STANDARDIZATION ON TEMPERATURE AND TIME FOR HOT WATER TREATMENT OF SELECTED CROPS AGAINST SEED BORNE DISEASES

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BY

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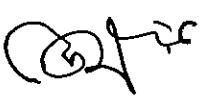

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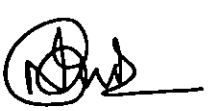

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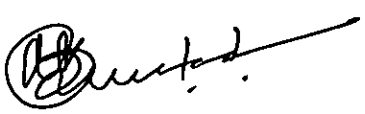

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*This is to certify that thesis entitled, "**STANDARDIZATION ON TEMPERATURE AND TIME FOR HOT WATER TREATMENTOF SELECTED CROPS AGAINST SEED BORNE DISEASES**" submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (M.S.) IN PLANT PATHOLOGY, embodies the result of a piece of bona fide research work carried out by MD. BABUL AKTER, Registration No.: 09-03520 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 source of information, as has been availed of during the course of this investigation has been duly acknowledged by him.

Dated: 26 May, 2016
Place: Dhaka, Bangladesh

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Dedicated to

My

Beloved Parents

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ABSTRACT

The experiment was conducted at the Central Laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, during July 2014 to December 2015. Effect of hot water treatment at different temperatures with varying durations on important seed-borne pathogens of rice, wheat, country bean, tomato and eggplant seeds were studied. Seeds dipping in hot water at 50 to 60° C for 5, 10 and 15 mins were evaluated for standardization of temperature and time for controlling seed-borne pathogens. Hot water treatment at 53 / 54°C for 15 mins gave the highest seed germination (90%) and completely eradicated seed infection by *Bipolaris oryzae* and *Fusarium* spp. of rice seed. Wheat seeds dipped in hot water at 51 / 52°C for 10 min yielded the maximum seed germination (90%) and reduced seed infection of *Bipolaris sorokiniana* and *Fusarium* spp. For country bean, 55 / 56°C for 15 mins was found effective with the highest seed germination (85%) and no seed infection of *Aspergilus niger.* In case of tomato and eggplant seeds 53 / 54°C for 15 mins gave the highest seed germination (88%, 90%, respectively) and no seed infection (0.0%). Hot water treated seeds sown in the pot soil gave 33.75%, 20%, 21.5%, 22.5% and 20% higher seed germination, respectively for rice, wheat, country bean, tomato and eggplant in comparison to untreated control. Therefore, hot water treatment of specific temperature may be suggested for controlling important seed-borne pathogens of rice, wheat, country bean, tomato and eggplant seeds for enhancing seed germination and reduction of seed infection.

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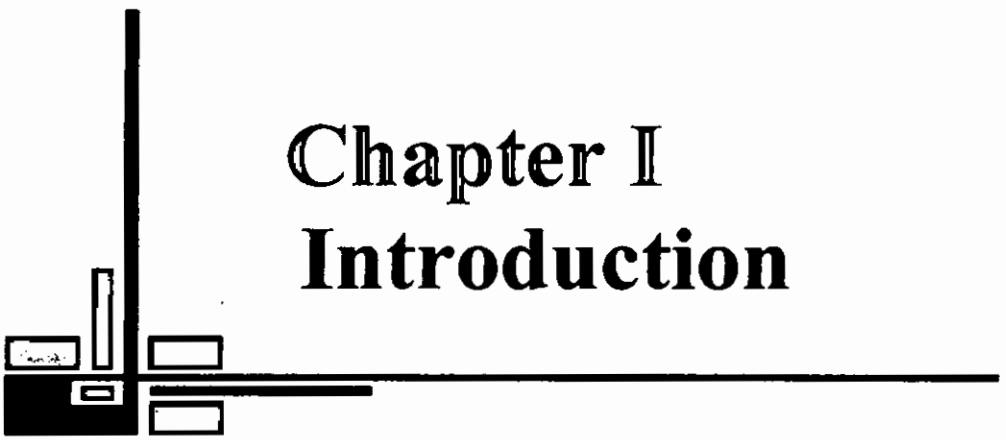
LIST OF ABBREVIATED TERMS

ABBREVIATION	FULL WORD
et al.	And others
BARI	Bangladesh Agricultural Research Institute
Cm^3	Centimeter cube
CV.	Cultivar
^0C	Degree centigrade
Etc.	Etcetera
Ed.	Edited
Eds.	Edition
G	Gram
J.	Journal
No.	Number
PDA	Potato Dextrose Agar
LSD	Least Significant Difference
DMRT	Duncan's New Multiple Range Test
%	Percent
RCBD	Randomized Completely Block Design
Res.	Research
SAU	Sher-e-Bangla Agricultural University
Viz.	Namely
Var.	Variety



Chapter I
Introduction

CHAPTER I

INTRODUCTION

Rice, wheat, country bean, tomato and eggplant are the most important crops of Bangladesh. Rice and wheat are the two principal cereal crops. Eggplant, tomato, and country bean are important vegetables. Rice and wheat are grown in 11.347 and 0.38 million hectares of the total land area, respectively (BBS, 2014).Tomato, eggplant and country bean are grown 23817.37, 28751.70 and 16588.87 hectares of the total land area, respectively (BBS, 2010).The average yield of rice and wheat in our country are 2.16 metric tons per hectare and 2.396 metric tons per hectare, respectively (BBS, 2014) .The average cereal yield is 4.406 metric tons / ha in the World(The World Bank 2015). The average yield of tomato, eggplant and country bean in our country are 7.99, 7.52 and 5.34 metric tons per hectare, respectively (BBS, 2014).The seed-borne diseases of these crops incur a huge loss every year in the country (Fakir, 2000b).

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 seed-borne (Fakir, 2000a).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 (Mia and Mathur, 1983; Legaspi *et al*,1985 and Fakir *et al.*, 1990). 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, 2000b).

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 (Razzaque *et al.*, 1992). 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 2.71 t/ha (FAO, 1999). 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 (Azhar *et al.*, 1972; Fakir, 1988). Considering 10% production loss of wheat suffered by this disease, approximately 17.50metric tons of wheat worth more than Tk.1400 million is lost annually in Bangladesh (Fakir, 2000b).

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables in terms of acreage, production, yield, commercial use and consumption. At present 6.10% (BBS, 2014) area is under tomato cultivation both in winter and summer. It is the most consumable vegetables crop next to potato and sweet potato occupying the top of the list of canned vegetables (Chowdhury, 1979). Its demand for both domestic and foreign markets has increased manifold due to its excellent nutritional and processing qualities (Hossain *et. al*, 1999). However, the yield of the crop is very low compared to those obtained in some advanced country (Sharfuddin and Siddique, 1985).

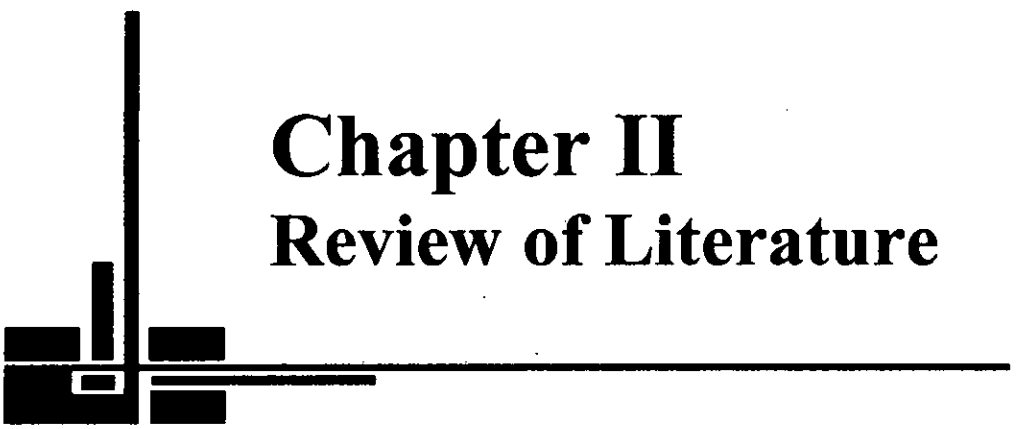
Eggplant also known as brinjal or aubergine (*Solanum melongena L.*) is an important low price summer vegetables. It belongs to family Solanaceae and contains 92.7% water, 4% carbohydrates, 1.4% protein, 1.3% fiber, 0.3% fats, 0.3% minerals and vitamin A in a negligible quantity (Tindall, 1978).The yield of eggplant is very low as compared to other Asian countries. Along with many other factors that contribute towards the poor yield in this region, seed-borne pathogens also constitute a major factor. As seed is a living tissue, storage conditions (moisture contents & temperature) directly affect its viability and germinability. More than 50 microorganisms are reported to be seed borne in nature in different vegetables seed lots (Richardson, 1990).

Common bean (*Lablab purpurescens L.*) is an annual leguminous herb popularly known as "seem". The green pods and developed unripe seeds are used as vegetables and the ripe seeds are used as pulse, "dhal", in India and to some extent in Bangladesh (Matin, 1989). There are various causes associated with lower yield of bean, where disease is considered as one of the most important factors of its yield reduction. Different phytopathogenic soil-borne as well as seed-borne fungi are responsible for disease development which attacks the plants during seedling to maturity stage. Out of different diseases of beans, anthracnose is considered as one of the most important. The disease anthracnose caused by *Colletotrichum lindemuthianum* is a serious disease causing considerable damage (Meah and Khan, 1987; Fakir *et al.*, 1991). In Bangladesh, Fakir (1980) observed 5% yield loss in beans due to the different diseases including anthracnose caused by *Colletotrichum lindemuthianum.*

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. 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 (Neergaard, 1979). 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 has been found very active in eliminating the seed infection by pathogenic fungi including *Phomopsis vexans*, increasing seed germination and reducing nursery diseases (Meah, 2003).

