STANDARDIZATION OF HOT WATER TREATMENT IN CONTROLLING MAJOR SEED BORNE PATHOGENS OF VEGETABLES

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STANDARDIZATION OF HOT WATER TREATMENT IN CONTROLLING MAJOR SEED BORNE PATHOGENS OF VEGETABLES

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CERTIFICATE

This is to certify that the thesis entitled, "***Standardization of Hot Water Treatment in Controlling Major Seed Borne Pathogens of Vegetables***" 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 **PLANT PATHOLOGY**, embodies the result of a piece of bona fide research work carried out by **NASRIN SULTANA Registration No. 17-08262** 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 duly been acknowledged.

Dated: 02/12/2019

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DEDICATED TO

MY

BELOVED PARENTS

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STANDARDIZATION OF HOT WATER TREATMENT IN CONTROLLING MAJOR SEED BORNE PATHOGENS OF VEGETABLES

ABSTRACT

The experiment was conducted to standardize the hot water in controlling major seed borne pathogens of selected vegetables during the period from September 2017 to March 2018, in 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 minutes against seed borne microflora were explored to standardize the temperature and time in controlling seed borne pathogens of bitter gourd, pumpkin, bottle gourd, cucumber, eggplant, chilli, tomato, lady´s fingers, radish and country bean seeds. The treatments were T_0 = Control, T_1 = 50 ºC for 5 minutes, T_2 = 52 ºC for 5 minutes, T_3 = 54 ºC for 5 minutes, T_4 =56 ºC for 5 minutes, T_5 = 58 ºC for 5 minutes and T_6 = 60 ºC for 5 minutes. Data were collected on % seed germination, % seed infection, % rotten seed and % dead seed. The experiments were conducted following Completely Randomized Design (CRD) and analyzed by computer package MSTAT-C. Significant differences were found in all the parameters. The highest seed germination in all the vegetables were recorded at the treatment where seeds were treated at 54 °C to 58°C for 5 minutes and the lowest were obtained at control. The lowest rotten seed of all the vegetables recorded in 60 °C and the highest rotten seeds obtained at T_0 (control). The highest dead seed of studied vegetables were recorded in T_6 (60 °C for 5 minutes) and the lowest were obtained at T_0 (control). The lowest infected seed of studied vegetables were recorded in treatment T_6 (60 °C for 5 minutes) and the highest infected seeds were obtained at T_0 (control). In most cases *Chaetomium* sp., *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. etc were prevalent in the seed samples. So, hot water seed treatment with 54 °C to 58°C for 5 minutes could be suggested as safe, pollution free and environmental friendly for management of seed borne pathogens of selected vegetables.

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LIST OF SYMBOL AND ABBREVIATIONS

ABBREVIATIONS	FULL WORD
%	Percentage
DAE	Department of Agricultural Extension
CV	Co efficient of variation
sp	species
Temp.	Temperature
e.g.	Exempli gratia (by way of example)
et al.	and others (at ell)
FAO	Food and Agricultural organization
cm	Centimeter
Mt	Metric ton
SAU	Sher-e-Bangla Agricultural University
J.	Journal
@	At the rate of
ml	Milliliter
CRD	Complete Randomized Design
LSD	Least significant difference
g	Gram
Kg	Kilogram
Xcc	*Xanthomonas campestris* pv. *campestris*
hr	Hour

CHAPTER I

INTRODUCTION

Bangladesh is predominantly an agriculture based country. But it has a huge deficit in vegetable production. Total annual vegetable production of Bangladesh is 1.6 million M tones in winter and 1.5 million M tones in summer season while the cultivated area of Bangladesh 0.47 million acres in winter and 0.65 million acres in summer season (BBS 2012). The consumption of vegetable in Bangladesh is about 50 g day^{-1} capita^{-1}, which is the lowest amongst the countries of South Asia and South Africa (Rekhi 1997). But dietitian recommended a daily allowance of 285 g vegetable for an adult person for a balance diet (Ramphall and Gill, 1990). Here people have been suffering from inadequate supply of vegetables since decades. As a result, chronic malnutrition is often seen in Bangladesh.

There are various causes associated with lower yield of vegetables, 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 lindernuthianum* is a serious disease causing considerable damage (Meah and Khan, 1987). In Bangladesh, Fakir (1980) observed 5% yield loss in beans due to the different diseases including anthracnose caused by *Colletotrichum lindemuthianum*.

