STANDARDIZATION OF WATER TEMPERATURE AND TIME IN CONTROLLING MAJOR SEED BORNE PATHOGENS OF RICE, WHEAT AND MAIZE

MD. KAUSER ALI

REGISTRATION NO: 15-06477

INSTITUTE OF SEED TECHNOLOGY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

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Approved by:

Prof. Dr. Md. Rafiqul Islam
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Supervisor

Prof. Abu Noman Faruq Ahmmed
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Co-supervisor

Prof. Dr. Md. Ismail Hossain
Chairman
Examination Committee
Director
Institute of Seed Technology

Institute of Seed Technology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207
PABX: 99110351 & 9144270-79

CERTIFICATE

This is to certify that the thesis entitled, **“Standardization of water temperature and time in controlling major seed borne pathogens of rice, wheat and maize”** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **Master of Science** in **Institute of Seed Technology,** embodies the result of a piece of research work carried out by Registration **No. 15-06477** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or sources of information, as have been availed of during the course of this investigation has duly been acknowledged.

Dated: June, 2022
Place: Dhaka, Bangladesh

Prof. Dr. Md. Rafiqul Islam
Supervisor
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Dhaka-1207

DEDICATED TO

MY

BELOVED PARENTS

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SAU, Dhaka **The Author**

Standardization of water temperature and time in controlling major seed borne pathogens of rice, wheat and maize

ABSTRACT

An investigation was conducted on hot water seed treatment of rice, wheat and maize seeds in controlling seed infection for proper germination during the period from July 2021 to December 2022, at the central laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207.Suitable temperature and time for seed treatment were determined. Irrespective to the cereal seeds, the temperature and time of treatment had shown significant effect on reduction of seed infection and increase of seed germination. The hot water treatment also had remarkable effect on rotten seeds, dead seeds and abnormal seedlings. The experimental treatments were T_1= Hot water at 52℃ for 5 and 10 min, T_2= Hot water at 54℃ for 5 and 10 min, T_3= Hot water at 56°C for 5 and 10 min, T_4= Hot water at 58℃ for 5 and 10 min, T_5= Hot water at 60℃ for 5 and 10 min and T_6= Control (Using normal water at ambient temperature (25±1°C)). Data were collected on % seed germination, % seed infection, % rotten seed and % dead seed. The experiments were conducted following Completely Randomized Design (CRD) with 4 replications. In case of rice seed, the highest seed germination (91%) and the lowest seed infection (0%) were noted at 56°C for 5 min while (94%) seed germination and (0%) seed infection were recorded at 54°C for 10 min. In case of wheat seed, the highest seed germination (88%) and the lowest seed infection (0%) were recorded at 54°C for 5 min while it was (90%) seed germination and (0%) seed infection at 52°C for 10 min. In case of maize seed, the highest seed germination and the lowest seed infection respectively were (82%) and (0%) at 58°C for 5 min while it was (84%) seed germination and (0%) seed infection at 56°C temperature for 10 min. In case of rice, *Fusarium spp* and in case of wheat & maize, *Bipolaris spp* was found. Irrespective to the cereal seeds, the seed infection was found to be reduced with the increase of temperature and time. The percent dead seed also increased with the increase of temperature. Thus, the rice seed should be treated with hot water at 56°C for 5 min or 54°C temperature for 10 min. In case of wheat seed, farmers are suggested to treat with hot water at 54°C for 5 min or 52°C temperature for 10 min while for maize seed, farmers are recommended to go through hot water at 60°C temperature for 5 min or 56°C temperature for 10 min for getting the highest seed germination minimising the seed infection.

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LIST OF ABBREVIATION AND ACRONYMS

AEZ	=	Agro-Ecological Zone
BARI	=	Bangladesh Agricultural Research Institute
BBS	=	Bangladesh Bureau of Statistics
FAO	=	Food and Agriculture Organization
et al.	=	And others
DAS	=	Days after Sowing
Mg	=	Milligram
kg	=	Kilogram
SAU	=	Sher-e-Bangla Agricultural University
SRDI	=	Soil Resources Development Institute
g	=	Gram
cm	=	Centimeter
wt	=	Weight
LSD	=	Least Significant Difference
^{0}C	=	Degree Celsius
NS	=	Not significant
Max	=	Maximum
Min	=	Minimum
%	=	Percent
NPK	=	Nitrogen, Phosphorus and Potassium
CV%	=	Percentage of Coefficient of Variance

CHAPPTER 1
INTRODUCTION

About 90% of all food crops grown on earth are propagated by seed. Important cereal crops like rice, wheat, maize, are attacked by devastating seed-borne diseases. So seed treatment is very important for preserving seed and good yield. Seed treatment or seed dressing with chemical or typically antimicrobial agents prior to planting are suggested (Koch and Roberts, 2014). In Bangladesh un-judicious use of chemical as seed treatment or insecticide is a big problem and it is polluting our environment day by day. Eco-friendly seed treatment with non-chemicals like *Trichoderma spp*, plant extracts, hot water treatment without causing any harmful effect on environment is crucially preferable.

Rice (*Oryza sativa*) is the first most important grain crops in Bangladesh. It suffers from more than 33 diseases, of which 17 are known to be seedborne (Fakir, 2000). Among the seed-borne pathogens *Alternaria pudwickii, Bipolaris oryzae, Curvularia lunata* and *Fusarium moniliforme* are considered as the most damaging and frequently transmitted by rice seeds (Mew and Gonzales, 2002 and Fakir, 2000). Apparently 10 % production loss of rice may be incurred annually due to seed borne diseases in the country. According to this guesstimate around 2.19 million tons of rice worth Tk. 13140 million is lost annually in Bangladesh (Fakir, 2000).

Wheat (*Triticum aestivum* L.) is the second most important grain crop in Bangladesh that plays a vital role in the national economy by reducing the volume of imported cereals (Perelló *et al.*, 2013). In spite of its importance, the yield of the crop in our country is low in comparison to the other countries of the world, where average yield estimated 4.5-7.0 t/ha (FAO, 2022). There are many constraints responsible for low yield of wheat in Bangladesh. Use of unhealthy or diseased seed is one of the major constraints. Wheat suffers from as many as 26 pathogens causing, 14 seed-borne diseases (Fakir, 2000a). Among them leaf spot, leaf blight and black point caused by *Bipolaris sorokiniana* has become a serious concern (Fakir, 2000). Considering 10% production loss of wheat suffered by this disease, approximately 17.50metric tons of wheat worth more than Tk. l400 million is lost annually in Bangladesh (Fakir, 2000).

Maize is the 2nd important cereal crop in the world as well as in Bangladesh, has a good potential, due to its low cost of production wide range of adaptability for both season cultivation and multipurpose use. The average yield of the crop in our country is lower in comparison with that of the world though its production area is increasing day by day. There are many causes of low maize yield of which diseases play a significant role. Moreover seed-borne diseases cause enormous losses both in storage as well as in the field. A total of 112 diseases are known to occur maize crops (USDA, 2019) and among them more than 70 are seed-borne. Important seed-borne diseases of maize are leaf spot, leaf blight, Collar rot, kernel rot, scutellum rot, seedling blight, anthracnose and head smut (Lizárraga-Paulín, *et al.,* 2013). In Bangladesh Fakir (2001) listed 11 seed-borne diseases on the crop. Seed health is a well-recognized factor in the modern agricultural science for desired plant population and good harvest. Seed treatment by chemicals is the best way to keep good seed health condition. But chemicals cause health hazard and environment pollution (Chapman and Harris, 1981).

Seed-borne diseases can be condensed by various control measures viz. use of chemical treatment, use of resistant varieties, cultural practices and physical treatment etc. Among the control measures, chemical treatment and use of resistant varieties are two most widely used practices for crop disease management. Among the practices used, seed treatment is the best way to control seed-borne diseases. Chemical seed treatment in general results in accumulation of harmful chemical residues in soil as well as in the plant product causing serious health hazard. Fungicide also causes environment pollution, obviously develop tolerance of the pathogen and also very costly (Kashiwagi, 2017).

Alternative means of seed treatment have drawn the attention of plant pathologists all over the world. In this outlook, use of hot water treatment in seed may become easy, less costly technology in controlling seed borne pathogens. Hot water treatment used since 1920 has been little used in practice (Forsberg, 2002). Then a modem Hot Water Treatment system was developed by Dr. Arnold Hara, UH Hilo Entomology in 1999. Hot water system showed a reduction in pesticide use by 80-90 %, a reduction in labour requirements and reduction in export rejection rates (Hara *et al.*, 2000). Hot Water seed treating plant was first used in Bangladesh by Prof. Dr. M. Bahadur Meah in vegetables (The Daily Star, August 3, 2003). The Hot Water Seed Treating Plant developed in the IPM lab, BAU has been found very effective in eliminating the seed infection by pathogenic fungi including Phomopsis vexans, increasing seed germination and reducing nursery diseases (Meah, 2003).

Hot water treatment with 50℃ temperature for 15 minutes gave good result for controlling seedborne fungi and maintaining better as well as safe germination of maize seeds (Rahman *et al.,* 2008). The present research was undertaken with the following objectives:

1. To determine the effect of hot water treatment in controlling seed infection of cereal crops Rice, Wheat and Maize.
2. To find out suitable water temperature and time for hot water treatment for rice, wheat and maize against seed infection.

CHAPTER 2
REVIEW OF LITERATURE

Hot water treatments of seed and plant material are classical thermophysical methods of plant protection. As early as the end of the 19th century the method was applied to control loose smut (*Ustilago nuda*) in cereals (Jensen, 1888). In the 1920s hot water treatment of cabbage seed to control black leg (*Phoma lingam*) was a standard method in USA (Walker, 1923). Further examples for application of hot water treatment were shown by Baker (1962), Gabrielson (1983), and Jahn *et al.* (2000). In the second half of the 20th century hot water treatment was displaced by the application of more effective chemicals. The method fell into oblivion and due to this the method was not extended to other fields and crops. In the light of current knowledge, practical application on a broad spectrum of crops is not possible. Hot water treatment gets more and more importance for organic farming and for the production of spices and medical plants (Trueman and Wick, 1996).

It could also become an alternative method for conventional farming especially in case of failure of chemicals permitted for seed treatment. On hot water treatment, an exact temperature has to be maintained throughout the application. Further, a decrease in temperature has to be avoided at the beginning of the treatment. It is necessary to determine the optimal parameters of hot water treatment and to develop a technology practicable for vegetables seed. Effective temperature treatment and duration have to be found out for every vegetable crop and the relevant pathogens. The principle is to eliminate the pathogens as far as possible without decreasing germination of seeds. In Bangladesh, hot water treatment of vegetable seeds pragmatically started by the farmers from 2003 (The Daily Star, August 3, 2003). But the research on hot water treatment of cereals are very limited in Bangladesh. Islam (2005) reported that hot water seed treatment at 56℃ for 15 minutes completely controlled *Phomopsis vexans* and increased seed germination by 53.5% over control. Hossain (2004) conducted an experiment on the control of *Phomopsis vexans* through hot water seed treatment. He found that seed treatment at 55℃ for 15 min completely controlled seed-borne *Phomopsis vexans* providing 87.0% seed germination.