In the above context, the present research was undertaken to standardize the temperature and duration for seed treatment of rice, wheat, eggplant, tomato and country bean.



Chapter II
Review of Literature

CHAPTER II

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 ^{0}C 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^{0}C for 15 mins 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^{0}C for 15 mins 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^{0}C for 10 mins yielded the maximum seed germination 84.0 % and completely eradicated seed infection by *Bipolaris sorokiniana* and *Fusarium* spp. whereas for jute seeds , 55- 56^{0}C for 15 mins 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 mins up to 53 °C for 10 to 30 mins. At higher temperature, however, treatment time must be lowered to avoid reduceing germination

of sensitive crops. In most cases efficacy of hot water treatments against *Alternaria 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 mins for brussels sprouts, cabbage, eggplant, tomato and spinach; 122°F (49.95°C) for 20

min for broccoli, cucumber, carrot, kale, cauliflower, chinese cabbage, kohlrabi and turnips; 122°F (49.95°C) for 15 mins for mustard and radish, 125°F (51.6°C) for 30 min for peppers and 118°F (47.73°C) for 30 mins 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 10^7 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.

Soaking seeds in normal water for 4 hr followed by hot water treatment at 52-54^{0}C for 10 mins 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 mins or 15 mins, and then stored at 5°C for 19 days followed by ripening at 20°C. Hot water treatment at 40°C for 15 mins 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 ^{0}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 ^{0}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 analyzed 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 pretreatment in order to germinate. In this research the effect of a) dry heating, at 30°C to 100°C for 10 to 60 min, b) chemical scarification with concentrated H_2SO4 for 15, 30, 60, 90 or 120 min, c) mechanical scarification for 5 sec, d) seed soaking in warm water (30°C to 100°C for one to six hours) and e) seed soaking in tap water for one to six days, on seed germination were examined. The most successful treatment was chemical scarification in concentrated H_2SO4 for 2 hours (germination percentage 99%). Soaking in 40 or 50°C warm water also resulted in high germination

percentages (86 and 91%). The germination obtained after soaking in tap water for two days was also satisfactory (73%).

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 30 s. 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

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

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 54°C caused a significant reduction in the *S. stanley* population and treatment at > or 57 °C reduced populations to < or = 1 CFU/g. However, treatment at > or = 54 ° C for 10 min caused a substantial reduction in viability of the seeds. Treatment at 57 or 60 ° 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 21^0 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

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 (15 S) 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 10-25 s 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 56 ° C 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 50°C 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 (50°C) 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 50^0C 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 55^0C 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 55^0C for 5 min significantly reduced ($P \leq 0.05$) decay development

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 prestorage 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 mins.

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

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.

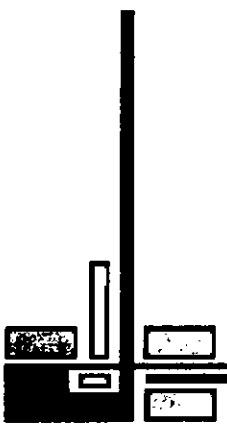
Koleva (1981) suggested that some strains infect wheat, oat, rye barley and triticale caused by bacterial leaf blight and controlled successfully by hot water treatment of seed at 53°C for 30 mins.

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

treatment at 50° C 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 III

Materials and Methods

CHAPTER III

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 and the pot experiments were conducted in the open space of the varenda of Central Laboratory.

3.2 Experiment period

The laboratory experiments and pot experiments were conducted during July 2014 to December 2015.

3.3 Collection of seed samples

Seed samples of rice, wheat, tomato, eggplant and country bean were collected from Satkhira district. One kilogram rice seeds, one kilogram wheat seeds, one kilogram country bean seeds, 100 grams tomato seeds and 100 grams eggplant seeds were collected from different areas of Satkhira district.

3.4 Target pathogens

Target pathogens of rice were *Bipolaris oryzae* and *Fusarium* spp. for country bean *Aspergilus niger*, for wheat *Bipolaris sorokiniana* and *Fusarium* spp. for tomato and eggplant *Phomopsis vexans.*

3.5 Selection of seed samples

For hundred seeds were taken randomly from each seed samples of rice, wheat, tomato and eggplant. In case of country bean, 100 seeds were taken randomly 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 hot water treatment of rice, wheat, tomato, eggplant and country bean seeds which has been developed at IPM Lab, BAU, Mymensingh.

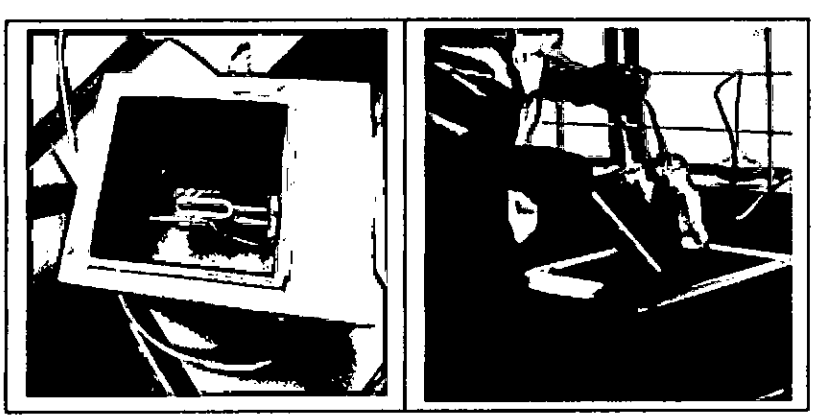


Plate 1. Hot water seed treating plant

3.7 Experiment

The seeds were treated with hot water at 50°C ,51°C, 52°C, 53°C, 54°C, 55°C, 56°C, 57°C, 58°C, 59°C with a control for different treatment periods, vis, 5min, 10 min and 15min. 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, 33 treatments were explored properly. Treating seeds at room 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 trialed in pot soil.

3.8 Hot water treatment procedure

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

a. About 2 liters water was poured in the hot water seed treating device.
b. Thermostat valve was adjusted to required temperature and switched on the power
c. Water was stirred by stick to heat the water uniformly.

d. Sufficient seeds of selected crops in a bag were dipped in hot water when the temperature reached to desired level.

e. The bag was stirred so that hot water comes in contact with each seed.

f. After required time, the bag was picked up and seeds were shade dried.

g. Then seeds were ready for evaluation.

3.9 Planting the treated seeds

After washing the plastic petridish, it was surface sterilized by 70% alcohol and allowed to aeration for sometime. Two filter papers soaked in sterile water and were set in the petridish. Twenty five (25) treated seeds were plated in each petridish except eggplant. Five (5) treated eggplant seeds were plated in each petridish. The petridishes were incubated at room 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.

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.

The collected data categorized into following parameters:

a) Category I: Germinated seeds

b) Category II: Abnormal germinated seeds

c) Category III: Dead seeds

d) Category IV: Rotten seeds

e) Category V: Infected seeds

1) Germinated seeds

Germination is generally associated with emergence of the radicle through the seed coat. 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.

2) Abnormal germinated seeds

According to ISTA (lnternational Seed Testing Association), abnormal germinated seeds 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 germinated seeds.

3) Dead seeds

According to ISTA (lnternational 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.

4) 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.

The following formulae were used for calculation of different category seeds:

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

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

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

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

$$\% \text{ Infected seed} = \frac{Number\ of\ pathogens\ in\ the\ seeds}{Number\ of\ total\ seeds\ in\ petridish} \times 100$$

3.11 Pot experiment

The treatment which shown better results in the laboratory experiments were re-evaluated through pot soil experiment.

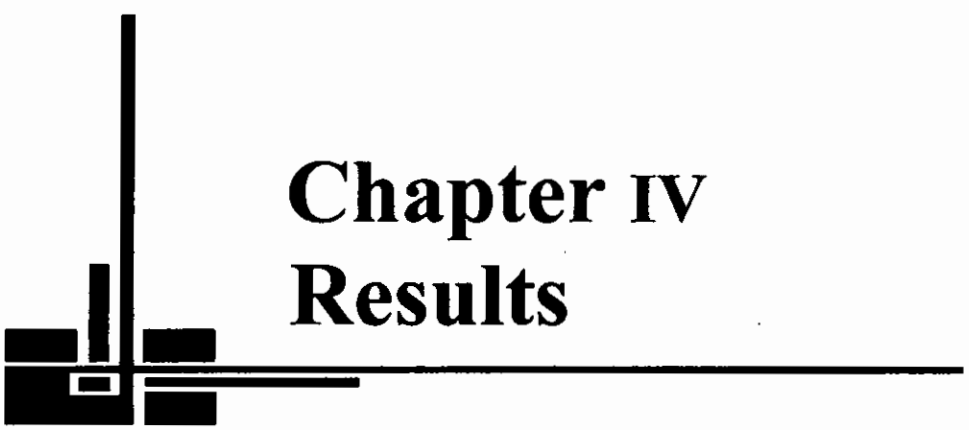
Rice seeds treated at 53~54° C for 15 min were sown in surface sterilized tray filled up with sterilized soil. One hundred seeds per tray (35X25 cm) were sown as treated and untreated conditions. Observation made for seed germination and other parameters. Similar experiments were done for eggplant (treated at56° C for 15 min), wheat seeds (treated at 51~52° C for 10 min) tomato seeds (treated at 53~54° C for 15min), eggplant seeds (treated at 54~55° C for 15 min).

3.12Experimental Design

The laboratory experiments were laid out in a Completely Randomized Design (CRD) with 4 replications. In the design 400 seeds were set up in 4 petridishes. Each petridish had 25 seeds for determining the germination, abnormal seed germination, dead seed, rotten seed and target pathogens percentage.The pot experiments were laid out in a Completely Randomized Design (CRD) with 4 replications. In the design 400 seeds were set up in the 4 pots. Each pot had 100 seeds. For eggplant 25 seeds were used for each pot for determining the germination, abnormal germination, dead seed, rotten seed and target pathogens percentage.

