EC treated seed. The present finding also supported by Singh (2011), who reported 66.6% germination was reduced in using Tilt 250 EC as seed treating chemicals.

Identification of seed-borne fungi from wheat seeds were done through standard blotter method. It was found that seed-borne fungi from the seeds may be destructive during germination of seeds or may be bringing about mortality soon after the emergence of seedlings, so it is necessary that seeds should be treated by chemicals before planting. Among seed-borne fungi *Alternaria* spp. was found most frequent among the identified fungi during incubation followed by *Bipolaris* spp,. *Aspergillus* spp, and *Curvularia* spp. Management of seed-borne fungi using fungicides helps in producing better emergence and vigorous of seedlings as seed protectants. The application of chemicals to the seed is a common and effective means for controlling the majority of seed-borne pathogens. From the result it is evident that seed treating chemical Tilt 250 EC had remarkable effect in reducing the association of fungi in case of BARI gom-26 (97%) and BARI gom-25 (95%) (Fig.4 & Fig. 5). Kakraliya et al. (2018) also found remarkable effect of fungicides, botanical and bio-agents against *Alternaria triticina* (89.72%) under field and *in vitro* conditions .Gupta et al. (2013) gave another report on the effect of seven fungicides namely propiconazole, hexaconazle, tricylazole, carbendazim, triadimefon, mancozeb and azoxystrobin on *Bipolaris oryzae* in laboratory. They also disclose fungicides were the most effective management of seed borne fungi. Neuben 72 WP also appeared as effective to control the seed-borne fungi of wheat. Neuben 72 WP reduced fungal association in case of BARI Gom-25 (93.25%) and BARI Gom-26 (89.75%) which is more or less in agreement with the findings of Pun et al. (2020); Suharti et al. (2020) and Hasan et al. (2012) findings. Fungicide Knowin 50 WP is effective in increasing germination of wheat seed. Tilt 250 EC is effective in reduction of fungal association.

CHAPTER V

SUMMERY AND CONCLUSION

5.1 Summary

Seeds stored by farmers for next growing season carry important seed borne fungi. In general storage condition with high temperature and high relative humidity of seed provided by farmer is the most favorable for the seed borne fungi resulting in poor germination. In Bangladesh the yield of wheat is low compared to other countries. The present study was conducted to detect seed borne fungi on wheat seed and evaluate the seed treating chemicals against seed borne fungi of wheat collected from RARS, Jashore. The research work carried out in the plant protection laboratory, Agrotechnology Discipline, Khulna University Khulna. The experiment conducted in completely randomized design (CRD) with two wheat varieties namely BARI Gom- 25 and BARI Gom-26. Four chemical fungicides Knowin 50 WP, Sunvit 50 WP, Neuben 72 WP and Tilt 250 EC were tested against seed borne fungi.

In case of seed germination between two wheat varieties, the highest germination percentage was occurred in BARI Gom-26. There was significant effect of chemical fungicides on germination percentage of wheat seed. All the fungicides increased seed germination percentage as compared to untreated control. The highest germination was 98.50% was observed when seeds were treated with Knowin 50 WP, which is 4.5% higher than the lowest germination found in Bari Gom-26 with control followed by Neuben 72 WP showed comparatively better performances than others fungicides, increased (3.75%) germination rate. The similar rate of germination increase (3.25%) found in Tilt 250 EC. And the lowest increase in germination rate was by sunvit 50 WP (2.50%). In case of BARI Gom-25, the highest germination was (98%) was observed when seed treated with Knowin 50 WP, which is 6% higher than the lowest germination found in Bari Gom-25 with control followed by Neuben 72 WP (97%) increased (5%) germination rate. Tilt 250 EC (96.5%) and sunvit 50 WP (96%) showed the lowest increase in germination rate.

The result of the present investigation reveals that variable associations of different fungi exist in the collected seed of wheat varieties which was preserved by the farmers for next year sowing. In blotter method four different fungi were identified and BARI Gom-26 showed lower fungal association than BARI Gom-25. The identified fungi were *Alternaria* spp., *Bipolaris* spp., *Curvularia* spp. and *Aspergillus* spp. The prevalence of different seed borne fungi was found to vary independently and individually in the wheat varieties.

The highest incidence of association was recorded by *Alternaria* spp. The maximum association by *Alternaria* spp. found in case of control in BARI Gom-25 (15.50%) and BARI Gom-26(12.75%), this is (2-7) % higher than other fungal association. With treatment the minimum association by *Alternaria* spp. was found in case of treated seed with Tilt 250 EC, which was (2.00%) in both BARI Gom-25 and Bari Gom-26. Tilt 250 EC redudced (10-13%) more fungal association by *Alternaria* spp. than other treatments. In case of both variety the lowest incidence of association observed by *Curvularia* spp. The maximum association by *Curvularia* spp. found in control, this included BARI Gom-25 (7.25%) and BARI Gom-26 (5.25%). that is also (3-5) % lower than other fungal association. The least amount of association by *Curvularia* spp. found in case seed treated with Tilt 250 EC, it was (0.00%) in both variety. Tilt 250 EC reduced (7-5%) more fungal association by *Curvularia* spp. than other treatments.