Zaman *et al.* (2009) carried out an experiment to determine the effect of different eco-friendly seed treatments against leaf blight (*Bipolaris sorokiniana*) of wheat under field condition. Twelve treatments were explored in these 12 experiments. Among the eco-friendly treatments the highest reduction of leaf infection over control was found in apparently healthy seeds treated with hot water in all the stages recorded. Kabir (2004) reported that hot water treatment of rice seeds at 53-54℃ for 15 min gave the highest seed germination (87.0 %) and completely eradicated seed infection of *Bipolaris oryzae, Alternaria padwickii* and *Fusarium spp*. Wheat seeds dipped in hot water at 51- 52℃ for 10 min yielded the maximum seed germination 84.0% and completely eradicated seed infection by *Bipolaris sorokiniana* and *Fusarium spp*. whereas for jute seeds , 55- 56℃ for 15 min was found effective with the highest seed germination (88.5%) and zero (0%) percent seed infection of *Colletrichum corchori, Macrophomina phaseolina* and *Botryodiplodia theobromae*.

Nega *et al.* (2003) found that seed borne pathogens could be reduced without significant losses of germination by hot water treatments at 50°C for 20 to 30 min up to 53°C for 10 to 30 min. At higher temperature, however, treatment time must be lowered to avoid reducing germination 9 of sensitive crops. In most cases efficacy of hot water treatments against *Alternaría* species (*A. dauci, A. radicina, A. alternata, A. brassicicola*) was high or (efficacy > 95 %). Treatment was also very efficient against Phoma species, *P. Ungarn, P. valerianella* (80-95 %). The reduction of *P. valerianella* on the seed of lamb's lettuce correlated in the first test year with the reduction of disease in the field. The number of spores in the pycnidia of *S. apiicola* and *S. petroselini* ware significantly reduced by hot water treatment.

Forsberg *et al.* (2002) attempted to develop the use of hot water, humid air for disinfestation of seed from pathogens towards high efficacy and high capacity applications was made by using techniques permitting uniform heat exposures for short periods. Using sufficient relative air humidity (> 90 %) and hot water, the treatments gave good sanitation effects. They conclude that the method of using hot water, humid air for sanitation of cereal seeds from pathogens has potential for practical use in larger scale.

Nesmith (2003) at Ohio State University reported that hot water treatment is effective against the major seed borne diseases of vegetables. He set up operative temperature of 122°F (49.95°C) for 25 min for brussels sprouts, cabbage, eggplant, tomato and spinach; 122°F (49.95°C) for 20 10 min for broccoli, cucumber, carrot, kale, cauliflower, Chinese cabbage, kohlrabi and turnips; 122°F (49.95°C) for l5 min for mustard and radish, l25°F (5l.6°C) for 30 min for peppers and l18°F (47.73°C) for 30 min for celery and lettuce.

Bari *et al.* (2003) studied the effect of hot-water treatment at various time and temperature regimes to scheme a decontamination process which is consistent with the recommendation of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) to diminish pathogens on seeds by 5log cfu/g. Alfalfa, mung bean and radish seeds were inoculated by immersion with more than 107 cfu/g of enterobacteria (*Salmonella senftenberg* W775, *S. bovismorbificans* and *Escherichia coli* O157:H–), dried and stored at 2°C. The numbers of salmonellae and E. coli O157:H– on these seeds remained unchanged during storage for 8 weeks. To achieve sprouting rates of more than 95%, time-temperature regimes were well-defined. The thermal treatment of contaminated mung bean (2–20 min for 55–80°C), radish and alfalfa seeds 0.5–8 min (53– 64°C) reduced all pathogens by more than 5log cfu/g.

Prabhu and Prasada (1970) reported that seed treatment of wheat with hot water effectively controlled seed borne inoculums, *Alternaria triticina* causing leaf blight. Seed treatment increased germination of seeds. 11 Soaking seeds in normal water for 4 hr followed by hot water treatment at 52-54℃ for 10 min was also effective. Jing *et al.* (2009) investigated the effects of hot water treatment in alleviating chilling injury and reducing ultrastructural damage of mature green cherry tomatoes (*Lycopersicun esculentum* cv). Mature-green cherry tomato fruits were treated in water at 40°C or 45°C for 5 min or 15 min, and then stored at 5°C for 19 days followed by ripening at 20°C. Hot water treatment at 40°C for 15 min increased tolerance of cherry tomato fruits to chilling stress, indicating as low outbreak of skin lesion, high color and low electrolyte leakage. Hot water treatment (40°C for 15 min) before storage alleviated chilling injury in cherry tomato fruits.

Raychoudhuri (1967) reported that hot water treatment of brinjal seeds at 50°C for 30 minutes helped in warding off the phomopsis blight or fruit rot infection by *Phomopsis vexans*. According to Raychoudhuri and Lele (1966) Phomopsis blight of brinjal also causes fruit rot and can be controlled by hot water treatment of seeds. It is eradicative aims at destroying diseases causing fungi and bacteria, which carried with the seeds. Hot water treatment (treating seeds at 50-52°C for 15-30 minutes) is an acceptable and standard practice and is recommended for chilli, brinjal, brassicas and cole crops.

Hussain *et al.* (2013) studied to evaluate mycofloral pathogenicity prevailing on corn (*Zea mays* L.) and indigenous management strategies in different districts of Azad Jammu & Kashmir (AJK) Pakistan. To reduce or eliminate the detrimental impacts of these species, four different management strategies were evaluated in experimental plot and results were analysed by LSD. The garlic extract treatment was the best with highest seed germination rate (85.75%), followed by Benomyl treatment (84.75%), hot water treatment (79%), and distilled water treatment (65%), respectively.

Merou *et al.* (2011) suggested that the seeds of *Albizia julibrissin* are dormant because of their hard seed coat and they need pre-treatment in order to germinate. Soaking in 40 or 50°C warm water also resulted in high germination percentages (86 and 91%). Jiskani (2002) described that the brown spot or blight of rice is a much more-wide spread and a common disease in almost all rice growing areas of the world. He prescribed that brown spot or blight of rice caused by *Helminthosporium oryzae* successfully controlled by hot water seed treatment at 54°C for 10 minutes.

James (1988) found hot water treatments have effectively been used on several agricultural crops to reduce or eliminate pathogens on seed while maintaining high levels of germinative capacity and to eliminate phytotoxic reactions (Baker 1956, Neergaard 1977, Walker 1969). A recent innovative approach to hot water treatments is the use of microwaves to heat water to the desired temperature (Lozano *et al.* 1986). Such a technique can be used to properly regulate exposure time and temperatures and is relatively easy to use commercially. Kohmann and Borja (2002) investigated the seedling growth and the number of variable fungal propagules retained on the container cavity walls as a result of different container cleaning treatments with a bath temperature of 60, 70, 80 or 95°C for 30s. The most frequently isolated fungi were *Paecilomyces sp*. and Penicillium sp. which are well-known saprophytes. Containers that were washed at 80°C had some organic 14 debris attached to the cavity walls, but no spores were visible. In used and unwashed containers fungal spores, hyphae and organic debris were found on the container cavity walls. Almost 60% of the seedlings grown in unwashed containers had dead or very stunted root systems but there was no additional effect of the warm-water treatment. In conclusion, hot water bath of at least 60°C was recommended.

Hermansen *et al.* (1999) studied the effects of hot water treatments on seed borne fungi, germination, emergence and yield of carrot. Seeds infected with *Aternaria dauci* were treated with hot water at temperatures ranging from 44 to 59°C at intervals of 5 for 5°C to 40 minutes. Different grades of healthy carrot seeds were treated at 50-55°C. Hot water treatment of seeds and seed treatment with the biological control agents had no effects on carrot yield and storage quality but reduced the incidence of the saprophyte *Ulocladium atrum* on the seeds. They included that hot water treatment is an alternative to fungicides to eradicate seed borne pathogens in carrots in organic farming systems.

Garcia-Jimenez *et al.* (2004) found to overcome *Dematophora necatrix* by the use of a hot-water treatment (HWT) of *Cyperus esculentus* tubers. Isolates of *D. necatrix* from *C. esculentus* showed sensitivity to temperatures above 34°C, indicating HWT could be used as a practical 15 way of destroying tuber-borne inoculum of this pathogen. Temperatures from 43°C to 64°C for three periods of time (10, 20 or 30 min) were applied to healthy tubers. These tubers tolerated temperatures of 55°C from 10 to 30 min without a reduction in sprouting. HWT at 53–55°C for 25–30 min was recommended to control tuber-borne inoculum.

Jaquette *et al.* (1996) studied the efficacy of chlorine and hot water treatments in killing *Salmonella stanley* inoculated onto alfalfa seeds. Treatment of seeds in water for 5 or 10 min at 54ºC caused a significant reduction in the *S. stanley* population and treatment at > or 57 ºC reduced populations to < or = 1 CFU/g. However, treatment at > or = 54 º C for 10 min caused a substantial reduction in viability of the seeds. Treatment at 57 or 60 º C for 5 min appears to be effective in killing *S. stanley* without substantially decreasing germinability of seeds. Storage of seeds for 8 to 9 weeks at 8 and 210 C resulted in reductions in populations of *S. stanley* of about 1 log10 and 2 log10 CFU/g, respectively.

Clear *et al.* (2002) determined Canada western red spring wheat (*Triticum aestivum*) (RS1, RS2) and Canada western amber durum wheat (AD1, AD2) were assessed after heating seed at 50 or 70°C for up to 14 days. RS2 and B2 with an initial incidence of 23 and 84% of *Fusarium graminearum*, respectively, were also heated at 60°C for 24 days and 16 80°C for 10 days. Germination rates in most samples were unaffected by the treatment times and temperatures sufficient to eradicate *F. graminearum*. They recommended that thermotherapy be applied to control national and international movements of *F. graminearum* and other heat-sensitive pathogens in germplasm used for research and breeding purposes.

Fallik *et al.* (2002) considered the effectiveness of a short pre-storage hot water rinsing and brushing on resistance to decline growth and chilling injury on pink tomato cv. 189 fruit that were reserved for 15 days at 5 or 12°C and 3 days at 22°C. He advocated that the alternative method of a very short (15S) HWRB (Hot Water Rinsing & Brushing) at 52°C for desirable tomatoes. This treatment prolonged storability well over 3 weeks at 5°C by minimizing chilling injury and increasing resistance against pathogen during storage.