3.13 Statistical Analysis of Data

All the recorded data were analyzed with the Analysis of Variance Technique and differences among treatment means compared with Tukey HSD with the help of a statistical computer packages (Statistic-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.



Chapter IV
Results

CHAPTER IV

RESULTS

4.1.1Hot water treatment of rice seeds at different temperatures for 15 mins

Rice seed dipped in hot water for different temperatures yielded significant differences in percent seed germination. The highest seed germination (90%) was recorded at temperature 53 – 54°C for 15 mins. Below or above this temperature level (53-54°C) reduced seed germination significantly. The lowest seed germination (72%) was obtained at 59 – 60°C which was significantly lower than control (78%) (Table 1, Figure 1).

Abnormal seed germination was found nil (0.0%) at 58-60°C. Below this temperature level (58-60°C), abnormal seed germination increased significantly. At room temperature (25±1°C) in control, abnormal seed germination (22%) was significantly higher than all other treatments (Table 1, Figure 1).

The highest dead seeds (34.0%) recorded at temperature 59-60°C.Below this temperature level (59-60°C) dead seeds decreased significantly.

Rotten seeds recorded nil (0.0%) at temperature 58-60°C which was statistically similar to that of temperatures 54-60°C. Temperature below this (54-60°C) increased the rotten seeds. The highest rotten seeds (22.0%) obtained at room temperature (25±1°C) in control (Table 1, Figure 1).

Seed infection of *Bipolaris oryzae* and *Fusarium* spp. in rice seeds recorded in different temperatures differed significantly. Percent seed borne infection of *Bipolaris oryzae* and *Fusarium* spp. were 22.0 and 25.0 %, respectively in untreated seeds at room temperature (25±1°C). Both the pathogenic infection decreased significantly with increase of temperature. Complete eradication of *Bipolaris oryzae* was obtained at 57-60°C and *Fusarium* spp obtained at 55-60°C (Table 1, Figure 1).

Table 1. Effect of hot water treatment at different temperature for 15 mins on seed germination and seed infection of rice seeds

Temperature (%)	Seed Germination (%)		Abnormal Seed Germination (%)		Dead Seed (%)		Rotten Seed (%)		Pathogens of *Bipolaris oryzae* (%)		*Fusarium* sp. (%)	
52	80.00	bc	14.00 (1.90)	b	16.00 (2.00)	d	6.00 (1.20)	b	10.00 (1.60)	b	6.00 (1.20)	b
53	88.00	a	8.00 (1.40)	c	2.00 (0.80)	e	1.00 (0.77)	c	4.00 (1.00)	c	2.00 (0.80)	c
54	**90.0**	a	6.00 (1.20)	c	0.00 (0.70)	f	0.00 (0.70)	c	2.00 (0.80)	cd	2.00 (0.80)	c
55	86.00	b	4.00 (1.00)	d	14.00 (1.90)	d	0.00 (0.70)	c	2.00 (0.80)	cd	0.00 (0.70)	c
56	82.00	bc	4.00 (1.00)	d	18.00 (2.11)	d	0.00 (0.70)	c	2.00 (0.80)	cd	0.00 (0.70)	c
57	80.00	c	3.00 (0.85)	d	20.00 (2.23)	c	0.00 (0.70)	c	0.00 (0.70)	d	0.00 (0.70)	c
58	74.00	d	0.00 (0.70)	e	26.00 (2.54)	b	0.00 (0.70)	c	0.00 (0.70)	d	0.00 (0.70)	c
59	72.00	e	0.00 (0.70)	e	28.00 (2.64)	b	0.00 (0.70)	c	0.00 (0.70)	d	0.00 (0.70)	c
60	72.00	e	0.00 (0.70)	e	**34.00** (2.91)	a	0.00 (0.70)	c	0.00 (0.70)	d	0.00 (0.70)	c
Control	78.00	cd	**22.00** (2.38)	a	22.00 (2.38)	c	**22.00** (2.38)	a	**22.00** (2.38)	a	**25.00** (2.51)	a
CV (%)	3.19		11.92		7.66		37.70		11.37		14.65	
Tukey HSD	6.3504		0.3440		0.3737		0.7103		0.2813		0.3469	
Level of Significance	**		**		**		**				**	

Figures in the parenthesis are the transformed values

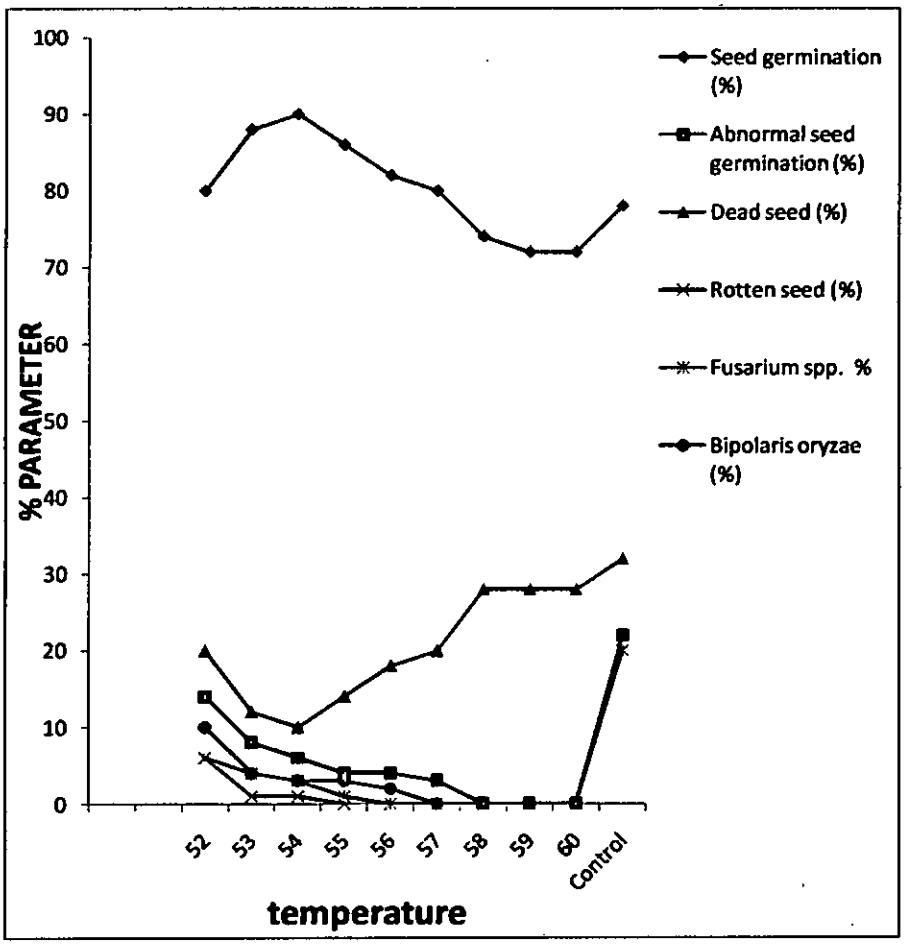


Figure 1. Effect of different temperature on different parameters of seed germination and seed infection treated for 15 mins by hot water of rice seed

4.1.2 Effect of time duration on seed germination and seed health of Rice in hot water treatment at 54^0 C

Seed germination and seed infection of rice recorded at 54^0C was found satisfactory treated for 15 mins. The germination was found the highest (90%) and abnormal seed, dead seed, rotten seed and incidence of *Bipolaris oryzae* and *Fusarium* spp. were found the lowest or minimum while the rice seed were treated at 54 ^{0}C for 15 mins (Table 2).

Table 2. Effect of treatment duration on seed germination and seed infection of rice seed treated at 54 ^{0}C

Time (Minutes)	Seed Germination (%)	Abnormal Seed Germination (%)	Dead Seed (%)	Rotten Seed (%)	% seed borne Pathogens of	
					Bipolaris oryzae	*Fusarium* sp.
5	86	9	9	3	5	4
10	88	8	6	0	4	4
15	90	6	0	0	2	2
Pot soil (15 mins)	87	7	13	3	4	3

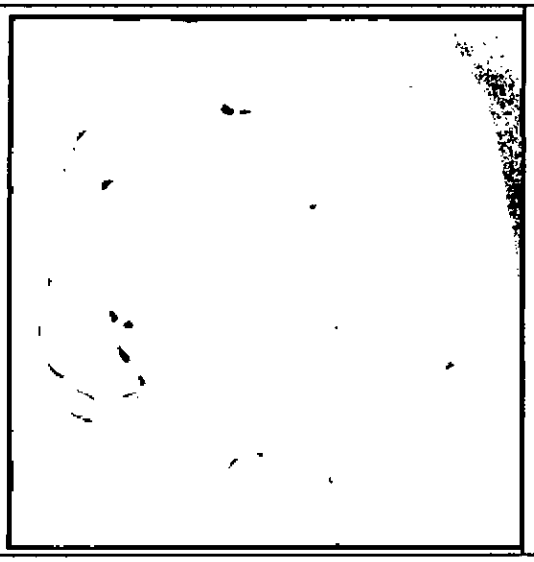


Plate 2. Germinated seedlings of rice treated at 54 ^{0}C for 15 mins in petridish

Plate 3. Germinated seedlings of rice from untreated control seed in petridish

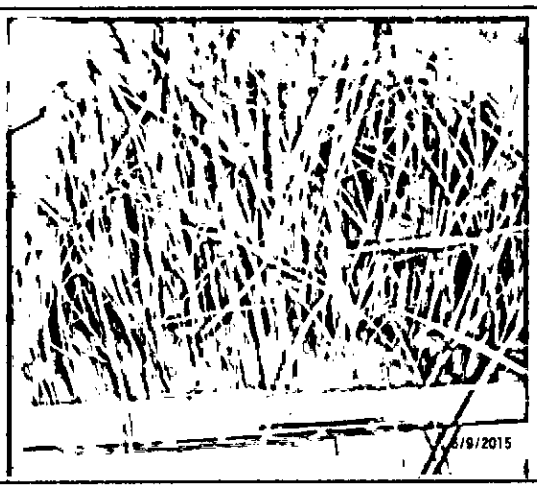


Plate 4. Rice seedling raised in pot soil from treated seeds by 54 ^{0}C for 15 mins

Plate 5. Rice seedlings raised in pot soil from untreated control seeds

4.2.1 Hot water treatment of wheat seeds at different temperatures for 10 mins

Wheat seed dipped in hot water for different temperatures yielded significant differences in percent seed germination. Highest seed germination (90%) was recorded at temperature 51/52°C for 10 mins. Below or above this temperature level (51-52 °C) reduced seed germination significantly. The lowest seed germination (60%) was obtained at 57/58°C which was significantly lower than control (65%) (Table 3, Figure 2).