Although, considerable amount of works have been done on the seed health particularly, about the transmission of fungi through the seeds of a number of vegetable crops like okra, tomato, chilli, radish, cucumber, bitter gourd, sweet gourd, brinjal and cabbage in the country (Fakir, 1980; Mridha *et al*., 1986; Sultana *et al*., 1988; Gupta *et al*., 1989; Valkonen and Koponen, 1990; Badul and Achar, 1998 and Krishra *et al*., 1998), very limited study has been carried out on seed-borne fungi or seed health of bottle gourd, indian spinach, red amaranth and spinach (Islam, 2005). Islam (2005) detected only a few fungi in the seeds of indian spinach, red amaranth and spinach collected from seed-stores of Mymensingh town. He recorded three species of pathogenic fungi viz. *Cercospora* sp. on Indian spinach, *Colletotrichum capsici* on red amaranth and *Fusarium* sp. on indian spinach, red amaranth and spinach. Most of the fungi were not identified to species level. Reports on the study on hot water treatments of bottle gourd are rare in the country.

Healthy or pathogen free seeds are considered as the vital factor for desired plant population and good harvest. Health of seeds can be affected by direct infection of pathogens or through contamination of seeds by pathogenic propagules as contamination in, on or with the seeds or as concomitant contamination (Rashid *et al*., 2000). Infection of seed by pathogenic organism and presence of propagules of pathogen in a seed lot is vitally important because infected seeds/seed lot may fail to germinate, cause infection to seedlings and growing plants. So, seed health is a determining factor for successful crop production.

Seed-borne diseases can be controlled by various control measures viz. use of chemical, botanical and bio-pesticides 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.

One of the possible alternatives would be the use of pesticides of plant origin, also known as botanical pesticides. Botanical products have been used by man since ancient times, especially in cultures with a strong herbal tradition (Yang and Tang, 1988). They have been reported to be effective against i.e. nematodes (Park *et al*. 2005) and fungi causing plant diseases (Vayias *et al*. 2006). Parts of the plants which are used for the pesticides are roots or rhizomes (i.e. derris) (Kardinan, 1995), vetiver (Zhu *et al*. 2001) and sweet flag (Schmidt and Streloke, 1994), flowers or buds (i.e. pyrethrum) (Athanassiou and Kavallieratos, 2005) and clove (Ho *et al*. 1994), seeds (i.e. neem) (Nathan, *et al*. 2006), castor bean (Niber, 1994). and yam bean (Kardinan, 1995), and leafs (i.e. patchouli) (Mardiningsih and Kardinan, 1995), betelvine (Wardhana *et al*. 2007) and tobacco (Opolot *et al*. 2006). Although the mechanisms of action of the botanical pesticides may differ greatly and are often not yet well understood, they have an advantage that they combine a wide range of toxic potencies hence reducing the chance of pests to develop resistances (Roger, 1997).

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 and less cost technology in controlling seed borne pathogens. Hot water treatment used since 1920 has been little used in practice (Neergaard, 1979). Then a modern 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). The hot water seed treating plant developed in the IPM lab has been found very active for eliminating the seed infection by pathogenic fungi, increasing seed germination and reducing nursery diseases (Meah, 2003).

Hot water seed treatment is a method that can use to eradicate, or at least reduce the level of pathogens (particularly bacterial pathogens), in vegetable seed. Some commercial vegetable seed companies routinely use this method to eradicate pathogens. Hot-water seed treatments are effective because hot water soaks into the seed for a brief time and kills disease-causing organisms, without killing the seed itself. Other common seed treatments (e.g., fungicide treatments) can also help reduce disease, but typically do not eliminate pathogens that have penetrated the seed coat.

Among the practices, seed treatment by hot water is the ecofriendly option for controlling seed borne pathogen. Hot water treatment of seed and plant material are classical thermophysical methods of plant protection. At the end of the 19th century, the method was applied to control loose smut (*Ustilago nuda*) in cereals (Jensen, 1888). In the second half of the 20th century hot water treatment was replaced by the application of more effective chemicals, but chemical pesticides are hazardous for ecological balance and human health. Thus, the present investigation was taken to fulfil the following objectives:

- To identify the seed borne microflora in selected vegetable seeds
- To standardize the temperature and time against major seed borne pathogens of vegetables

CHAPTER II
REVIEW OF LITERATURE

Hot water treatments of seed and plant material are classical the morphophysical 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°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°C for l5 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°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°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°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. brawcicola*) 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 l22°F (49.95°C) for 25 mins for brussels sprouts, cabbage, eggplant, tomato and spinach; l22°F (49.95°C) for 20 min for broccoli, cucumber, carrot, kale, cauliflower, chinese cabbage, kohlrabi and turnips; l22°F (49.95°C) for 15 mins for mustard and radish, l25°F (5l.6°C) for 30 min for peppers and ll8°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 (N ACMCF) 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 senflenberg* W775, S *bovisrnorbificans* and Escherichia coli Ol57:H), dried and stored at 2 °C. The numbers of salmonellae and E. coli Ol57: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.

Jing *et al*. (2009) investigated the effects of hot water treatment in alleviating chilling injury and reducing ultrastructural damage of mature-green cherry tomatoes (*Lycoperscun 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.