Fallik (2004) summarized the latest developments in hot water immersion treatment (HWT) and hot water rinsing and brushing (HWRB) technologies. These treatments kill pathogens that cause surface decay, while maintaining fruit quality during prolonged storage and marketing. The physiological responses of cultivars of different fruit species to heat treatments vary according to season, growing location, soil type, production practices and fruit maturity. In general, higher the temperature, the shorter the treatment in order to avoid heat damage. HWT is applied at temperatures between 43 and 53°C for periods of several minutes up to 2 hours for quarantine treatments, while HWRB is employed commercially for 10-25s at temperatures between 48 and 63°C. The time and temperature of exposure that benefits fresh harvested quality depends on cultivar, fruit maturity, fruit size and condition during the growing season. Both HWT and HWRB inhibit ripening, reducing decay incidence and in several commodities induce resistance against pathogens and against chilling injuries.

Eissenberg *et al.* (1983) suggested that heat or antibody treatment decreases attachment to L cells and promotes the fusion of Chlamydia containing phagosomes with lysosomes in macrophages. Elementary Bodies (EB) envelopes heated to 56ºC for 15 min were consistently found in ferritin-labeled phagolysome as early as 30 min. EB envelope material occurred in the absence of phagolysome fusion. The data add credence to the belief that the spontaneous breakdown or autolytic enzyme release of EB envelope components must occur preparatory to the conversion of EB to reticulate bodies.

Lal *et al.* (2002) studied the effect of postharvest water dipping treatments and storage conditions on shelf life and quality of ber (*Ziziphus mauritiana* Lamk). Fresh fruits of ber 'Umran' were dipped hot (50°C) water for 5 min and packed under different storage containers i.e. corrugated fibre board boxes, sealed polythene bags and perforated polythene bags. Control fruits were packed without dipping treatment. Result showed that postharvest water dipping at 50ºC for 5 min significantly increased the shelf life and maintained the quality of ber fruits, particularly late in the storage period. They suggested that postharvest fruit dipping in hot water (50ºC) for five minutes followed by packaging in sealed polythene bags can enhance the shelf life and quality of per fruits.

Animashaun (2015) studied focused on the effect of hot water dipping as a nonchemical method to control the black mould disease caused by *Alternaria alternata* on red tomatoes. Hot water dip at 50°C for 5 or 10 min was carried out on *Alternaria alternata* spore suspension (in-vitro), the results showed a significant ($P \leq 0.05$) reduction in germination of spores after 48 h. The hot water temp was increased to 50 and 55ºC and inoculated fruits were immersed for 5 min in separate hot water bath. In this trial the result showed that dipping artificially inoculated fruit at 50 or 55ºC for 5 min significantly reduced ($P \leq 0.05$) decay development 19 caused by *A. alternata*. Splitting was observed on the pericarp (skin) at the point of inoculation of fruits before hot water treatment at 55°C for 5 min.

The hot water treatment of the tomatoes had the following effects on the attributes of quality. This study has shown that pre storage hot water treatment may be a useful non-chemical method of controlling *A. alternata* postharvest disease pathogen without adverse consequence on the fruit quality. Khaleduzzaman (1996) deliberated hot water treatment of wheat seeds at 49°C, 52°C, 55°C and 61°C respectively for 5 and 10 min in controlling seed borne infection. Hot water treatment at 52-55°C for 10 min gave highest control of *Aternaria tenuis, Aspergillus flavus, Aspergillus niger, Bipolaris sorokiniana,* and *Fusarium spp*. and expanded the percentage of seed germination.

Swarup *et al.* (1993) informed that wheat gall nematode caused by *Anguin tritici* efficiently controlled by hot water treatment of 54°C for 10 min. Strandberg and White (1989) deliberated the tolerance of carrot seeds to heat treatments that could eradicate seed-borne pathogens. They perceived that germination and emergence of seedlings from seeds treated in hot water at 35, 40, 45, 50 or 55°C from 4-20 minutes were not 20 affected, but seeds treated at 60℃ for 8 minutes or more were affected adversely. At 45 and 50°C, treatment durations as long as 48 minutes did not affect emergence, but > 20 minutes at 55℃ reduced emergence. Prolonged treatment and the higher temperatures were particularly effective in reducing populations of *Alternaria dauci.*

Singh (1983) studied the method of hot water treatment as soaking of eggplant seeds in water at 20 - 30°C for 4-6 hr. Then seeds were dipped in water at 49°C for 2 min, shaded by dehydrating before planting. There are chances of reduction in germination if there is an increase temperature or duration of soaking of the seeds. Koleva (1981) suggested that some strains infect wheat, oat, rye barley and triticale caused by bacterial leaf blight and controlled successfully by hot water treatment of seed at 53°C for 30 min. Lambat *et al.* (1974) studied seventeen seed lots of sugar beet, from crops raised from exotic material in Kalpa valley and Srinagar, showed a high incidence of

Pleospora betae, Cercospora beticola and *Verticillium sp*. when tested by the moist blotter method. They recommended that prewashing the seeds in running water for 2 hours followed by hot water 21 treatment at 50ºC for 20 min and drying in the sun (35-40 deg) for 6-8 h was very effective in controlling *P. betae*.

Suryanarayana *et al.* (1963) recommended that hot water treatment of rice seeds at 50°C for 15 min effectively eliminated seed infection of *Alternaria pudwickii.* Hiremath and Hedge (1981) prescribed that seed treatment of rice at 52°C for 10 min for controlling seedling blight.

CHAPTER 3
MATERIALS AND METHODS

3.1 Experimental site

The in vitro experiments were conducted in the Central Laboratory of the Department of Plant Pathology at Sher-e-Bangla Agricultural University

3.2 Experiment period

The laboratory experiments were conducted during July 2021 to December 2022.

3.3 Collection of seed samples

Seed samples of rice and wheat were collected from Bangladesh Agricultural Development Corporation (BADC). One kilogram rice seeds and one kilogram wheat seeds were collected from Bangladesh Agricultural Development Corporation (BADC). Maize seed were collected from Savar local market.

3.4 Pathogen identification

Pathogen infection of rice, wheat and maize were identified by using stereo-microscope and pathogen *Bipolaris spp* for wheat & Maize and *Fusarium spp* for rice by using compound microscope.

3.5 Selection of seed samples

For two hundred seeds were taken randomly from each seed samples of rice, wheat & maize so that maximum incidence of seed borne infection may be available.

3.6 Hot Water Seed Treating Device

Hot water seed treating device was used for treatment of rice, wheat, and maize seeds. The seed treating device was developed at IPM Lab, BAU, Mymensingh.

3.7 Selection of Temperature and Time

The seeds were treated with hot water at 52℃, 54℃, 56℃, 58℃, 60℃ and with a control (ambient temperature) for different treatment periods, viz, 5 min and 10 min. The experiments were conducted following Completely Randomized Design (CRD) with 4 replications. The seeds were wrapped loosely in cotton bag and placed in a hot water device that constantly held the water at the recommended temperature. For each crop, treatments were explored properly. Treating seeds at ambient temperature (25±1°C) served as control. Experiment with temperature and time combination that yielded the best result in term of seed germination and seed infection were repeated. Temperature and time combination that yielded the best result was trailed in pot soil.

3.7.1 Treatments of the experiments:

1. T_1= Hot water at 52℃ for 5 and 10 min
2. T_2= Hot water at 54℃ for 5 and 10 min
3. T_3= Hot water at 56°C for 5 and 10 min
4. T_4= Hot water at 58℃ for 5 and 10 min
5. T_5= Hot water at 60℃ for 5 and 10 min
6. T_6= Control (Using normal water at ambient temperature (25±1°C))

3.8 Hot water treatment procedure

Following steps were followed for hot water treatment for each crop seeds.

a. About 2 litters of water was poured in the hot water seed treating device.
b. Thermostat valve was adjusted to required temperature and switched on the power. Water was stirred by stick to maintain uniformly.
c. Sufficient seeds of selected crops in a bag were dipped in hot water when the temperature reached to desired level.
d. The bag was stirred so that hot water comes in contact with each seed.
e. After required time, the bag was picked up and seeds were shade dried.
f. Then seeds were ready for evaluation.

3.9 Planting of treated seeds

Plastic petridish were used. It was surface sterilized by 70% alcohol and allowed to aeration for some time. Two filter papers soaked in sterile water and were set in the petridish. Twenty-five (25) treated seeds were plated in each petridish. The petridishes were incubated at ambient temperature (25±1°C) for 7-10 days. After 7 days, seed germination, abnormal seed germination, dead seeds, rotten seeds and target pathogens were recorded from each petridish. 25 Rice seeds, 25 Wheat seeds and Maize 10 seeds were plated in each peteidish, treatments were replicated 4 times.

3.10 Data collection

After 7 days of seed placement, for each treatment, 5 parameters were examined. The parameters were seed germination, abnormal seed germination, dead seed, rotten seed and percent seed infection by target pathogens.

1. Percent seed germination
2. Percent rotten seed
3. Percent dead seed
4. Percent abnormal seedling
5. Percent infected seedling

1) Germinated seeds

Germinated seeds were counted following the method of the International Seed Testing Association (ISTA, 2004) defines germination as "the emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether it is able to develop further into a satisfactory plant under favourable conditions". A well-developed root system including a primary root, a well-developed and intact hypocotyl without damage to the conducting tissues an intact plumule with a well-developed green leaf within or emerging through the coleoptile or an intact epicotyl with a normal plumule bud are shown in germinated seeds.

$$\% \text{ Germination seed} = \frac{\text{Number of seedling}}{\text{Number of total seeds in petridish}} \times 100$$

2) Rotten seeds

According to ISTA (International Seed Testing Association), rotten seeds are those whose seedlings or seeds with lesions affect the conducting tissues of the epicotyl, hypocotyl or root. Seedlings with any of the essential structures so diseased or decayed that normal development is prevented, called rotten seeds. 5) Infected seeds The seeds which carry pathogen inside or outside the seed with any part of seed are called pathogen borne seeds.

$$\% \text{ Rotten seed} = \frac{\text{Number of rotten seeds}}{\text{Number of total seeds in petridish}} \times 100$$

3) Dead seeds

According to ISTA (International Seed Testing Association), seeds which are not viable or remain hard at the end of the test period because they have not absorbed water due to an impermeable seed coat. Seeds, other than hard seeds, which remain firm and apparently viable after the appropriate treatment or dormancy are classified as fresh ungerminated seeds and must be reported them as dead seeds.

$$\% \text{ Dead seed} = \frac{\text{Number of dead seeds}}{\text{Number of total seeds in petridish}} \times 100$$

4) Percent abnormal seedling

According to ISTA (International Seed Testing Association), abnormal seedlings are those which do not show the capacity for continued development into normal plants. Seedlings with no cotyledons, seedlings with constrictions, splits, cracks or lesions which affect the conducting tissues of the epicotyle, hypocotyle or root; seedling without a primary root are shown, are called abnormal seedling.

$$\% \text{ Abnormal seedling} = \frac{\text{Number of abnormal seedlings}}{\text{Number of total seeds in petridish}} \times 100$$

5) Percent infected seedling

The seedlings which carry pathogen inside or outside with any part of seedlings are called infected seedling. Percentage of infected seedlings were measured.

$$\% \text{ Infected seedling} = \frac{\text{Number of infected seedlings}}{\text{Number of total seeds in petridish}} \times 100$$

3.11 Experimental Design

The laboratory experiments were laid out in a Completely Randomized Design (CRD) with 4 replications. In the design 200 seeds were set up in 8 petridish. For rice and wheat, each petridish had 25 seeds and for maize,10 seeds were plated in 20 petridish for determining the germination, abnormal seed germination, dead seed, rotten seed and target pathogens percentage.