Abnormal seed germination was found nil (0.0%) at 56-58°C.Below this temperature level (56-58°C), abnormal seed germination increased significantly. At room temperature (25±1°C), abnormal seed germination (22%) was significantly higher than all other treatments (Table 3, Figure 2).

The highest dead seed (40.0%) was recorded at temperature 58 °C. Below this temperature level dead seeds decreased significantly (Table 3, Figure2).

Rotten seeds recorded nil (0.0%) at temperature 58 °C which was statistically similar to that of temperatures 51 – 58 °C. Temperature below 51°C increased the rotten seeds. The highest rotten seeds (22.0%) obtained at room temperature (25±1°C) (Table 3, Figure 2).

Seed infection of *Bipolaris sorokiniana* and *Fusarium* spp. in wheat seeds differed significantly in different temperature (Table 3, Figure 2). Percent seed borne infection of *Bipolaris sorokiniana* and *Fusarium* spp. were 22.0 and 24.0 %, respectively in untreated seeds at room temperature (25±1°C). Both the pathogenic infection decreased significantly with the increase of temperature. Complete eradication of *Bipolaris sorokiniana* and *Fusarium* spp. obtained at 55-58°C (Table 3, Figure 2).

4.2.2 Effect of treatment duration on seed germination and seed health of wheat in hot water treatment at 51 ^{0}

Seed germination and seed infection of wheat recorded at 51^{0}C was found satisfactory while treated for 10 mins. The germination was found the highest (90%) and abnormal seed, dead seed, rotten seed and incidence of *Bipolaris sorokiniana* and *Fusarium* sp. were found the lowest or minimum while the wheat seeds treated at 51^{0}C for 10 mins (Table 4)

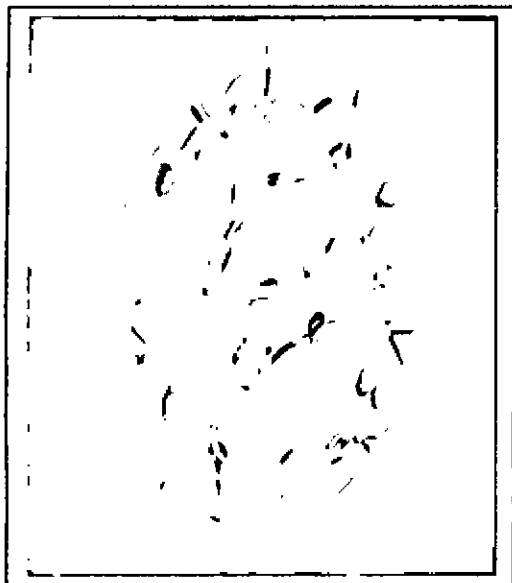


Plate 6. Germinated seedlings of wheat treated at 54 ^{0}C for 15 mins in petridish

Plate 7. Germinated seedlings of wheat from untreated control seed in petridish

Plate-8. Seedlingsof wheat raised in the pot soil from seed treated at 51°C for 10 mins (left) and untreated (right)

Table 3. Effect of hot water treatment at different temperature for 10 mins on seed germination and seed infection of wheat seeds

Temperature (^{0} C)	Seed Germination (%)		Abnormal Seed Germination (%)		Dead Seed (%)		Rotten Seed (%)		Pathogens of *Bipolaris sorokinian a* (%)		*Fusarium* spp. (%)	
50	85.00	ab	(1.20) 6.00	b	(1.60) 10.00	d	(1.20) 6.00	b	(1.60) 10.0	b	(1.30) 7.00	b
51	**90.00**	**a**	(1.20) 6.00	b	(0.80) 2.00	e	(0.77) 1.00	c	(0.80) 2.00	c	(0.85) 3.0	c
52	89.00	a	(1.00) 4.00	c	(1.00) 4.00	e	(0.70) 0.00	c	(1.00) 4.00	c	(0.77) 1.00	c
53	84.00	ab	(1.00) 4.00	c	(1.60) 10.00	d	(0.07) 0.00	c	(0.80) 2.00	cd	(0.80) 2.00	c
54	82.00	bc	(1.00) 4.00	c	(2.11) 18.00	c	(0.07) 0.00	c	(0.80) 2.00	d	(0.80) 2.00	c
55	80.00	c	(1.00) 4.00	c	(2.23) 20.00	c	(0.07) 0.00	c	(0.70) 0.00	d	(0.70) 0.00	d
56	70.00	d	(0.70) 0.00	d	(2.74) 30.00	b	(0.70) 0.00	c	(0.70) 0.00	d	(0.70) 0.00	d
57	62.00	e	(0.70) 0.00	d	(3.31) 38.00	a	(0.70) 0.00	c	(0.70) 0.00	d	(0.70) 0.00	d
58	60.00	e	(0.70) 0.00	d	(3.38) **40.00**	a	(0.70) 0.00	c	(0.70) 0.00	d	(0.70) 0.00	d
Control	65.00	e	(2.38) **22.0**	a	(2.96) 35.00	a	(2.38) **22.00**	a	(2.38) **22.0**	a	(2.47) **24.00**	a
CV (%)	4.46		10.50		8.85		12.64		10.03		11.11	
Tukey HSD	8.2612		0.2964		0.4759		0.2801		0.2428		0.2587	
Level of Significance	**		**		**		**		**		**	

Figures in the parenthesis are the transformed values

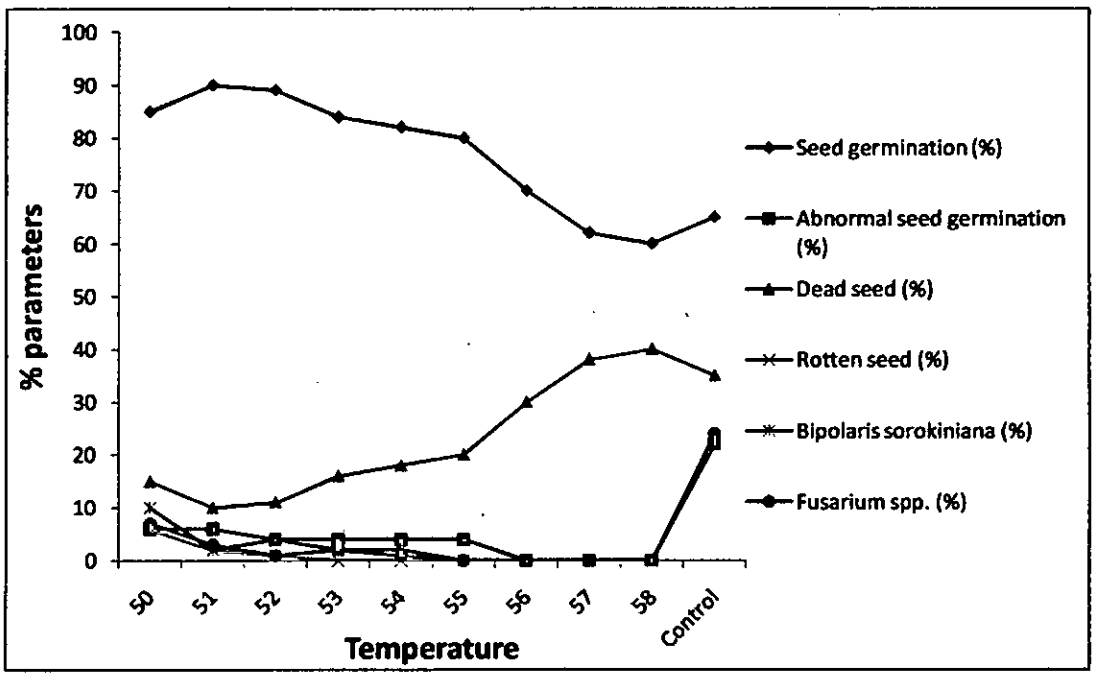


Figure 2. Effect of different temperature on different parameters of wheat seed germination and seed infection for treating 10 mins by hot water

Table 4. Effect of treatment duration on seed germination and seed infection of wheat seed treated at 51^{0}C

Time (Mins)	Seed Germination (%)	Abnormal Seed Germination (%)	Dead Seed (%)	Rotten Seed (%)	% seed borne Pathogens	
					Bipolaris sorokiniana	*Fusarium* sp.
5	80.0	8.0	10.0	3.0	8.0	10.0
10	90 .0	6 .0	2.0	1.0	2.0	3.0
15	85.0	7.0	2.0	1.0	3.0	6.0
Pot soil (10 mins)	87.0	7.0	8.0	3.0	2.0	7.0

4.3.1 Hot water treatment of country bean seeds at different temperatures for 15 mins

Country bean seed dipped in hot water for different temperatures yielded significant differences in percent seed germination. The highest seed germination (85%) was recorded at temperature 55°C for 15 mins. Below or above this temperature (55 °C) reduced seed germination significantly. The lowest seed germination (55%) was obtained at 58°C which was significantly lower than control (60%) (Table 5, Figure 3).