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_2SO_4 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_2SO_4 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.

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.

Garcia-Jimenez *et al*.(2004) found to overcome *Dematophora necatrix* by the use of a hot-water treatment (HWT) of *Cyperus esculentustubers*. Isolates of *D. necatrix from C. eseulentus* 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.

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 l5 days at 5 or 12°C and 3 days at 22°C. He advocated that the alternative method of a very short (l5 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.

Lal *et al*. (2002) studied the effect of postharvest water dipping treatments and storage conditions on shelf life and quality of ber (*Ziziphus rnauritiana* 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 non chemical 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 (PS0.05) reduction in germination of spores after 48 h. The hot water temp was increased to 50 and 55°C 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°C for 5 min significantly reduced (P5005) 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 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.

CHAPTER III
MATERIALS AND METHODS

3.1 Experimental site

A set of in vitro experiments were conducted in the Central Laboratory of the Department of Plant Pathology at Sher-e-Bangla Agricultural University, Dhaka.

3.2 Experiment period

The laboratory experiments were conducted during September 2018 to March 2019.

3.3 Collection of seed samples

Seed samples of cucumber, bottle gourd, bitter gourd, pumpkin, tomato, eggplant, chilli, ladys finger, raddish and country bean were collected from Siddique Bazar, Dhaka. Total 500 gram seed each of cucumber, pumpkin, bottle gourd, bitter gourd, country bean and lady´s finger, and 100 gram seed each of tomato, eggplant, chilli and raddish seeds were collected from Siddique Bazar, Dhaka.

3.4 Selection of seed samples

In case of large seeds of country bean, pumpkin, bottle gourd, bitter gourd, cucumber and lady´s finger 400 seeds were taken randomly from each seed sample. And in case of small seeds of tomato, chilli, eggplant and raddish 800 seeds were taken randomly from each seed sample so that maximum incidence of seed borne infection may be available.

3.5 Hot Water Seed Treating Device

Hot water seed treating device was used for hot water treatment of cucumber, bottle gourd, bitter gourd, eggplant, chilli, raddish, lady´s finger, tomato, eggplant and country bean seeds which has been developed at IPM Lab, BAU, Mymensingh.

Figure 01: Hot Water Treating Device

3.6 Experiment

The seeds were treated with hot water at 50°C, 52°C, 54° C, 56 ° C, 58 ° C, 60 ° C with a control for the treatment period 5 min. The seeds were wrapped loosely in cotton bag and placed in a hot water device that constantly held the water at the recommended temperature. Treating seeds at room temperature (25±1°C) served as control.

3.7 Procedure of Hot water treatment

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.

3.8 Plating the treated seeds

After washing the plastic petridish, it was surface sterilized by 70% alcohol and allowed to aeration for sometimes. Two filter papers soaked in sterile water and were set in the petridishes. Twenty five (25) treated seeds of each of tomato, chilli, eggplant and raddish were plated in each petridish, and ten (10) treated seeds of each of bottle gourd, bitter gourd, pumpkin, country bean, cucumber and lady's finger were plated in each petridish. The petridishes were incubated at room temperature (25°C) for 7-10 days. After 10 days, seed germination, dead seeds, rotten seeds and infected seeds were recorded from each petridish. Some pathogens were also identified by stereomicroscope from each petridish.

Figure 02: Plating the treated seeds

3.9 Data collection

After 10 days of seed placement, for each treatment, 4 parameters were examined. The parameters were germinated seed, dead seed, rotten seed and percent seed infection.

The collected data categorized into following parameters:

a) Category I: Germinated seeds

b) Category II: Dead seeds

c) Category III: Rotten seeds

d) Category IV: 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 seen in germinated seeds.

2) Infected seeds

The seeds which carry pathogen inside or outside the seed with any part of seed were treated as pathogen borne seeds/infected seed.

3) 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 part of the essential structures may be diseased or decayed that normal development is inhibited, known as rotten seeds.

4) 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 reported them as dead seeds.

The calculation of germinated seed, dead seed, rotten seed and infected seed were done by following formula:

i. % Germination seed = $\frac{\text{Number of germinated seed}}{\text{Number of total seeds planted/inspected}} \times 100$

ii. % Infected seed = $\frac{\text{Number of infected seeds}}{\text{Number of total seeds planted/inspected}} \times 100$

iii. % Rotten seed = $\frac{\text{Number of rotten seeds}}{\text{Number of total seeds planted/inspected}} \times 100$

iv. % Dead seed germination = $\frac{\text{Number of dead seeds}}{\text{Number of total seeds planted/inspected}} \times 100$

3.10 Statistical Analysis of Data

The data for characters under the present study were statistically analyzed by following Completely Randomized Design (CRD). The analysis of variance was performed and means were compared by Least Significant Difference (LSD) test at 5% level of probability for interpretation of results (Gomez and Gomez, 1984).