3.12 Statistical Analysis of Data

All the recorded data were analysed with the Analysis of Variance Technique and differences among treatment means compared with LSD with the help of a statistical computer packages (Statistix-10). Arcsine transformation was done for data regarding germination percentage, dead seed and square root transformation was done for data regarding abnormal germination and rotten seeds.

Plate 01. Application of hot water treatment.

CHAPTER IV
RESULTS

4.1 Hot water treatment of rice seeds at different temperatures

4.1.1 Hot water treatment of rice seeds at different temperatures for 5 min

Germinated seed (%)

Rice seed dipped in hot water for 5 min at different temperatures showed significant differences regarding seed germination. The highest seed germination (91%) was recorded at temperature 56°C for 5 min. The lowest seed germination (75%) was obtained at 60°C which was significantly lower than control (76%) (Table 4.1.1).

Rotten Seed (%)

Rotten seeds recorded 9.50% at temperature 54°C. Temperature below 54°C increased the rotten seeds (10.33%). The highest rotten seeds (12%) obtained at control treatment (T_6) (Table 4.1.1)

Dead Seed (%)

The highest dead seeds (25%) recorded at temperature 60°C. Lowest dead seed was observed at 54℃ (5.50%). Dead seeds gradually increased with the increase of temperature from 54°C to 60°C.

Abnormal Seedlings (%)

Abnormal seed germination was found nil (0.0%) at 58°C. Below 58°C abnormal seed germination increased significantly. Abnormal seed germination was significantly higher (20%) at temperature (25±1°C) in control than all other treatments (Table 4.1.1).

Infected Seedlings (%)

Seed infection in rice seeds recorded in different temperatures differed significantly. Percent seed infection was 25%, in ambient temperature (25±1°C). The pathogenic infection decreased significantly with the increase of temperature. Complete control of seed infection was obtained at 58°C and 60°C.

Table 4.1.1 Effect of hot water treatment at different temperature for 5 min on seed germination and infection of rice seedlings

Treatments	Germinated seed (%)	Rotten Seed (%)	Dead Seed (%)	Abnormal Seedlings (%)	Infected Seedlings (%)
T_1	77.67cd	10.33ab	12.00b	15.00b	10.00b
T_2	85.00b	9.50b	5.50d	5.67c	2.50d
T_3	91.00a	0.00d	9.00c	5.00c	0.00cd
T_4	88.50ab	0.00cd	11.50bc	0.00d	0.00e
T_5	75.00d	0.00cd	25.00a	0.00d	0.00e
T_6	76.00cd	12.00a	12.00b	20.00a	25.00a
$LSD_{(0.05)}$	4.85	3.15	3.30	3.62	5.91
CV%	6.39	10.04	9.83	7.44	7.03
Level of Sig.	**	**	**	*	*

[T_1= Hot water at 52°C for 5 min, T_2= Hot water at 54°C for 5 min, T_3= Hot water at 56°C for 5 min, T_4= Hot water at 58°C for 5 min, T_5= Hot water at 60°C for 5 min and T_6= Control (Using normal water at ambient temperature (25±1°C) for 5 min]

4.1.2 Hot water treatment of rice seeds at different temperatures for 10 min

Germinated seed (%)

Rice seed dipped in hot water for 10 min at different temperatures showed significant differences regarding seed germination. The highest seed germination (94%) was recorded at temperature 54°C for 10 min. The lowest seed germination (75.33%) was obtained at control. The seed germination gradually decreases with the increase of temperature from 54°C. (Table 4.1.2).

Rotten Seed (%)

Rotten seeds recorded nil (0.0%) at temperature 58°C which was statistically similar to that of temperatures 60℃. Temperature below 58°C increased the rotten seeds. The highest rotten seeds (9.0%) obtained at ambient temperature (25±1°C) in control (Table 4.1.2).

Dead Seed (%)

The highest dead seeds (22%) recorded at temperature 60°C. Below this temperature level dead seeds decreased gradually and it was the lowest (20%) at 54°C.

Abnormal Seedlings (%)

Abnormal seed germination was found nil (0.0%) at 58°C and 60°C. Below 56°C temperature, abnormal seed germination increased significantly. At ambient temperature (25±1°C) in control, abnormal seed germination was significantly higher 15% than all other treatments.

Infected Seedlings (%)

Seed infection in rice seeds recorded in different temperatures differed significantly. Percent seed infection was 20%, at ambient temperature (25±1°C). Both the pathogenic infection decreased significantly with increase of temperature. Complete eradication of seed infection was obtained at 58℃ and 60°C.

Table 4.1.2 Effect of hot water treatment at different temperature for 10 min on seed germination and infection of rice seedlings

Treatments	Germinated seed (%)	Rotten Seed (%)	Dead Seed (%)	Abnormal Seedlings (%)	Infected Seedlings (%)
T_1	80.00de	5.00bc	15.00b	10.00b	8.00b
T_2	94.00a	4.00c	2.00d	5.00c	0.00c
T_3	89.00b	6.00bc	3.00c	5.33c	0.00c
T_4	84.50c	0.00d	15.50b	0.00d	0.00c
T_5	78.00e	0.00d	22.00a	0.00d	0.00c
T_6	75.33f	9.00a	15.67b	15.00a	20.00a
$LSD_{(0.05)}$	3.90	2.01	1.60	4.03	5.92
CV%	8.41	7.55	7.55	9.28	9.01
Level of Sig.	**	*	**	*	*

[T_1= Hot water at 52℃ for 10 min, T_2= Hot water at 54℃ for 10 min, T_3= Hot water at 56°C for 10 min, T_4= Hot water at 58℃ for 10 min, T_5= Hot water at 60℃ for 10 min and T_6= Control (Using normal water at ambient temperature (25±1°C) for 10 min]

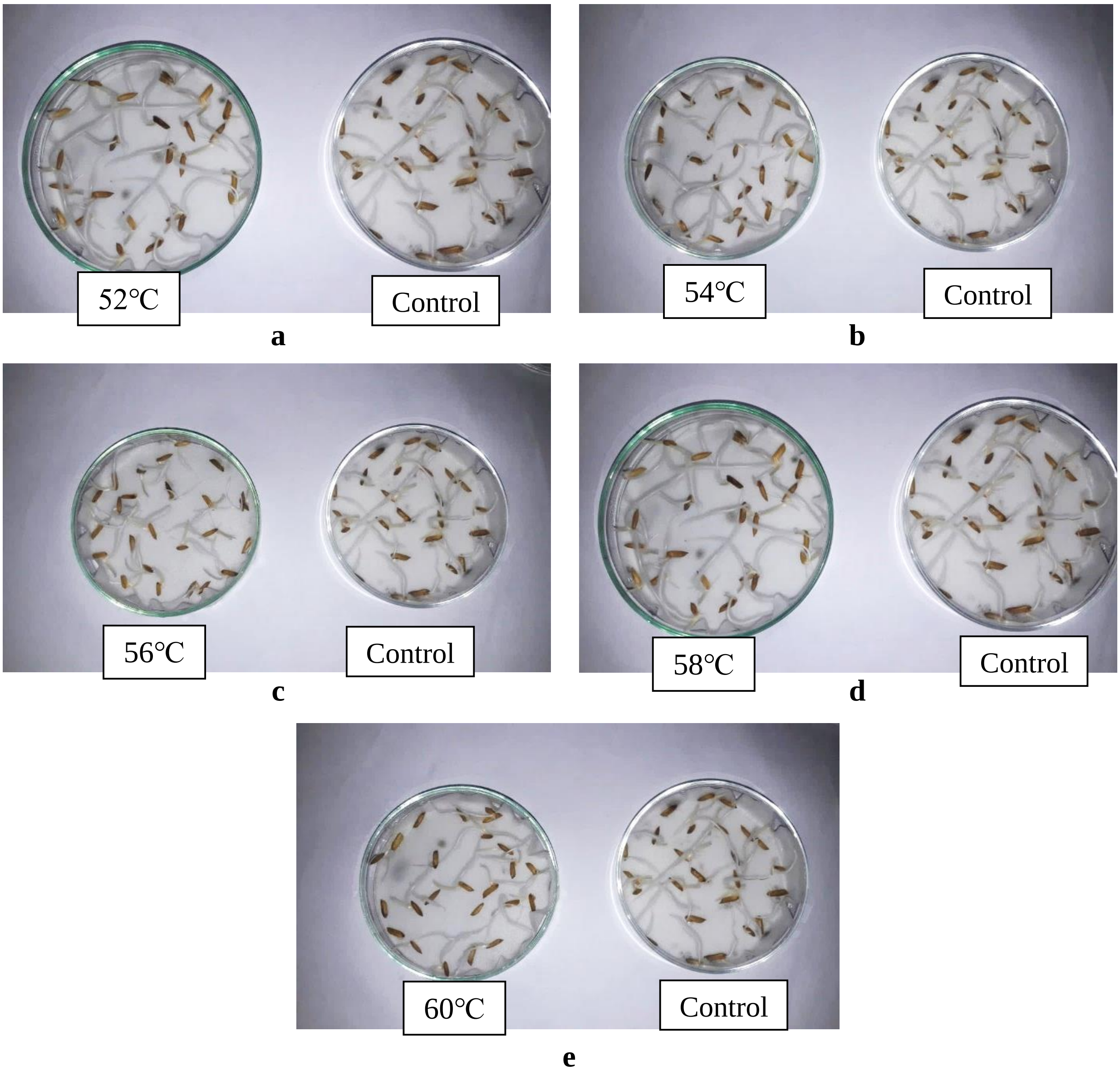


Plate 02. a. Hot water treatment of rice seeds at 52°C temperature;
b. Hot water treatment of rice seeds at 54°C temperature;
c. Hot water treatment of rice seeds at 56°C temperature;
d. Hot water treatment of rice seeds at 58°C temperature;
e. Hot water treatment of rice seeds at 60°C temperature.