The maximum association by *Bipolaris* spp. found in case of control, which was in BARI Gom-25 (12.50%) and BARI Gom-26 (11.25%). The minimum association by *Bipolaris* spp. was found in case seed treatment with Tilt 250 EC, which was in BARI Gom-25 (1.00%) and in BARI Gom-26 (0%). Tilt 250 EC reduced (6-4%) more fungal association by *Bipolaris* spp. than other treatments. In case of *Aspergillus* spp. the maximum association by *Aspergillus* spp found in case of control, which was in BARI Gom-25 (13.25%) and BARI Gom-26 (8.25%). The minimum association by *Aspergillus* spp. found in case of seed treated with Tilt 250 EC, which was in BARI Gom-25 (2.00%) and Bari Gom-26 (1.00%).

In respect to efficacy, among the tested fungicides Tilt 250 EC was the best in controlling the major seed borne fungi of wheat. In case of *Bipolaris* spp. and *Curvularia* spp. Tilt 250 EC reduced 99.50- 100 % fungal association and in case of *Alternaria* spp. and *Aspergillus* spp. it reduced 98-99 % fungal association. After that Neuben 72 WP fungicide showed better performance than others.

5.2 Conclusion

- All the fungicides used for the study showed significantly increased seed germination percentage following blotter method.

- Knowin 50 WP was the most effective fungicide for increasing germination percentage in BARI Gom-26 (98.50%) and BARI Gom-25 (98%) among the tested fungicide, which indicated 4.5-6.0 % higher than control.

- In blotter method, the identified fungi were *Alternaria* spp., *Bipolaris* spp., *Curvularia* spp. and *Aspergillus* spp. and the association of fungi were higher in BARI Gom-25.

- Among the fungi, *Alternaria* spp. was the most prevalent and *Curvularia* spp. was the least prevalent in both varieties of wheat seed.

- Tilt 250 EC showed remarkable effect in reducing fungal association in BARI Gom-26 (97%) and BARI Gom-25 (95%), which was 2.75-13.5 % higher than other fungicides.

CHAPTER VI

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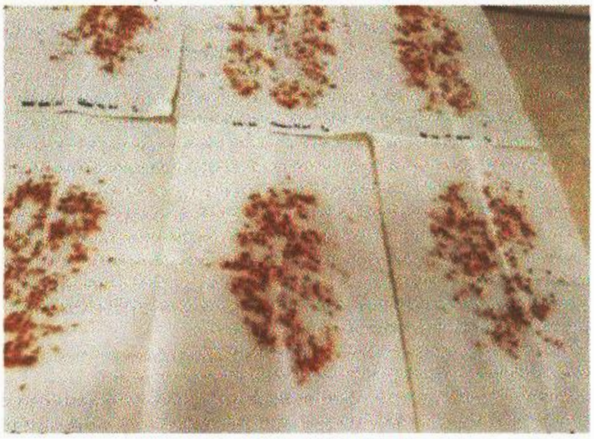
APENDIX

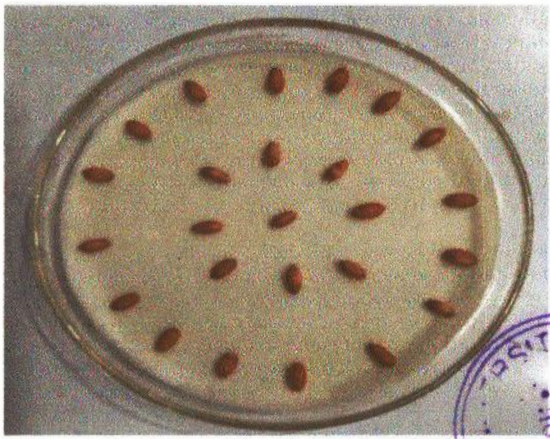
SOME PICTORIAL VIEW OF RESEARCH

Collection of sample

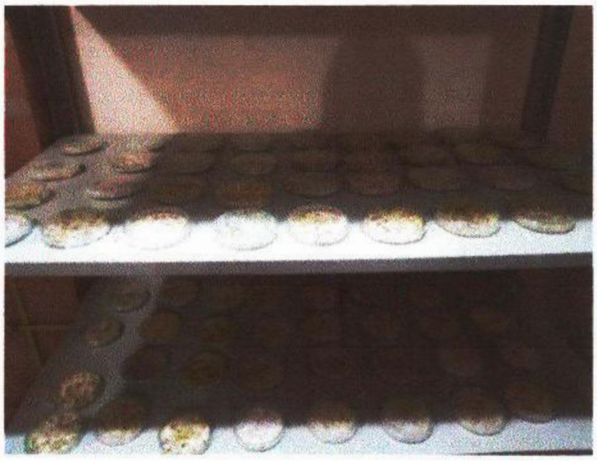
Preparation of fungicidal solutions



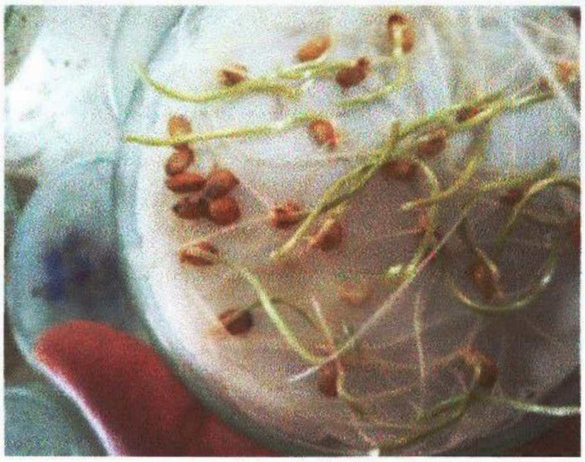
Seed treatment with chemicals & drying



Plating of seeds



Incubation for 7days



Germination test of treated seed