CHAPTER IV
RESULTS AND DISCUSSION

4.1.1 Effect of hot water treatment on different parameters of bitter gourd seed at different temperature

The effect of hot water treatment on different parameters of bitter gourd seed found to be differed significantly at different temperature. The parameters were % seed germination, % seed infection, % rotten seed and % dead seed.

In case of % seed germination, the highest seed germination (78.5%) was recorded in treatment T_3 where seeds were treated at 54°C temperature for 5 minutes which was statistically similar with T_2 (75.5%) where seeds were treated with 52°C and T_4 (78.0%) where seeds were treated with 56°C temperature for 5 minutes. The lowest seed germination (44%) was obtained in control treatment where the seeds were treated with normal water at room temperature (Table 1).

In case of seed infection, the lowest infected seed (2.5%) was recorded in treatment T_6 (60°C) for 5 minutes and the highest infected seed (38.0%) was obtained in control treatment (T_0) (Table 1).

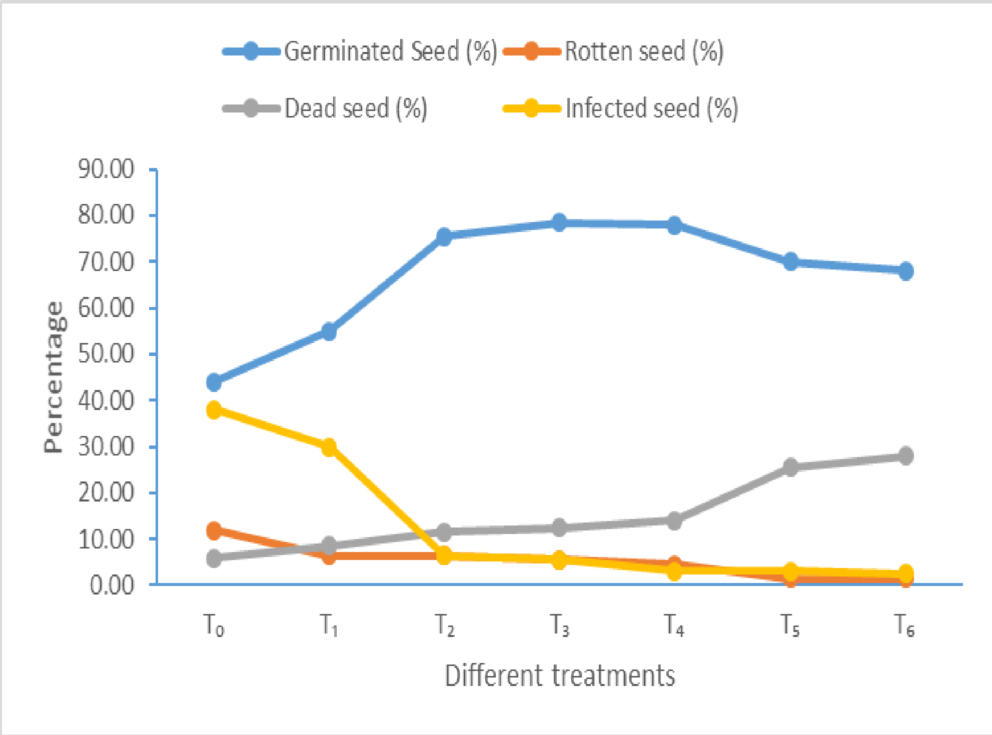
In case of rotten seed, the lowest rotten seed (1.5%) was obtained in treatment T_6 where seeds were treated at 60°C temperature for 5 minutes which was statistically similar with T_5 (1.5%) where seeds were treated in 58°C temperature. It was recorded that temperature laid below 58 °C increased the rotten seeds. The highest rotten seed (12.0%) recorded in control (Table 1).

In case of dead seed, the highest dead seed (28.0%) was recorded in treatment T_6 where seeds were treated at 60°C temperature for 5 minutes and that was statistically similar to treatment T_5 (25.5%) where seeds were treated at 58°C temperature for 5 minutes, The lowest dead seed (6.0%) was obtained in control (Table 1).

Table 1. Effect of hot water treatments on different parameters of bitter gourd seed at different temperature for 5 minutes

Treatment	Germinated seed (%)	Infected Seed (%)	Rotten Seed (%)	Dead Seed (%)
T_0	44.00 d	38.00 a	12.00 a	6.00 d
T_1	55.00 c	30.00 b	6.50 b	8.50c d
T_2	75.50 a	6.50 c	6.50 b	11.50 bc
T_3	78.50 a	5.50 cd	5.50 b	12.50 bc
T_4	78.00 a	3.00 cd	4.50 b	14.00 b
T_5	70.00 b	3.00 cd	1.50 c	25.50 a
T_6	68.00 b	2.50 d	1.50 c	28.00 a
CV (%)	4.81	9.99	10.61	12.45
LSD $_{(0.05)}$	4.73	3.52	2.44	4.10

T_0 = Control, T_1 = 50 ºC for 5 mins., T_2 = 52 ºC for 5 mins.,
T_3 = 54 ºC for 5 mins., T_4 = 56 ºC for 5 mins., $T_{5,}$ = 58 ºC for 5 mins.,
T_6 = 60 ºC for 5 mins.