4.2 Hot water treatment of wheat seeds at different temperatures

4.2.1 Hot water treatment of wheat seeds at different temperatures for 5 min

Germinated seed (%)

Wheat seed dipped in hot water for 5 min at different temperatures yielded significant differences regarding seed germination. The highest seed germination (88%) was recorded at temperature 54°C for 5 min. The lowest seed germination (63.67%) was obtained at 60°C (Table 4.2.1).

Rotten Seed (%)

Lowest rotten seeds recorded (0%) at temperature 58°C which was similar to that of temperature 60°C. Temperature below 58°C increased the rotten seeds. The highest rotten seeds (20%) obtained at ambient temperature (25±1°C) (Table 4.2.1).

Dead Seed (%)

The highest dead seeds (36.33 %) recorded at 60°C temperature. The lowest dead seed (10%) was recorded at 52°C temperature. Dead seeds gradually increased with the increase of temperature from 52°C to 60°C. (Table 4.2.1).

Abnormal Seedlings (%)

Abnormal seed germination was found nil (0.0%) at 60°C. Below 60°C abnormal seed germination increased significantly. At ambient temperature (25±1°C), abnormal seed germination was significantly higher (22%) than all other treatments (Table 4.2.1).

Infected Seedlings (%)

Seed infection in wheat seeds recorded in different temperatures differed significantly. Percent seed infection of *was* 25%, in untreated control at ambient temperature (25±1°C). The pathogenic infection decreased significantly with the increase of temperature. Complete control of seed infection was obtained at 54°C to 60°C temperature (Table 4.2.1).

Table 4.2.1 Effect of hot water treatment at different temperature for 5 min on seed germination and infection of wheat seedlings

Treatments	Germinated seed (%)	Rotten Seed (%)	Dead Seed (%)	Abnormal Seedlings (%)	Infected Seedlings (%)
T_1	85.33ab	3.67b	10.00e	4.67bc	2.00bc
T_2	88.00a	2.00c	11.00de	6.00bc	0.00c
T_3	82.00b	1.00a	17.00c	2.50cd	0.00c
T_4	70.00c	0.00d	29.00b	2.00cd	0.00c
T_5	63.67de	0.00d	36.33a	0.00d	0.00c
T_6	68.00e	20.00b	12.00d	22.00a	25.00a
$LSD_{(0.05)}$	3.75	1.01	3.81	2.61	3.05
CV%	5.03	7.94	7.25	9.83	9.07
Level of Sig.	**	*	**	*	*

[T_1= Hot water at 52°C for 5 min, T_2= Hot water at 54°C for 5 min, T_3= Hot water at 56°C for 5 min, T_4= Hot water at 58°C for 5 min, T_5= Hot water at 60°C for 5 min and T_6= Control (Using normal water at ambient temperature (25±1°C) for 5 min]

4.2.2 Hot water treatment of wheat seeds at different temperatures for 10 min

Germinated seed (%)

Wheat seed dipped in hot water for 10 min at different temperatures showed significant differences regarding seed germination. The highest seed germination (90%) was recorded at temperature 52°C for 10 min. The lowest seed germination (65.33%) was obtained at 60°C which was significantly lower than control (72%) (Table 4.2.2).

Rotten Seed (%)

Rotten seeds recorded nil (0.00%) at temperature 52℃ to 60°C. The highest rotten seeds (18%) obtained at ambient temperature (25±1°C) in control (Table 4.2.2).

Dead Seed (%)

The highest dead seeds (34.67%) recorded at 60℃ temperatures. The lowest dead seed was observed 10% at control and 52°C. Dead seeds gradually decreased with the decrease of temperature from 60°C to 52°C (Table 4.2.2).

Abnormal Seedlings (%)

Abnormal seed germination was found nil (0.0%) at 56°C, 58°C and 60°C. Below 56°C abnormal seed germination increased significantly. Abnormal seed germination was significantly higher (20%) at ambient temperature (25±1°C) in control than all other treatments (Table 4.2.2).

Infected Seedlings (%)

Seed infection in wheat seeds recorded in different temperatures differed significantly. Percent seed infection of was 22%, in untreated control at ambient temperature (25±1°C). The pathogenic infection decreased significantly with the increase of temperature. Complete control of seed infection was obtained at 52℃ to 60°C temperature (Table 4.2.2).

Table 4.2.2 Effect of hot water treatment at different temperature for 10 min on seed germination and infection of wheat seedlings

Treatments	Germinated seed (%)	Rotten Seed (%)	Dead Seed (%)	Abnormal Seedlings (%)	Infected Seedlings (%)
T_1	90.00a	0.00c	10.00e	4.00b	0.00b
T_2	86.00b	0.00c	14.00d	4.00b	0.00b
T_3	78.50c	0.00c	21.50c	0.00c	0.00b
T_4	70.00d	0.00c	30.00b	0.00c	0.00b
T_5	65.33ef	0.00c	34.67a	0.00c	0.00b
T_6	72.00f	18.00a	10.00de	20.00a	22.00a
$LSD_{(0.05)}$	4.72	2.90	2.41	4.00	5.91
CV%	8.04	8.55	8.77	7.30	7.48
Level of Sig.	**	*	**	*	*

[T_1= Hot water at 52°C for 10 min, T_2= Hot water at 54°C for 10 min, T_3= Hot water at 56°C for 10 min, T_4= Hot water at 58°C for 10 min, T_5= Hot water at 60°C for 10 min and T_6= Control (Using normal water at ambient temperature (25±1°C) for 10 min]

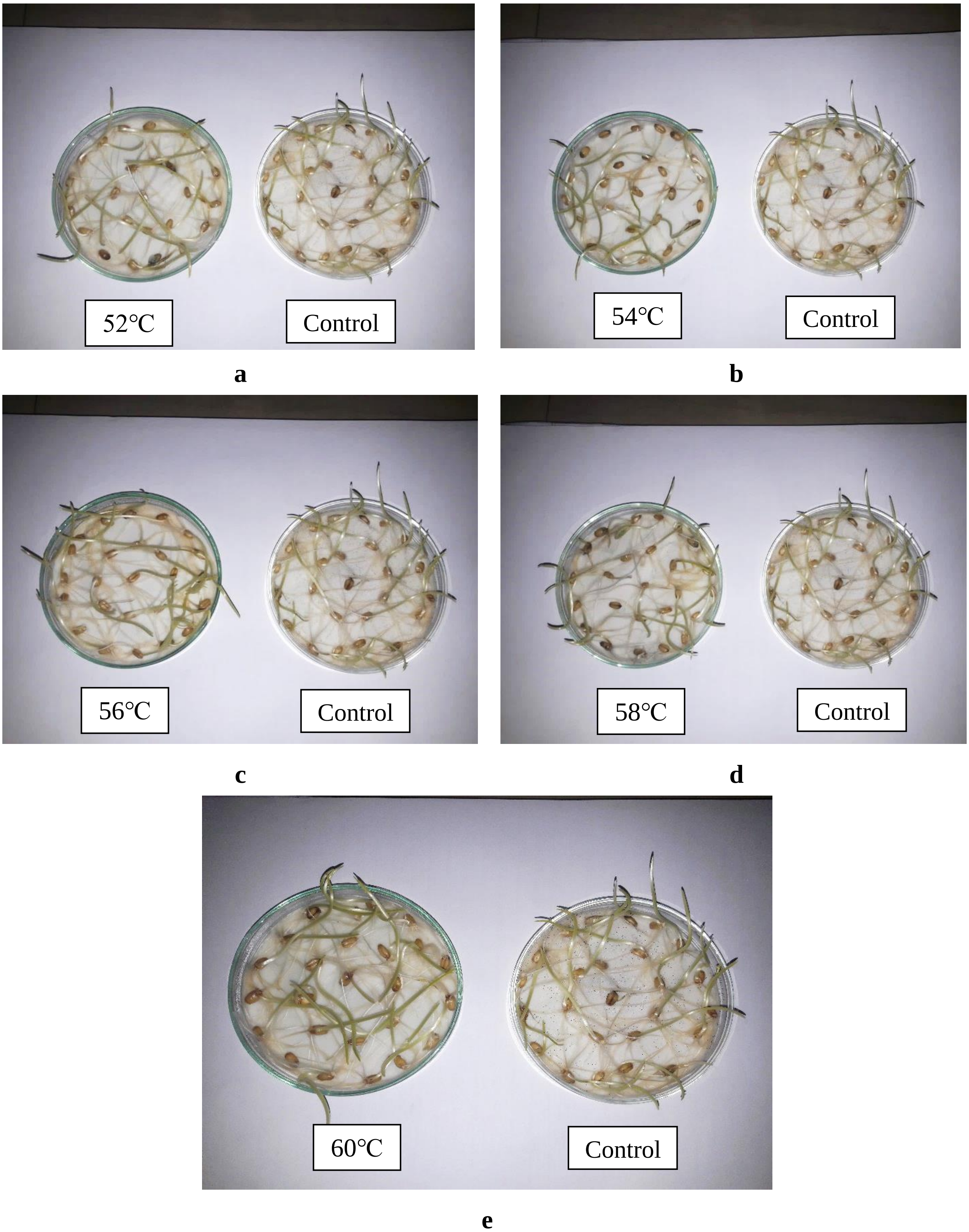


Plate 03. a. Hot water treatment of wheat seeds at 52°C temperature;
b. Hot water treatment of wheat seeds at 54°C temperature;
c. Hot water treatment of wheat seeds at 56°C temperature;
d. Hot water treatment of wheat seeds at 58°C temperature;
e. Hot water treatment of wheat seeds at 60°C temperature.

4.3 Hot water treatment of maize seeds at different temperatures

4.3.1 Hot water treatment of maize seeds at different temperatures for 5 min

Germinated seed (%)

Maize seed dipped in hot water for 5 min at different temperatures showed significant differences regarding seed germination. The highest seed germination (82%) was recorded at temperature 56°C to 60°C for 5 min. The lowest seed germination (68.67%) was obtained at control (Table 4.3.1).

Rotten Seed (%)

Rotten seeds recorded nil (0.00%) at temperature 54°C to 58°C. The highest rotten seeds (9.33%) obtained at ambient temperature (25±1°C) (Table 4.3.1).

Dead Seed (%)

The highest dead seeds (22%) recorded at temperature (25±1°C) in control. Dead seeds gradually increased with the increase of temperature from 52°C to 54°C (Table 4.3.1).