Abnormal seed germination was found nil (0.0%) at 58°C and below up 56 °C temperature, abnormal seed germination increased significantly. At room temperature (25±1°C) in control, abnormal seed germination (40%) was significantly higher than all other treatments (Table 5, Figure 3).

The highest dead seeds (40.0%) were recorded at temperature 58°C. Below this temperature level, dead seeds decreased gradually up to 10 % in 54 -58 °C temperatures then it increased gradually up 35 % in 50- 58 °C temperature (Table 5, Figure 3).

Rotten seeds recorded nil (0.0%) at temperature 55°C to 58°C. Temperature below (55 °C) increased the rotten seeds. The highest rotten seeds (60.0%) obtained at room temperature (25±1°C) in control (Table 5, Figure 3).

Seed infection of *Aspergilus niger* in country bean seeds recorded in different temperature differed significantly (Table 5).Percent seed-borne infection of *Aspergilus niger* was 35.0 % in untreated control at room temperature (25±1°C). The pathogenic infection decreased significantly with increase of temperature. Complete eradication of *Aspergilus niger* obtained at 55-58°C. (Table 5, Figure 3).

Table 5. Effect of hot water treatment at different temperature for 15 mins on seed germination and seed infection of country bean seeds

Temperature (°C)	Seed Germination (%)		Abnormal Seed Germination (%)		Dead Seed (%)		Rotten Seed (%)		(%) Pathogens of *Aspergillus niger*	
50	65.0	c	(2.96) 35.00	a	(2.96) 35.00	b	(2.96) 35.00	b	(4.85) 50.00	b
51	65.0	c	(2.96) 35.00	a	(2.96) 35.00	b	(2.96) 35.00	b	(1.60) 10.00	c
52	70.0	b	(2.74) 30.00	ab	(2.74) 30.00	c	(2.74) 30.00	b	(1.60) 10.0	c
53	75.0	b	(1.18) 28.00	a	(1.70) 11.00	d	(2.51) 25.00	c	(1.10) 5.00	d
54	77.5	ab	(2.64) 28.00	ab	(1.60) 10.00	d	(1.60) 10.0	d	0.80 2.00	d
55	**85.0**	a	(1.60) 10.00	bc	(1.60) 10.00	d	(0.70) 0.00	e	(0.70) 0.00	e
56	80.0	ab	(0.70) 0.00	c	(1.90) 14.00	d	(0.70) 0.00	e	(0.70) 0.00	e
57	78.0	ab	(0.70) 0.00	c	(2.74) 30.00	c	(0.70) 0.00	e	(0.70) 0.00	e
58	55.0	d	(0.70) 0.00	c	(3.38) **40.00**	a	(0.70) 0.00	e	(0.70) 0.00	e
Control	60.0	cd	(3.38) **40.00**	a	(2.96) 35.00	b	(5.87) **60.0**	a	(6.23) **35.0**	a
CV (%)	23.9		13.39		12.82		15.77		12.81	
Tukey HSD	41.481		0.3680		0.3463		0.3876		0.3049	
Level of Significance	**		**		**		**		**	

Figures in the parenthesis are the transformed values

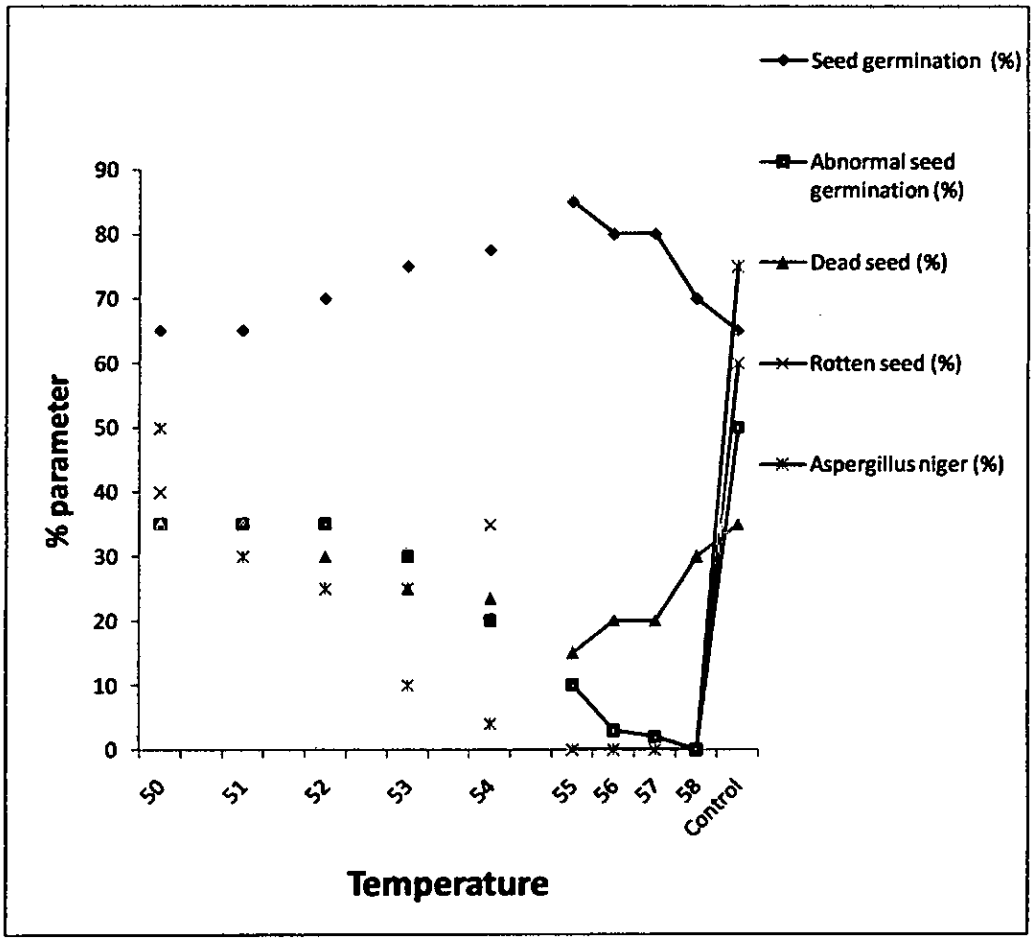


Figure 3. Effect of different temperature on different parameters of country bean seed germination and seed infection treated for 15 mins by hot water.

Table 6. Effect of time duration on seed germination and seed infection of country bean seeds treated at 55 ^{0}C

Time (Minutes)	Seed Germination (%)	Abnormal Seed Germination (%)	Dead Seed (%)	Rotten Seed (%)	Pathogens of *Aspergilus niger* (%)
5	78	15.0	22.0	3.0	4.0
10	82	10.0	15.0	0.0	0.0
15	85	10.0	10.0	0.0	0.0
Pot soil (10 mins)	83	13.0	17.0	5.0	4.0

4.3.2 Effect of time duration on seed germination and seed health of country bean in hot water treatment at 55^0 C

Seed germination and seed infection of country bean recorded at 55^0C was found satisfactory treated for 15 mins. The germination was found the highest (85%) and abnormal seed, dead seed, rotten seed and incidence of *Aspergilus niger* was found the lowest or minimum while the country bean seed were treated at 55^0C for 15mins (Table 6).

Plate-9. Germinated seedlings of country bean treated at 54 ^{0}C for 15 mins in petridish

Plate-10. Germinated seedlings of country bean from untreated control seed in petridish

Plate-11. Country bean seedling raised in pot soil from treated seeds by 54 ^{0}C for 15 mins

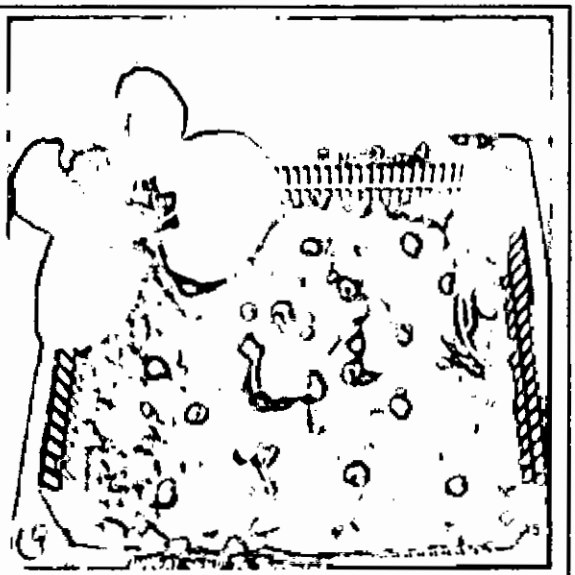


Plate-12. Country bean seedlings raised in pot soil from untreated control seeds

4.4.1 Hot water treatment of tomato seeds at different temperatures for 15 mins

Tomato seed dipped in hot water for different temperatures yielded significant differences in percent seed germination. Highest seed germination (88%) was recorded at temperature 52°C and 53°C for 15 mins. Below or above this temperature (52°C -53°C) reduced seed germination significantly. The lowest seed germination (42 %) was obtained at 58°C which was significantly lower than control (70%) (Table 7, Figure 4).

Abnormal seed germination was found nil (0.0%) at 56°C. Below this temperature 58 °C, abnormal seed germination increased significantly. At room temperature (25±1°C), abnormal seed germination (22%) was significantly higher than all other treatments (Table 7, Figure 4).

The highest dead seeds (58.0 %) were recorded at temperature 58°C. Below this temperature (58 ^{0}C) dead seeds decreased significantly (Table 7, Figure 4).

Rotten seeds recorded nil (0.0%) at temperature 58°C which was statistically similar to that of temperatures 55-57°C.Temperature below this (55 °C) increased the rotten seeds. The highest rotten seeds (10.0%) obtained at room temperature (25±1°C) (Table 7, Figure 4).