T_0=Control

T_1= 50 ºC for 5 mins

T_2 = 52 ºC for 5 mins

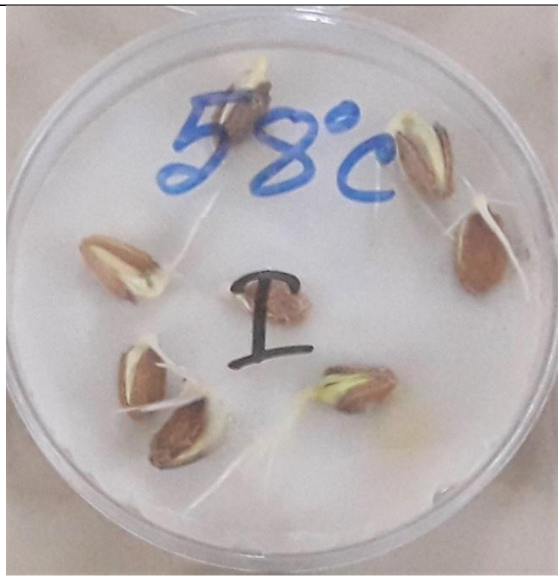
T_3 = 54 ºC for 5 mins

T_4 =56 ºC for 5 mins

$T_{5,}$ =58 ºC for 5 mins

T_6 =60 ºC for 5 mins

Graph 1. Effect of different hot water treatment on different parameter of bitter gourd seed

	
Figure 03. Germinated seedlings of bitter gourd from untreated control seed	**Figure 04**. Germinated seedlings of bitter gourd treated at 54 ºC for 5 minutes