Abnormal Seedlings (%)

Abnormal seed germination was found nil (0.0%) at 56°C to 60°C. Below 56°C abnormal seed germination increased significantly. At ambient temperature (25±1°C), abnormal seed germination was significantly higher (22%) than all other treatments (Table 4.3.1).

Infected Seedlings

Seed infection in maize seeds recorded in different temperatures differed significantly. Percent seed borne infection was 30%, at ambient temperature (25±1°C). The pathogenic infection decreased significantly with the increase of temperature. Complete control of seed infection was obtained at 60°C (Table 4.3.1).

Table 4.3.1 Effect of hot water treatment at different temperature for 5 min on seed germination and infection of maize seedlings

Treatments	Germinated seed (%)	Rotten Seed (%)	Dead Seed (%)	Abnormal Seedlings (%)	Infected Seedlings (%)
T_1	78.01	1.67cd	16.33d	8.00b	10.00b
T_2	81.50ab	0.00d	18.50cd	5.33c	10.33b
T_3	82.00ab	0.00d	18.00c	0.00d	5.00c
T_4	82.00b	0.00d	18.00b	0.00d	2.33d
T_5	82.00c	0.00d	18.00b	0.00d	0.00e
T_6	68.67bc	9.33a	22.00cd	22.00a	30.00a
$LSD_{(0.05)}$	3.88	1.88	3.68	3.41	3.19
CV%	8.10	6.01	6.90	7.03	7.91
Level of Sig.	**	*	*	**	**

[T_1= Hot water at 52°C for 5 min, T_2= Hot water at 54°C for 5 min, T_3= Hot water at 56°C for 5 min, T_4= Hot water at 58°C for 5 min, T_5= Hot water at 60°C for 5 min and T_6= Control (Using normal water at ambient temperature (25±1°C) for 5 min]

4.3.2 Hot water treatment of maize seeds at different temperatures for 10 min

Germinated seed (%)

Maize seed dipped in hot water for 10 min at different temperatures showed significant differences regarding seed germination. The highest seed germination (84%) was recorded at temperature 54°C and 56°C for 10 min. The lowest seed germination (67%) was obtained at ambient temperature (25±1°C) (Table 4.3.2).

Rotten Seed (%)

Rotten seeds recorded nil (0.00%) at temperature 52°C to 60°C. The highest rotten seeds (15%) obtained at ambient temperature (25±1°C) in control (Table 4.3.2).

Dead Seed (%)

The highest dead seeds (22%) recorded at 60°C temperature. Below 60°C temperature dead seeds decreased significantly (Table 4.3.2).

Abnormal Seedlings (%)

Abnormal seed germination was found nil (0.0%) at 54°C to 60°C temperature. Below 54°C temperature abnormal seed germination increased significantly. abnormal seed germination was significantly higher (18%) at ambient temperature (25±1°C) in control, than all other treatments (Table 4.3.2).

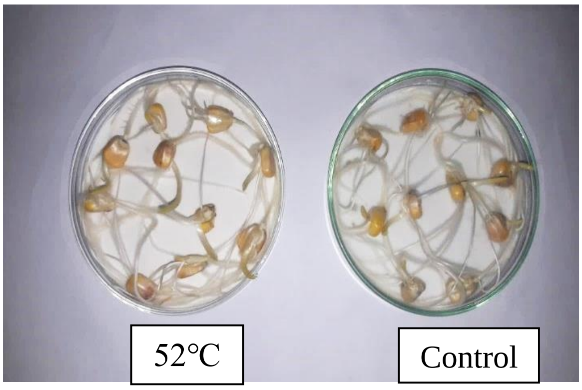
Infected Seedlings (%)

Seed infection in maize seeds recorded in different temperatures differed significantly. Percent seed infection was 23% in untreated seeds at ambient temperature (25±1°C). The pathogenic infection decreased significantly with increase of temperature. Complete eradication of seed infection was obtained at 58℃ and 60°C (Table 4.3.2).

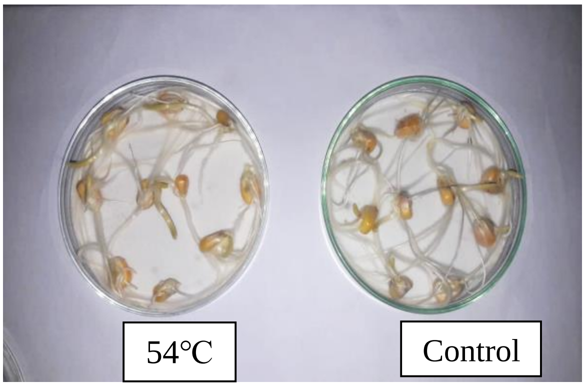
Table 4.3.2 Effect of hot water treatment at different temperature for 10 min on seed germination and infection of maize seedlings

Treatments	Germinated seed (%)	Rotten Seed (%)	Dead Seed (%)	Abnormal Seedlings (%)	Infected Seedlings (%)
T_1	83.67ab	0.00c	16.33bc	5.67b	8.67bc
T_2	84.00a	0.00c	16.00c	0.00c	3.00cd
T_3	84.00a	0.00c	16.00c	0.00c	0.00d
T_4	81.50ab	0.00c	18.50b	0.00c	0.00d
T_5	78.00b	0.00c	22.00a	0.00c	0.00d
T_6	67.00c	15.00a	18.00b	18.00a	23.00a
$LSD_{(0.05)}$	4.81	2.09	2.88	3.15	6.19
CV%	9.30	10.13	8.94	8.71	7.34
Level of Sig.	**	**	**	*	*

[T_1= Hot water at 52℃ for 10 min, T_2= Hot water at 54℃ for 10 min, T_3= Hot water at 56°C for 10 min, T_4= Hot water at 58℃ for 10 min, T_5= Hot water at 60℃ for 10 min and T_6= Control (Using normal water at ambient temperature (25±1°C) for 10 min]



a



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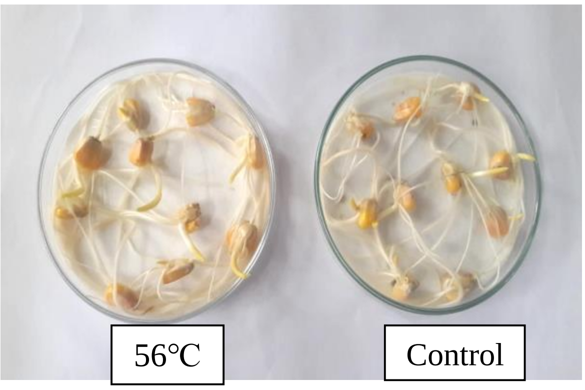

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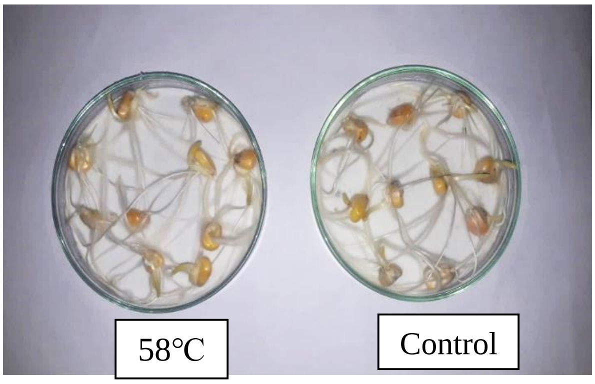

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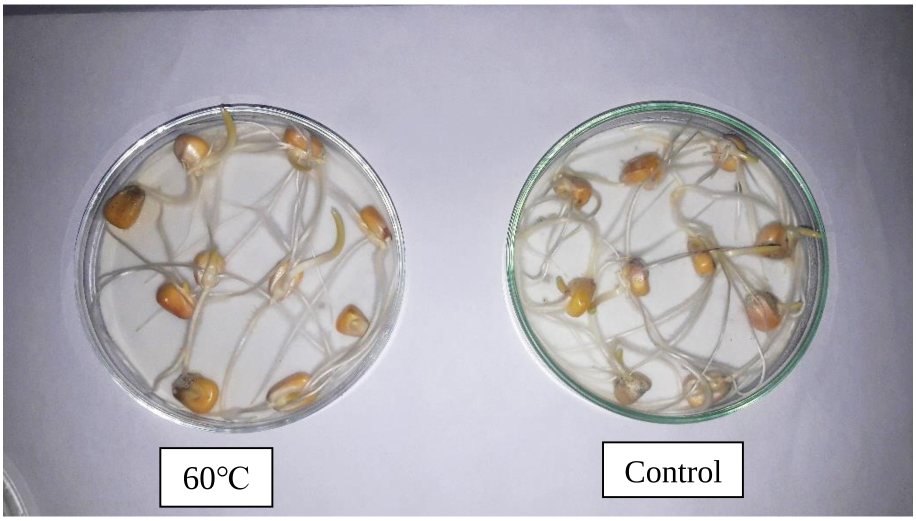
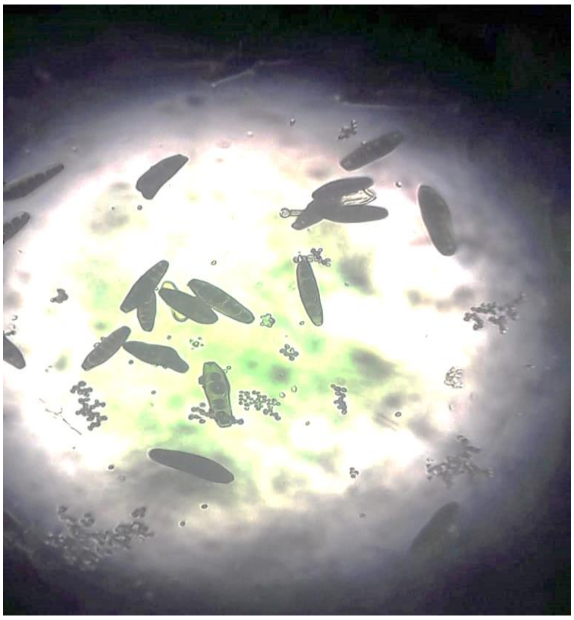


E

Plate 04. a. Hot water treatment of maize seeds at 52°C temperature;
b. Hot water treatment of maize seeds at 54°C temperature;
c. Hot water treatment of maize seeds at 56°C temperature;
d. Hot water treatment of maize seeds at 58°C temperature;
e. Hot water treatment of maize seeds at 60°C temperature.