Seed infection of *Fusarium* spp. of tomato seeds recorded in different temperature differed significantly. Percent seed-borne infection of *Fusarium* spp. was 18.0 % in untreated seeds at room temperature (25±1°C).The pathogenic infection decreased significantly with increase of temperature. Complete eradication of *Fusarium* spp. obtained at 56-58°C (Table 7, Figure 4).

Table 7. Effect of hot water treatment at different temperature for 15 mins on seed germination and seed infection of tomato seeds

Temperature (%)	Seed Germination (%)		Abnormal Seed Germination (%)		Dead Seed (%)		Rotten Seed (%)		Pathogens of *Fusarium spp.*(%)	
50	76.00	bc	(1.90) 14.00	b	(2.47) 24.00	bc	(1.20) 6.00	b	(1.20) 6.00	b
51	80.00	b	(1.20) 6.00	c	(2.23) 20.00	cd	(0.77) 1.00	c	(0.80) 2.00	bc
52	**88.00**	a	(1.00) 4.00	c	(1.80) 12.00	d	(0.70) 0.00	c	(0.80) 2..00	bc
53	**88.00**	a	(1.00) 4.00	c	(1.80) 12.00	d	(0.70) 0.00	c	(0.77) 1.00	c
54	82.00	b	(1.20) 6.00	c	(2.11) 18.00	c	(0.7) 0.00	c	(0.80) 2.00	bc
55	76.00	bc	(1.00) 4.00	c	(2.47) 24.00	bc	(0.70) 0.00	c	(0.80) 2.00	bc
56	70.00	c	(0.70) 0.00	d	(2.74) 30.00	b	(0.70) 0.00	c	(0.70) 0.00	c
57	48.00	d	(0.70) 0.00	d	(2.23) 20.00	a	(0.70) 0.00	c	(0.70) 0.00	c
58	42.00	d	(0.70) 0.00	d	(5.55) **58.00**	a	(0.70) 0.00	c	(0.70) 0.00	c
Control	70.00	d	(2.38) **22.00**	a	(2.74) 30.0	b	(1.60) **10.00**	a	(2.11) **18.00**	a
CV (%)	5.55		10.49		9.12		17.68		15.92	
Tukey HSD	10.041		0.2964		0.5145		0.3795		0.3945	
Level of Significance	**		**		**		**		**	

Figures in the parenthesis are the transformed values

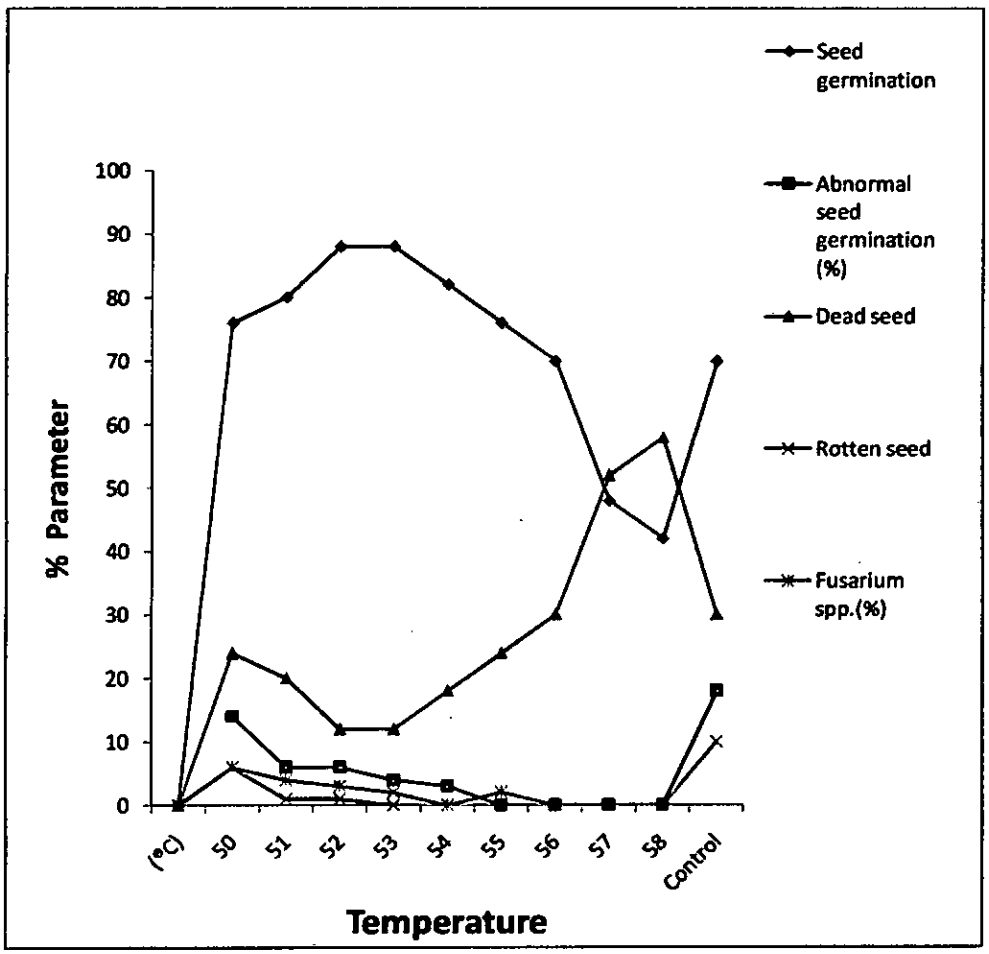


Figure 4. Effects of different temperature on different parameters of tomato seed germination and seed infection for treating 15 mins by hot water

4.4.2 Effect of treatment duration on seed germination and seed health of tomato due to hot water treatment at 52 ^{0}C

Seed germination and seed infection of tomato recorded at 52^{0}C was found satisfactory treated for 15 mins. The germination was found the highest (88%) and abnormal seed, dead seed, rotten seed and incidence of *Aspergilus niger* was found the lowest or minimum while the tomato seed were treated at 52^{0}C for 15 mins (Table 8).

Table 8. Effect of time duration on seed germination and seed infection of tomato seeds treated at 52 ^{0}C

Time (Minutes)	Seed Germination (%)	Abnormal Seed Germination (%)	Dead Seed (%)	Rotten Seed (%)	Pathogens of *Aspergilus niger* (%)
5	78	10 .0	18.0	3.0	4.0
10	85	8.0	15.0	0.0	3.0
15	88	4.0	12.0	0.0	2.0
Pot soil (10 mins)	85	15.0	17.0	5.0	4.0

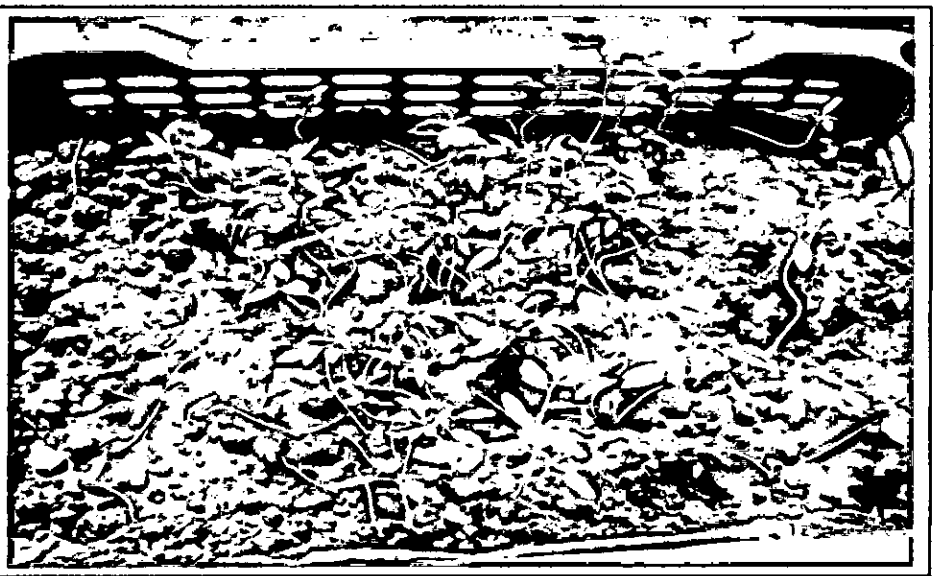


Plate 13. Tomato seedling raised in pot soil from treated seeds at 54 ^{0}C for 15 mins

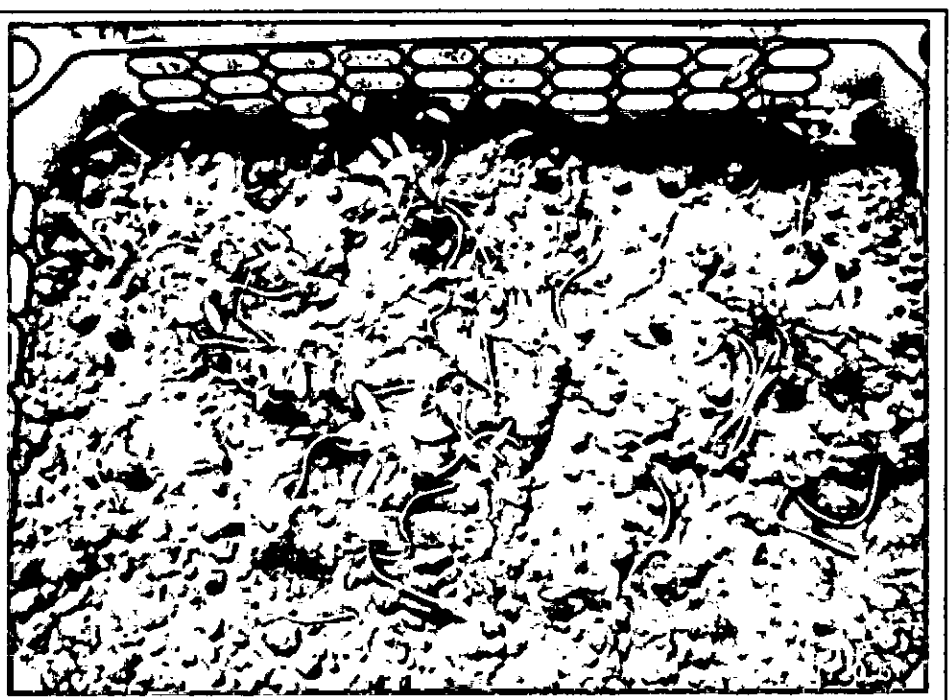


Plate14. Tomato seedlings raised in pot soil from untreated seeds

4.5.1 Hot water treatment of eggplant seeds at different temperatures for 15 mins

Eggplant seeds dipped in hot water at different temperatures yielded significant differences in percent seed germination. The highest seed germination (90%) was recorded at temperature 54°C for 15 mins. Below or above this temperature (54 °C) reduced seed germination significantly. The lowest seed germination (30 %) was obtained at 61°C which was significantly lower than control (70%) (Table 9, Figure 5).