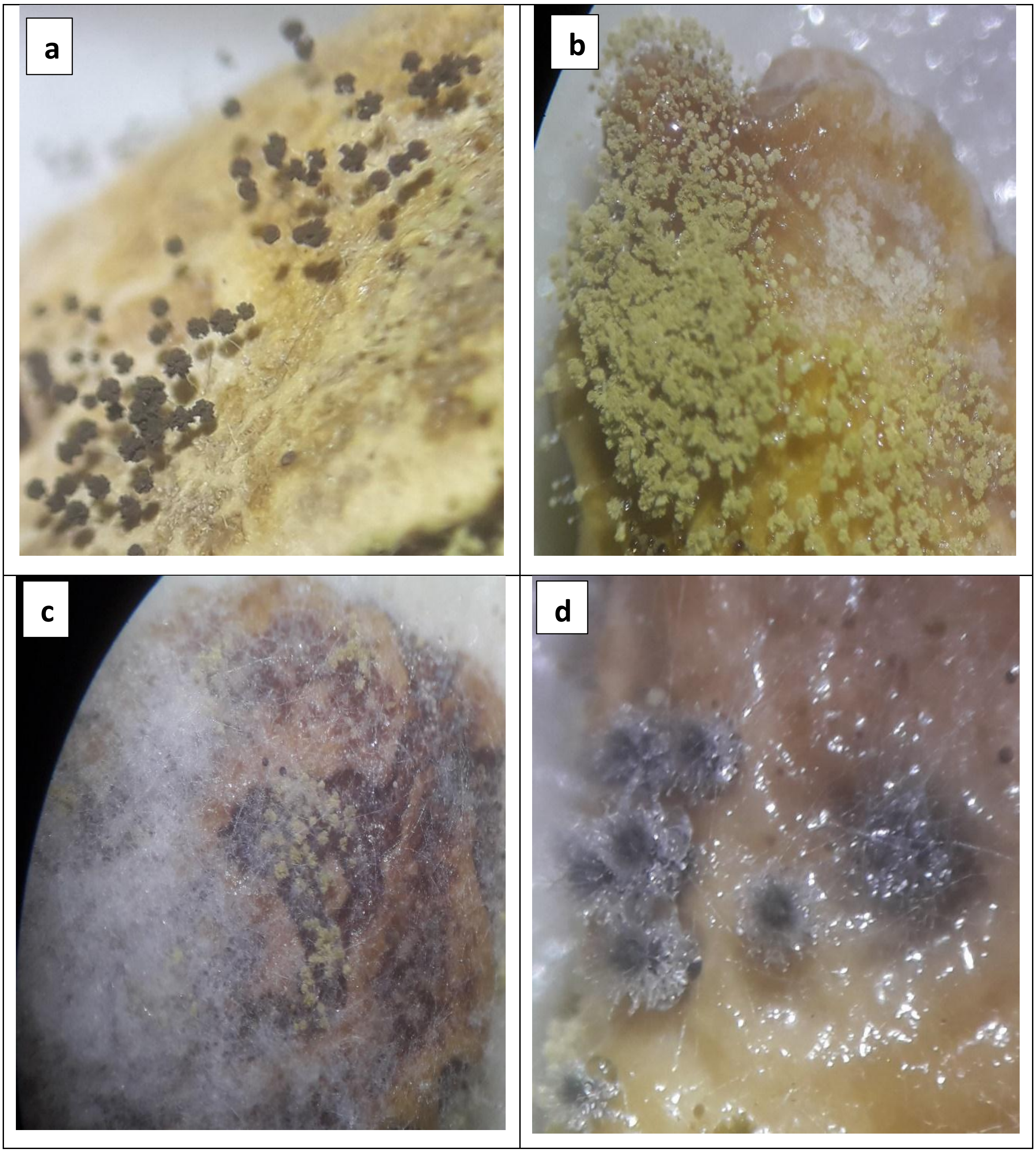


Figure 05 (a-d): Stereo microscopic view of pathogens on Bitter gourd seed, (a) *Aspergillus niger*, (b) *Aspergillus flavus*, (c) *Fusarium* sp., (d) *Chaetomium* sp.

4.1.2 Effect of hot water treatment on different parameters of pumpkin seed at different temperature

The effect of hot water treatment on different parameters of sweet gourd seed found to be differed significantly at different temperature. The parameters were % seed germination, % seed infection, % rotten seed and % dead seed.

In case of % seed germination, the highest seed germination (80.0%) was recorded in treatment T_3 (54°C) temperature for 5 minutes which was statistically similar with T_4 (77.5%) where seeds were treated with 56°C for 5 minutes. The lowest seed germination (52.0%) was obtained in control where the seeds were treated with normal water at room temperature (Table 2).

In case of seed infection, the lowest infected seed (1%) was recorded at treatment T_6 where seeds were treated in 60°C temperature for 5 minutes which was statistically similar with T_4 (2.5%) where seeds were treated with 56°C and T_5 (1.5%) where seeds were treated with 58°C temperature for 5 minutes. The highest infected seed (32.0 %) was obtained in control (T_0) (Table 2).

In case of rotten seed, the lowest rotten seed (1.0%) was recorded in treatment T_5 (58 °C) and T_6 (60 °C) for 5 minutes. Temperature below 58°C increased the rotten seed. The highest rotten seed (10.0%) obtained in control where the seeds were treated with normal water at room temperature (Table 2).

In case of dead seed, the highest dead seed (26.0%) was recorded at treatment T_6 where seeds were treated in 60°C temperature for 5 minutes which was statistically similar with T_5 (25.0%) where seeds were treated with 58°C temperature for 5 minutes.and the lowest dead seed (6.0%) was obtained in control (T_0) (Table 2).

Table 2. Effect of hot water treatment on different parameters of pumpkin seed at different temperature

Treatment	Germinated seed (%)	Infected Seed (%)	Rotten Seed (%)	Dead Seed (%)
T_0	52.00 e	32.00 a	10.00 a	6.00 d
T_1	67.00 d	20.50 b	5.50 b	7.00 d
T_2	71.50 cd	13.00 c	4.50 b	11.00 c
T_3	80.00 a	8.00 d	2.50 c	12.00 c
T_4	77.50 ab	2.50 e	2.50 c	17.50 b
T_5	72.50 bc	1.50 e	1.00 d	25.00 a
T_6	72.00 cd	1.00 e	1.00 d	26.00 a
CV (%)	4.45	9.40	15.30	11.61
LSD $_{(0.05)}$	5.02	3.52	1.43	2.54

T_0 = Control, T_1 = 50 ºC for 5 mins., T_2 = 52 ºC for 5 mins.,
T_3 = 54 ºC for 5 mins., T_4 = 56 ºC for 5 mins., $T_{5,}$ = 58 ºC for 5 mins.,
T_6 = 60 ºC for 5 mins.