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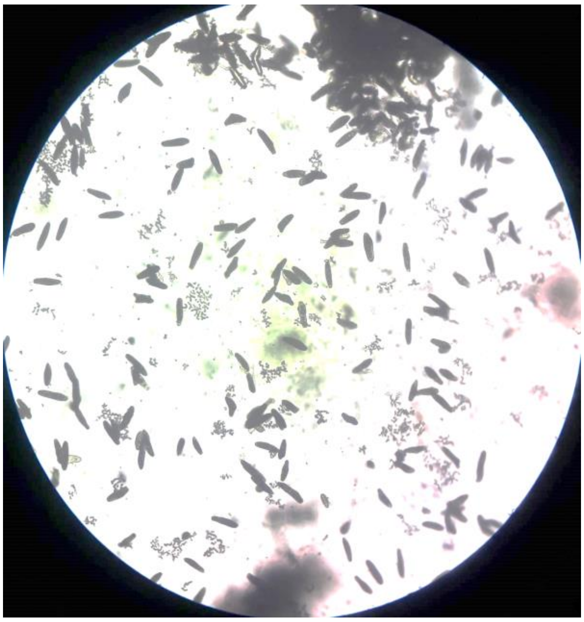
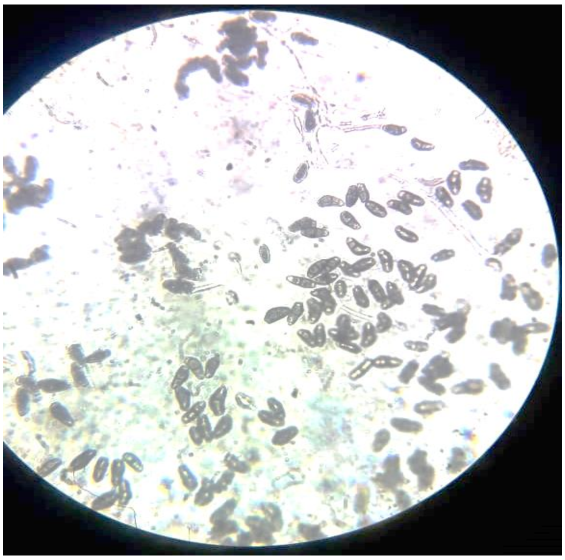


B



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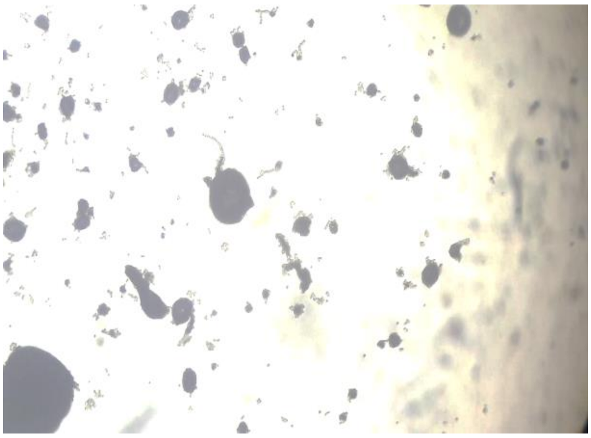

D

Plate 05. a. b. c. Pathogen found in wheat and maize seeds (*Bipolaris spp*); d. Pathogen found in rice seeds (*Fusarium spp*)

CHAPTER V

DISCUSSION

The investigation was conducted on water seed treatment of rice, wheat and maize seeds in controlling seed infection for proper germination during the period from July 2021 to December 2022, at the central laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 to determine the suitable temperature and time for seed treatment.

The rice seed dipped in hot water for 5 min at different temperatures showed significant differences regarding seed germination. The highest seed germination (91%) was recorded at temperature 56°C for 5 min. The lowest seed germination (75%) was obtained at 60°C which was significantly lower than control (76%). Rotten seeds recorded 9.50% at temperature 54°C. Temperature below 54°C increased the rotten seeds (10.33%). The highest rotten seeds (12%) obtained at 60°C temperature. The highest dead seeds (25%) recorded at temperature 60°C. Lowest dead seed was observed at 52°C (5.50%). Dead seeds gradually increased with the increase of temperature from 54°C to 60°C. Abnormal seed germination was found nil (0.0%) at 58°C. Below 58°C abnormal seed germination increased significantly. Abnormal seed germination was significantly higher (20%) at ambient temperature (25±1°C) than all other treatments. Seed infection in rice seeds recorded in different temperatures differed significantly. Percent seed infection was 25%, in untreated control at ambient temperature (25±1°C). The pathogenic infection decreased significantly with the increase of temperature. Complete control of seed infection was obtained at 58°C and 60°C. Rice seed dipped in hot water for 10 min at different temperatures showed significant differences regarding seed germination. The highest seed germination (94%) was recorded at temperature 54°C for 10 min. The lowest seed germination (75.33%) was obtained at control. The seed germination gradually

decreases with the increase of temperature from 54°C. Rotten seeds recorded nil (0.0%) at temperature 58°C which was statistically similar to that of temperatures 60°C. Temperature below 58°C increased the rotten seeds. The highest rotten seeds

(9.0%) obtained at ambient temperature (25±1°C). The highest dead seeds (22%) recorded at temperature 60°C. Below this temperature level dead seeds decreased gradually and it was the lowest (20%) at 54°C. Abnormal seed germination was found nil (0.0%) at 58°C and 60°C. Below 56°C temperature, abnormal seed germination increased significantly. At ambient temperature (25±1°C) in control, abnormal seed germination was significantly higher 15% than all other treatments. Seed infection in rice seeds recorded in different temperatures differed significantly. Percent seed infection was 20%, at ambient temperature (25±1°C). Both the pathogenic infection decreased significantly with increase of temperature. Complete eradication of *seed infection* was obtained at 58°C and 60°C. The results of the present investigation keep in with the report of Legaspi *et al.* (1985), Jayaweera *et al.* (1988) and Fakir *et al*. (l990) in case of rice seeds in Bangladesh. Islam (2005) also reported 56°C temperature for 15 min. found to be effective for controlling *Phomopsis vexans* of eggplant seed by hot water treatment.

The wheat seed dipped in hot water for 5 min at different temperatures yielded significant differences regarding seed germination. The highest seed germination (88%) was recorded at temperature 54°C for 5 min. The lowest seed germination (63.67%) was obtained at 60°C. Lowest rotten seeds recorded (0%) at temperature 58°C which was similar to that of temperature 60°C. Temperature below 58°C increased the rotten seeds. The highest rotten seeds (20%) obtained at ambient temperature (25±1°C). The highest dead seeds (36.33 %) recorded at 60°C temperature. The lowest dead seed (10%) was recorded at 52°C temperature. Dead seeds gradually increased with the increase of temperature from 52°C to 60°C. Abnormal seed germination was found nil (0.0%) at 60°C. Below 60°C abnormal seed germination increased significantly. At ambient temperature (25±1°C), abnormal seed germination was significantly higher (22%) than all other treatments. Seed infection in wheat seeds recorded in different temperatures differed significantly. Percent seed infection of *was* 25%, at ambient temperature (25±1°C). The pathogenic infection decreased significantly with the increase of temperature. Complete control of seed infection was obtained at 54°C to 60°C temperature. Wheat seed dipped in hot water for 10 min at different temperatures showed significant differences regarding seed germination. The highest seed germination (90%) was recorded at temperature 52°C for 10 min. The lowest seed germination (65.33%) was obtained at 60°C which was significantly higher than control (64%). Rotten seeds recorded nil (0.00%) at temperature 52℃ to 60°C. The highest rotten seeds (18%) obtained at ambient temperature (25±1°C). The highest dead seeds (34.67%) recorded at 60℃ temperature. The lowest dead seed was observed 10% at control and 52°C. Dead seeds gradually decreased with the decrease of temperature from 60°C to 52°C. Abnormal seed germination was found nil (0.0%) at 56°C, 58°C and 60°C. Below 56°C abnormal seed germination increased significantly. Abnormal seed germination was significantly higher (20%) at ambient temperature (25±1°C) than all other treatments. Seed infection in wheat seeds recorded in different temperatures differed significantly. Percent seed infection of was 22%, at ambient temperature (25±1°C). The pathogenic infection decreased significantly with the increase of temperature. Complete control of seed infection was obtained at 52℃ to 60°C temperature. Kahn (1977) and Khaleduzzaman (1996) reported 52-54° C as effective temperature in eradicating seed borne fungi from wheat seeds treated for 10 min. Winter *et al.* (1996) however found 52° C and 5-10 min treatment effective in eliminating *Helminthosporium sativum* and *Drechslera teres* from barley seeds. But deferent findings were found by Winter *et al.* (2001), Swarup *et al.* (1993), Koleva (1981), Prabhu and Prusada (1970), Bever, (1951), Bedi (1957) and Dean (1964), Winter *et al*. (2001) stated that *Fusarium graminearum*, *Tilletia carries*, *Gerlachia nivalis* and *Septoria nodorum* were eliminated by treating at 45°C for 2 hours with Skim milk powder. Prabhu and Prasada (1970) reported the elimination of Alternaria spp. at 52-54°C for 10 min while seed borne infection of loose smut was eliminated at 55.5ºC for 10 minutes (Bever, 1951; Bedi, 1957; Dean, 1969). *Bipolaris sorokiniana* and *Fusarium spp*. recorded in wheat seeds as 22% and 24% of seed infection which are in agreement with Fakir (2000) who listed 24 seed borne pathogens of wheat seeds along with these two fungi.

The maize seed dipped in hot water for 5 min at different temperatures showed significant differences regarding seed germination. The highest seed germination (82%) was recorded at temperature 56°C to 60°C for 5 min. The lowest seed germination (68.67%) was obtained at control. Rotten seeds recorded nil (0.00%) at temperature 54°C to 58°C. The highest rotten seeds (9.33%) obtained at ambient temperature (25±1°C). The highest dead seeds (22%) recorded at temperature (25±1°C) in control. Dead seeds gradually increased with the increase of temperature from 52°C to 54°C. Abnormal seed germination was found nil (0.0%) at 56℃ to 60°C. Below 56°C abnormal seed germination increased significantly. At ambient temperature (25±1°C), abnormal seed germination was significantly higher (22%) than all other treatments. Seed infection in maize seeds recorded in different temperatures differed significantly. Percent seed borne infection was 30%, in untreated control at ambient temperature (25±1°C). The pathogenic infection decreased significantly with the increase of temperature. Complete control of seed infection was obtained at 60°C. Maize seed dipped in hot water for 10 min at different temperatures showed significant differences regarding seed germination. The highest seed germination (84%) was recorded at temperature 54℃ and 56°C for 10 min. The lowest seed germination (67%) was obtained at ambient temperature (25±1°C) in control. Rotten seeds recorded nil (0.00%) at temperature 52°C to 60°C. The highest rotten seeds (15%) obtained at ambient temperature (25±1°C). The highest dead seeds (22%) recorded at 60°C temperature. Below 60°C temperature dead seeds decreased significantly. Abnormal seed germination was found nil (0.0%) at 54°C to 60°C temperature. Below 54°C temperature abnormal seed germination increased significantly. Abnormal seed germination was significantly higher (18%) at ambient temperature (25±1°C) in control, than all other treatments. Seed infection in maize seeds recorded in different temperatures differed significantly. Percent seed infection was 23% at ambient temperature (25±1°C). The pathogenic infection decreased significantly with increase of temperature. Complete eradication of seed infection was obtained at 58℃ and 60°C. Similar results were also obtained from different crops by Winter *et al.* (1994) and Verzignasis *et al.* (1997). In the present study, considering both pathogenic incidence and germination parentages of the seeds at 58℃ temperature for 10 min. was effective for controlling seed borne microflora of maize. This result closely agreed with the report of Nega *et al.* (2003) and Muniz (2001).