Abnormal seed germination was found nil (0.0%) at 59°C. Below this temperature 59 °C, abnormal seed germination increased significantly. At room temperature (25±1°C), abnormal seed germination (22%) was significantly higher than all other treatments (Table 9, Figure 5).

The highest dead seeds (70.0 %) recorded at temperature 60 and 61°C. Below this temperature (60-61^{0}C) dead seeds decreased significantly. (Table 9, Figure 5).

4.5.1 Hot water treatment of eggplant seeds at different temperatures for 15 mins

Eggplant seeds dipped in hot water at different temperatures yielded significant differences in percent seed germination. The highest seed germination (90%) was recorded at temperature 54°C for 15 mins. Below or above this temperature (54 °C) reduced seed germination significantly. The lowest seed germination (30 %) was obtained at 61°C which was significantly lower than control (70%) (Table 9, Figure 5).

Abnormal seed germination was found nil (0.0%) at 59°C. Below this temperature 59 °C, abnormal seed germination increased significantly. At room temperature (25±1°C), abnormal seed germination (22%) was significantly higher than all other treatments (Table 9, Figure 5).

The highest dead seeds (70.0 %) recorded at temperature 60 and 61°C. Below this temperature (60-61°C) dead seeds decreased significantly. (Table 9, Figure 5).

Rotten seeds recorded nil (0.0%) at temperature between 54°C -61°C. Temperature below this (54 °C) increased the rotten seeds. The highest rotten seeds (10.0%) obtained at room temperature (25±1°C) (Table 9, Figure 5).

Seed infection of *Phomopsis vexans* eggplant seeds recorded in different temperature differed significantly. Percent seed borne infection of *Phomopsis vexans* was 22.0 % in untreated seeds at room temperature (25±1°C). The pathogenic infection decreased significantly with increase of temperature. Complete eradication of *Phomopsis vexans* obtained at 55-58°C. (Table 9, Figure 5).

Table 9. Effect of hot water treatment at different temperature for 15 mins on germination and infection of eggplant seeds

Temperature (^{0}C)	Seed Germination (%)		Abnormal Seed Germination (%)		Dead Seed (%)		Rotten Seed (%)		Pathogen of *Phomopsis vexans.(%)*	
53	88.00	ab	(1.85) 13.00	b	(1.80) 12.00	d	(1.00) 4.00	b	(1.10) 5.00	b
54	**90.00**	a	(1.20) 6.00	c	(1.60) 10.00	d	(0.70) 0.00	c	(0.80) 2.00	bc
55	87.00	ab	(1.10) 5.00	c	(1.85) 13.00	d	(0.70) 0.00	c	(0.70) 0.00	c
56	74.00	c	(1.00) 4.00	cd	(2.54) 26.00	d	(0.70) 0.00	c	(0.70) 0.00	c
57	60.00	d	(1.20) 6.00	c	(3.38) 40.00	c	(0.70) 0.00	c	(0.70) 0.00	c
58	54.00	d	1.00 4.00	cd	(4.45) 46.00	c	(0.70) 0.00	c	(0.70) 0.00	c
59	42.00	e	(0.70) 0.00	d	(5.74) 58.00	b	(0.70) 0.00	c	(0.70) 0.00	c
60	30.00	f	(0.70) 0.00	d	(6.05) **70.00**	a	(0.70) 0.00	c	(0.70) 0.00	c
61	30.00	f	(0.70) 0.00	d	(6.05) **70.00**	a	(0.70) 0.00	c	(0.70) 0.00	c
Control	70.00	c	(2.38) **22.00**	a	(2.74) 30.00	cd	(2.17) **19.0**	a	(2.38) **22.0**	a
CV (%)	4.68		13.40		9.65		13.06		12.52	
Tukey HSD	6.5310		0.3794		0.6681		0.2771		0.2846	
Level of Significance	**		**		**		**		**	

Figures in the parenthesis are the transformed values

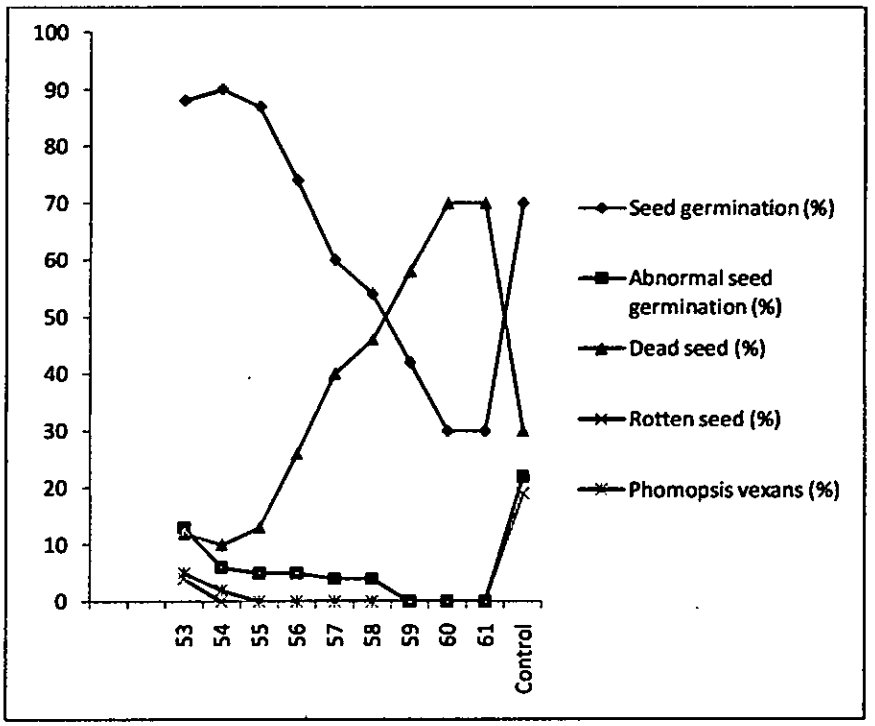


Figure 5. Effect of different temperature on different parameters of seed germination and seed infection for treating eggplant seeds 10 mins by hot water

Plate-15. Germinated seedlings of eggplant treated at 54 ^{0}C for 15 mins in petridish

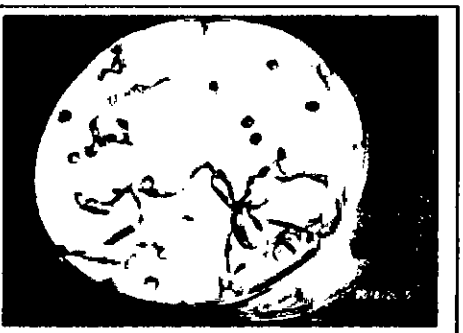


Plate-16. Germinated seedlings of eggplant from untreated control seed in petridish

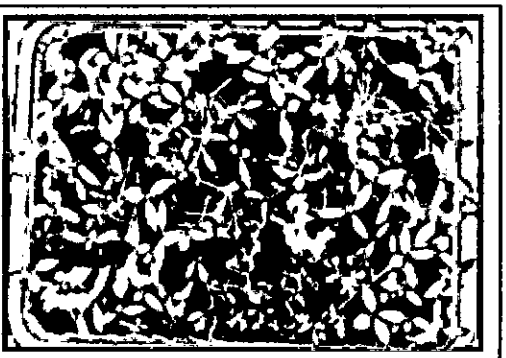


Plate-17. Eggplant seedlings raised in pot soil from untreated control seeds

Plate 18. Eggplant seedling raised in pot soil from treated seeds at 54 ^{0}C for 15

Table 10. Effect of treatment duration on seed germination and seed infection of eggplant seeds treated at 54 ^{0}C

Time (Minutes)	Seed Germination (%)	Abnormal Seed Germination (%)	Dead Seed (%)	Rotten Seed (%)	Pathogens of *Phomopsis vexans.* (%)
5	78	12.0	16.0	3.0	4.0
10	85	8.0	13.0	0.0	1.0
15	90	6.0	8.0	0.0	0.0
Pot soil (15 mins)	85	13.0	10.0	3.0	4.0

4.5.2 Effect of treatment duration on germination and seed health status of eggplant seed due to hot water treatment at 54 ^{0}C

Seed germination and seed infection of eggplant recorded at 54 ^{0}C was found satisfactory when treated for 15 mins. The germination was found the highest (90%) and abnormal seed, dead seed, rotten seed and incidence of *Phomopsis vexans* ware found the lowest or minimum when the eggplant seeds were treated at 54 ^{0}C for 15 mins (Table 10).

4.6 Pot experiment

Rice seed germination was recorded 87.0 % in pot soil as against 59.5% in untreated control while dead seeds percentage was 13.0% in treated seeds and 40.5% in untreated seeds (Fig. 2).

Seed germination of wheat recorded 87.0 % in pot soil as against 62% in untreated rice while dead seeds percentage was 13.0% in treated seeds and 38 % in untreated seeds (Fig. 4).