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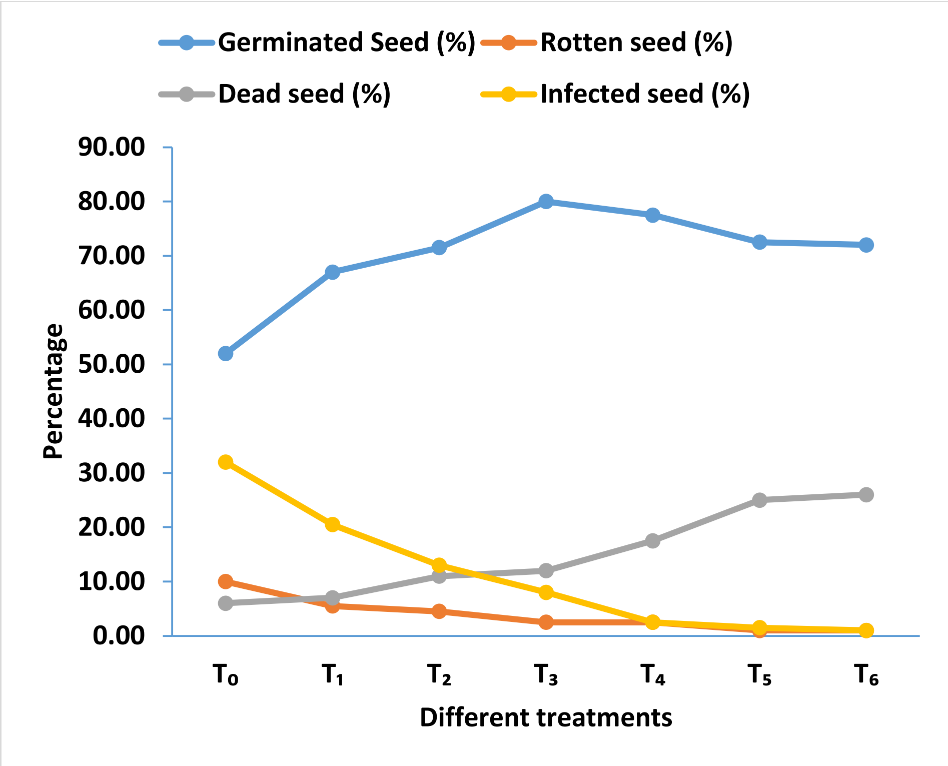

T_0=Control

T_1= 50 ºC for 5 mins

T_2 = 52 ºC for 5 mins

T_3 = 54 ºC for 5 mins

T_4 =56 ºC for 5 mins

$T_{5,}$ =58 ºC for 5 mins

T_6 =60 ºC for 5 mins

Graph 2. Effect of different hot water treatment on different parameter of pumpkin seed

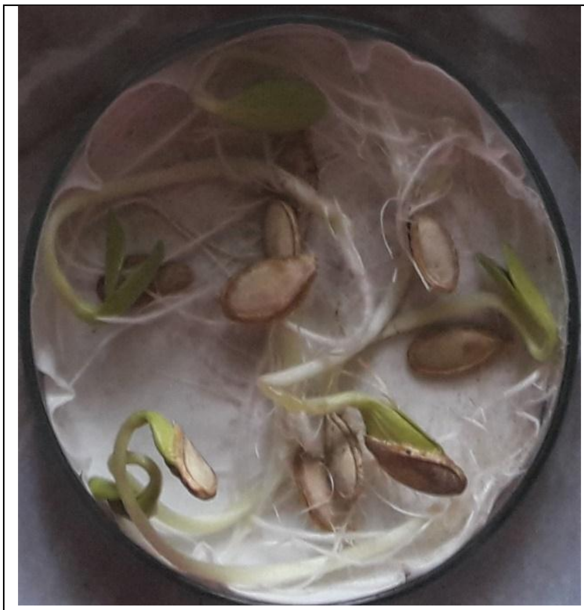	
Figure 06. Germinated seedlings of pumpkin from untreated control seed	**Figure 07**. Germinated seedlings of pumpkin treated at 54 ºC for 5 minutes

Figure 08: Stereo microscopic view of pathogen on pumpkin seed, *Aspergillus niger*

4.1.3 Effect of hot water treatment on different parameters of bottle gourd seed at different temperature

The effect of hot water treatment on different parameters of bottle gourd seed found to be differed significantly at different temperature. The parameters were % seed germination, % seed infection, % rotten seed and % dead seed.

In case of % seed germination, the highest seed germination (82.5%) was recorded in treatment T_4 (56°C) for 5 minutes which was statistically similar with T_2 (76.5%) where seeds were treated with 52°C, T_3 (82.0%) where seeds were treated with 54°C and T_5 (79.0%) where seeds were treated with 58°C temperature for 5 minutes. The lowest seed germination (60.0%) was obtained in control where the seeds were treated with normal water at room temperature (Table 3).

In case of seed infection, the lowest infected seed (2.0%) was recorded at treatment T_6 (60 °C) and T_5 (58 °C) for 5 minutes which was statistically similar with T_4 (3.5%) where seeds were treated with 56°C and T_3 (6.0%) where seeds were treated with 54°C the highest infected seed (28.0 %) was obtained in control where the seeds were treated with normal water at room temperature (Table 3).