CHAPTER VI

SUMMARY AND CONCLUSION

In case of rice, hot water for 5 min at different temperatures showed significant differences on seed germination. The highest seed germination (91%) was recorded at temperature 56°C for 5 min. The lowest seed germination (75%) was obtained at 60°C which was significantly lower than control (76%). Rotten seeds recorded (9.50%) at 54°C temperature. Temperature below 54°C increased the rotten seeds (10.33%). The highest rotten seeds (12%) obtained at 60℃ temperature. The highest dead seeds (25%) recorded at temperature 60°C. Lowest dead seed was observed at 52℃ (5.50%). Dead seeds gradually increased with the increase of temperature from 54°C to 60°C. Abnormal seed germination was found nil (0.0%) at 58°C. Below 58°C abnormal seed germination increased significantly. Abnormal seed germination was significantly higher (20%) at ambient temperature (25±1°C) than all other treatments. Seed infection in rice seeds recorded in different temperatures differed significantly. Complete control of seed infection was obtained at 58℃ and 60°C.

Rice seed dipped in hot water for 10 min at different temperatures showed significant differences regarding seed germination. The highest seed germination (94%) was recorded at temperature 54°C for 10 min. The lowest seed germination (75.33%) was obtained at control. The seed germination gradually decreased with the increase of temperature from 54°C. Rotten seeds recorded nil (0.0%) at temperature 58°C which was statistically similar to that of temperatures 60℃. Temperature below 58°C increased the rotten seeds. The highest rotten seeds (9.0%) obtained at ambient temperature (25±1°C) in control. The highest dead seeds (22%) recorded at temperature 60°C. Below this temperature level dead seeds decreased gradually and it was the lowest (20%) at 54°C. Abnormal seed germination was found nil (0.0%) at 58°C and 60°C. Below 56°C temperature, abnormal seed germination increased significantly. Complete eradication of *seed infection* was obtained at 58℃ and 60°C.

For wheat seed, hot water for 5 min at different temperatures yielded significant differences regarding seed germination. The highest seed germination (88%) was recorded at temperature 54°C for 5 min. The lowest seed germination (63.67%) was obtained at 60°C. Lowest rotten seeds recorded (0%) at temperature 58°C which was similar to that of temperature 60°C. Temperature below 58°C increased the rotten seeds. The highest rotten seeds (20%) obtained at ambient temperature. The highest dead seeds (36.33 %) recorded at 60°C temperature. The lowest dead seed (10%) was recorded at 52°C temperature. Dead seeds gradually increased with the increase of temperature from 52°C to 60°C. Abnormal seed germination was found nil (0.0%) at 60°C. Below 60°C. Abnormal seed germination increased significantly. Complete control of seed infection was obtained at 54℃ to 60°C temperature.

Wheat seed treated with hot water for 10 min at different temperatures showed significant differences regarding seed germination. The highest seed germination (90%) was recorded at temperature 52°C for 10 min. The lowest seed germination (65.33%) was obtained at 60°C which was significantly higher than control (64%). Rotten seeds recorded nil (0.00%) at temperature 52℃ to 60°C. The highest rotten seeds (18%) obtained at ambient temperature (25±1°C) in control. The highest dead seeds (34.67%) recorded at 60℃ temperature. The lowest dead seed was observed 10% at control and 52°C. Dead seeds gradually decreased with the decrease of temperature from 60°C to 52°C. Abnormal seed germination was found nil (0.0%) at 56°C, 58°C and 60°C. Below 56°C abnormal seed germination increased significantly. Complete control of seed infection was obtained at 52℃ to 60°C temperature.

For maize seed, hot water for 5 min at different temperatures showed significant differences regarding seed germination. The highest seed germination (82%) was recorded at temperature 56°C to 60°C for 5 min. The lowest seed germination (68.67%) was obtained at control. Rotten seeds recorded nil (0.00%) at temperature 54°C to 58°C. The highest rotten seeds (9.33%) obtained at ambient temperature (25±1°C). The highest dead seeds (22%) recorded at temperature (25±1°C) in control. Dead seeds gradually increased with the increase of temperature from 52°C to 54°C.

Abnormal seed germination was found nil (0.0%) at 56°C to 60°C. Below 56°C abnormal seed germination increased significantly. Complete control of seed infection was obtained at 60°C.

Maize seed treated with hot water for 10 min at different temperatures showed significant differences regarding seed germination. The highest seed germination (84%) was recorded at temperature 54°C and 56°C for 10 min. The lowest seed germination (67%) was obtained at ambient temperature (25±1°C). Rotten seeds recorded nil (0.00%) at temperature 52°C to 60°C. The highest rotten seeds (15%) obtained at ambient temperature (25±1°C). The highest dead seeds (22%) recorded at 60°C temperature. Below 60°C temperature dead seeds decreased significantly. Abnormal seed germination was found nil (0.0%) at 54°C to 60°C temperature. Below 54°C temperature abnormal seed germination increased significantly. Complete eradication of seed infection was obtained at 58°C and 60°C.

In conclusion, the rice seed should be treated with hot water at 56°C for 5 min or 54°C temperature for 10 min. In case of wheat seed, farmers are suggested to treat with hot water at 54°C for 5 min or 52°C temperature for 10 min while for maize seed the farmers are recommended to go through with hot water at 60°C temperature for 5 min or 56°C temperature for 10 min for getting the highest seed germination minimising the seed infection.

CHAPTER VII

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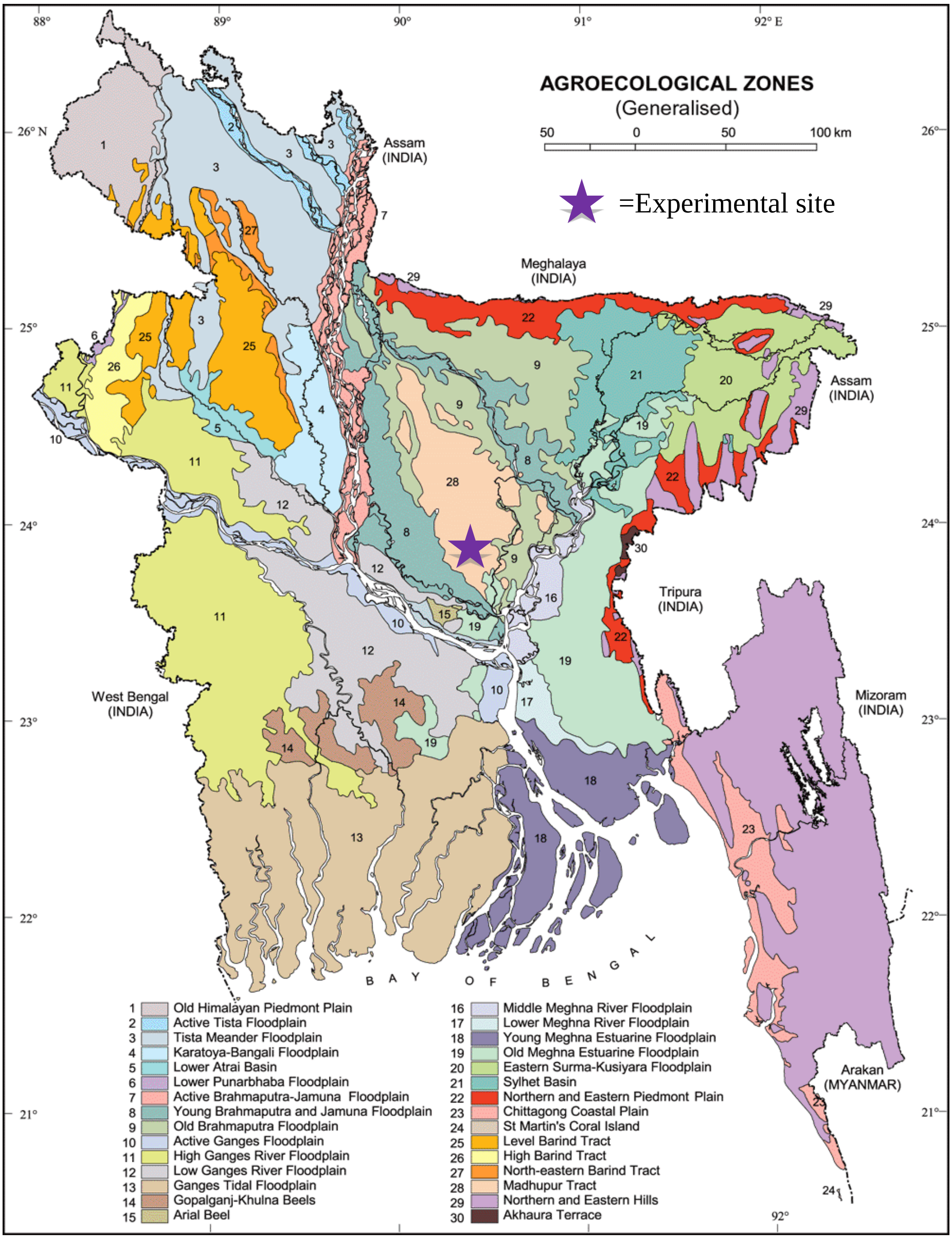
APPENDICES

Appendix I. Map showing the experimental site under study



Appendix II. Monthly meteorological information during the period from November, 2021 to April, 2022

Year	Month	Air temperature (^0C)		Relative humidity (%)	Total rainfall (mm)
		Maximum	Minimum		
2021	November	28.10	11.83	58.18	47.00
	December	25.00	9.46	69.53	00.00
2022	January	25.20	12.80	69.00	00.00
	February	27.30	16.90	66.00	39.00
	March	31.70	19.20	57.00	23.00
	April	33.50	25.90	64.50	119.00

Meterological Centre, Agargaon, Dhaka (Climate Division)