Country bean seed germination was recorded 82.0 % in pot soil as against 62% in untreated country bean while dead seeds percentage was 18.0% in treated seeds and 48 % in untreated seeds (Fig. 6).

Seed germination was recorded 85.0 % in pot soil as against 68% in untreated tomato while dead seeds percentage was 15.0% in treated seeds and 32 % in untreated seeds (Fig. 8).

Seed germination was recorded 86.0 % in pot soil as against 70% in untreated eggplant while dead seeds percentage was 14.0% in treated seeds and 30 % in untreated seeds (Fig. 10).

Chapter v
Discussion

CHAPTER V

DISCUSSION

5.1 Hot water treatment of rice seeds

Rice treated with hot water at 53/54°C for 15 mins yielded seed germination 90% and completely eradicated seed-borne infection of *Bipolaris oryzae* and *Fusarium* spp. Jiskani (2002), IRRI (1938) and Mohanty (1975) reported that 53-54°C was found effective temperature in eliminating *Bipolaris oryzae* from rice seeds. The effective time of seeds treatment with hot water reported by previous researches was 15 mins. The differences in the endings may be attributed to use different cultivars and machine used for seed treatment.

The seed infection of *Bipolaris oryzae* and *Fusarium* spp. *were* recorded 22.5 and 25 % in rice seeds. The findings are in agreement with Fakir (2000 a) who listed these two fungi along with others (41) as seed-borne pathogens of rice in Bangladesh. The results also in agreement with Sova *et al.* (1983) who reported 2% seed infection of rice by *Bipolaris oryzae* and also with Mia and Mathur (1983) reporting as high as 88.5% seed infection of rice by *Bipolaris oryzae.* However, the results of the present investigation differed with those reports in respect of level of seed infection by different pathogens. Crop cultivars, seed lots, time of collection of seeds and storage conditions could be considered as some of

the factors for the differences. In the present investigation, *Bipolaris oryzae* detected as major seed-borne pathogens of rice which agree with the report of Legaspi *et al.* (1985), Jayaweera *et al.* (1988) and Fakir *et al.* (1990) who recorded *Bipolaris oryzae* as a predominant pathogen in rice seeds in Bangladesh.

5. 2. Hot water treatment of wheat seeds

Hot water treatment of wheat seeds at 51/52° C for 10 mins yielded seed germination 90% and reduced seed-borne infection of *Bipolaris sorokiniana* and *Fusarium* spp. But the findings Kahn (1977) and Khaleduzzaman (1996) who reported 52-54° C as effective temperature in eradicating seed borne fungi from wheat seeds treated for 10 mins. Winter *et al.* (1996) however found 52° C and 5-10 mins 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 mins while seed borne infection of loose smut was eliminated at 55.5°C 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 (2000a) who listed 24 seed borne pathogens of wheat seeds along with these two fungi.

5.3 Hot water treatment of country bean seeds

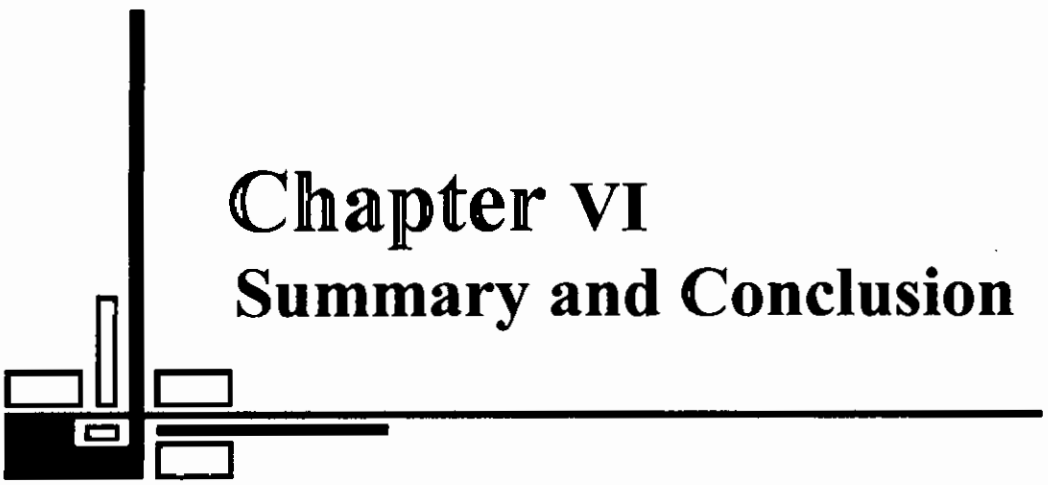
Dipping country bean seeds in hot water at 55°C for 15 mins yielded seed germination 85% and completely eradicated seed-borne infection of *Aspergillus niger*. *Aspergillus niger* was recorded in country bean seeds as 22% of seed infection. As the seed coat of country bean is thick and hard. The seedwall tolerate higher temperature that helps to eradicate the seed-borne pathogens. Research reports were hardly available in Bangladesh with hot water treatment of country bean seeds.

5.4 Hot water treatment of tomato seeds

Hot water treatment of tomato seeds at 52-53° C for 15 mins yielded seed germination 88% and reduced seed borne infection of *Fusarium* spp. Tomato seeds seemed to be delicate to hot water treatment. Thus, report on hot water treatment of tomato seeds are rare in Bangladesh.

5.5 Hot water treatment of eggplant seeds

Hot water treatment of eggplant seeds at 54 ° C for 15 mins yielded seed germination 90% and reduced seed borne infection of *Phomopsis vexans.* Earlier studies support the present findings to some extent (Hossain, 2004; Meah, 2003; Prabhu and Prasada, 1970; Raychoudhury, 1967; Raychoudhury and Lele 1966; Islam, 2005). Hossain (2004) obtained 100% control of *Phomopsis vexans* and 87.0 % seed germination treating seed at 55 ^{0}C for 15 mins. Prabhu and Prasada(1970) reported seed treatment at 52- 54 ^{0}C for 10 mins after soaking seeds in normal water for 4 hr was effective in controlling seed borne *Alternaria triticina.* According to Raychoudhury (1967), hot water treatment of eggplant seeds (50 ^{0}C for 30 mins) was found effective in warding off the *Phomopsis* blight and fruit rot infection by *Phomopsis vexans.* Raychoudhury and Lele (1966), recommended hot water seed treating at 50- 52 ^{0}C for 15 – 30 mins for eggplant, chilli, brassicas and cole crops for destroying the seed-borne pathogens. Islam (2005) reported that hot water seed treatment at 56 ^{0}C for 15 mins completely controlled *Phomopsis vexsans* and increased seed germination by 53.8% over control.



Chapter VI
Summary and Conclusion

CHAPTER VI

SUMMARY AND CONCLUSION

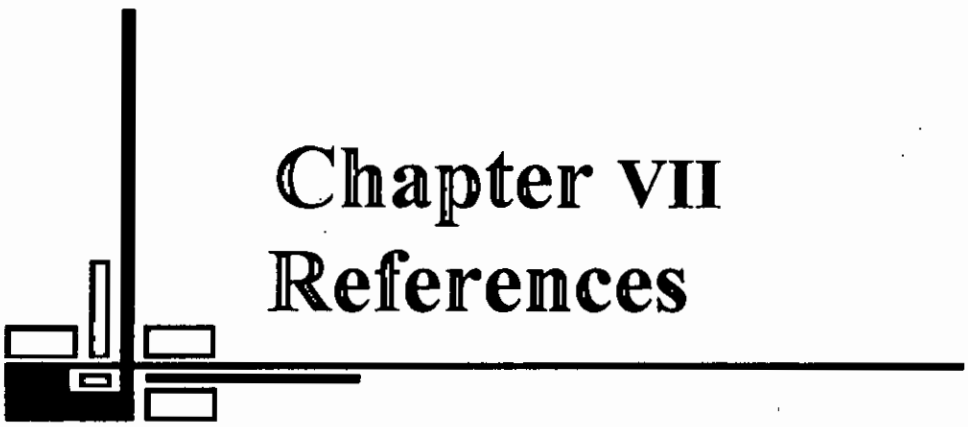
Hot water seed treatment of rice, wheat, country bean, tomato and eggplant seeds in eradicating seed borne infection and maximum seed germination was conducted during the period from July 2014 to December 2015, at the central laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207. Effect of dipping seed in hot water at 50 to 60°C for 5, 10 and 15 mins studied to standardize its effect in controlling seed borne infection of rice, wheat, country bean, tomato and eggplant seeds considering seed germination (%), abnormal seed germination (%),dead Seeds (%), and rotten seeds (%). Standard blotter technique followed for this work.

Hot water treatment of rice seeds was found effective at 53/54°C for 15 min in eliminating completely seed-borne infection of *Bipolaris oryzae* and *Fusarium* spp. with the highest seed germination (90%). For wheat seeds 51/52°C for 10 min was found effective against *Bipolaris sorokiniana* and F*usarium* spp. with maximum seed germination 90.0%. For country bean, 55 / 57°C for 15 mins was found effective with highest seed germination (85%) and zero percent seed infection of *Aspergilus niger.* In case of tomato and eggplant seeds 53 / 54°C for 15

mins gave the highest seed germination (88%, 90%, respectively) and the lowest seed infection (0.0%).

Hot water treated seeds sown in the pot soil gave 33.75%, 20%, 21.5%, 22.5% and 20% higher seed germination, reepectively for rice, wheat, country bean, tomato and eggplant in comparison to untreated control.

The management of seed-borne infection might play a vital role on their production. Organic agriculture, a concept of pollution free crop cultivation in Bangladesh as elsewhere in the world may be benefitted through adopting technology like hot water treatment of seeds for management of seed borne pathogens. Further researchers are desired to re-check the efficacy of hot water treatment of rice, wheat, country bean, tomato and eggplant seeds against seed-borne pathogens and to take step to transfer the technology to the farmers.



Chapter VII
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CHAPTER VII

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