In case of rotten seed, the lowest rotten seed (0.5%) was recorded at treatment T_6 (60 °C), T_5 (58 °C) and T_4 (56 °C) for 5 minutes which was statistically similar with T_3 (1.5%) where seeds were treated in 54°C temperature. Temperature below 56°C increased the rotten seeds. The highest rotten seed (8.0%) obtained in control (T_0) (Table 3).

In case of dead seed, the highest dead seed (22.0%) was recorded at treatment T_6 (60°C) for 5 minutes which was statistically similar with (18.5%) where seeds were treated in 58°C temperature for 5 minutes and the lowest dead seed (4.0%) was obtained in control where the seeds were treated with normal water at room temperature (Table 3).

Table 3. Effect of hot water treatment on different parameters of bottle gourd seed at different temperature

Treatment	Germinated seed (%)	Rotten Seed (%)	Dead Seed (%)	Infected Seed (%)
T_0	60.00d	8.00a	4.00c	28.00a
T_1	67.50c	3.00b	5.00c	24.50a
T_2	76.50ab	2.50bc	6.00c	15.00b
T_3	82.00ab	1.50d	10.50b	6.00c
T_4	82.50a	0.50d	11.00b	3.50c
T_5	79.00ab	0.50d	18.50a	2.00c
T_6	75.50b	0.50d	22.00a	2.00c
CV (%)	6.13	10.35	12.34	9.82
LSD $_{(0.05)}$	6.73	1.39	4.42	5.07

T_0 = Control, T_1 = 50 ºC for 5 mins., T_2 = 52 ºC for 5 mins., T_3 = 54 ºC for 5 mins., T_4 = 56 ºC for 5 mins., T_5, = 58 ºC for 5 mins., T_6 = 60 ºC for 5 mins.

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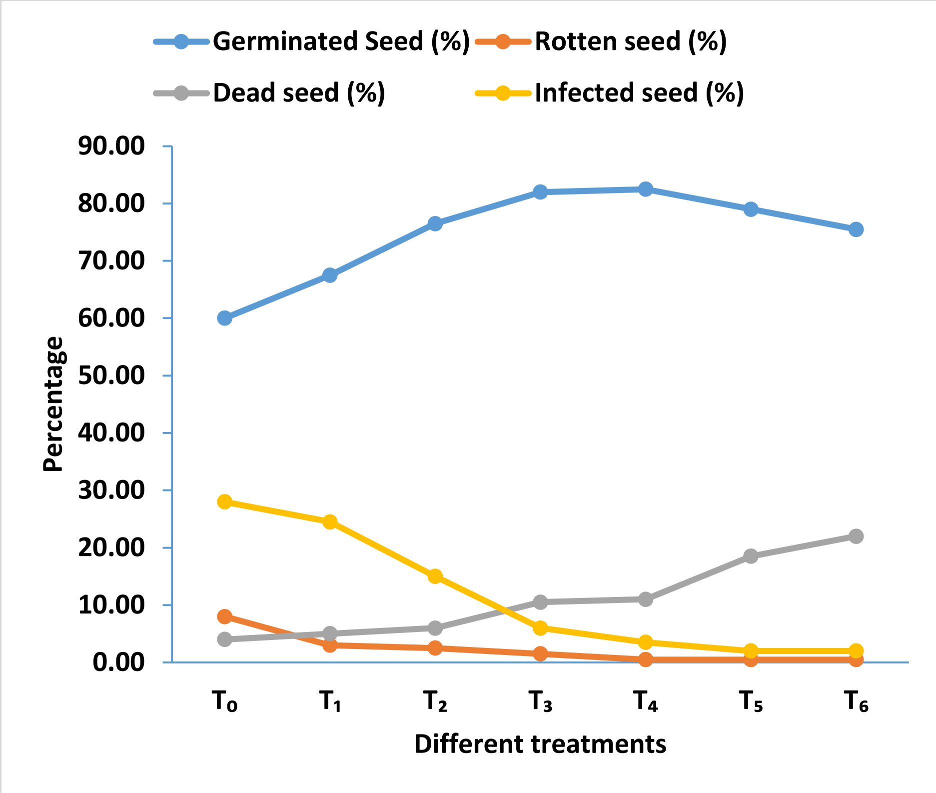

T_0=Control

T_1= 50 ºC for 5 mins

T_2 = 52 ºC for 5 mins

T_3 = 54 ºC for 5 mins

T_4 =56 ºC for 5 mins

$T_{5,}$ =58 ºC for 5 mins

T_6 =60 ºC for 5 mins

Graph 3. Effect of different hot water treatment on different parameter of bottle gourd seed

<table>
<tr><td></td><td></td></tr>
<tr><td>Figure 09. Seeds of bottle gourd from untreated control seed</td><td>Figure 10. Germinated seedlings of bottle gourd treated at 56ºC for 5 minutes</td></tr>
</table>

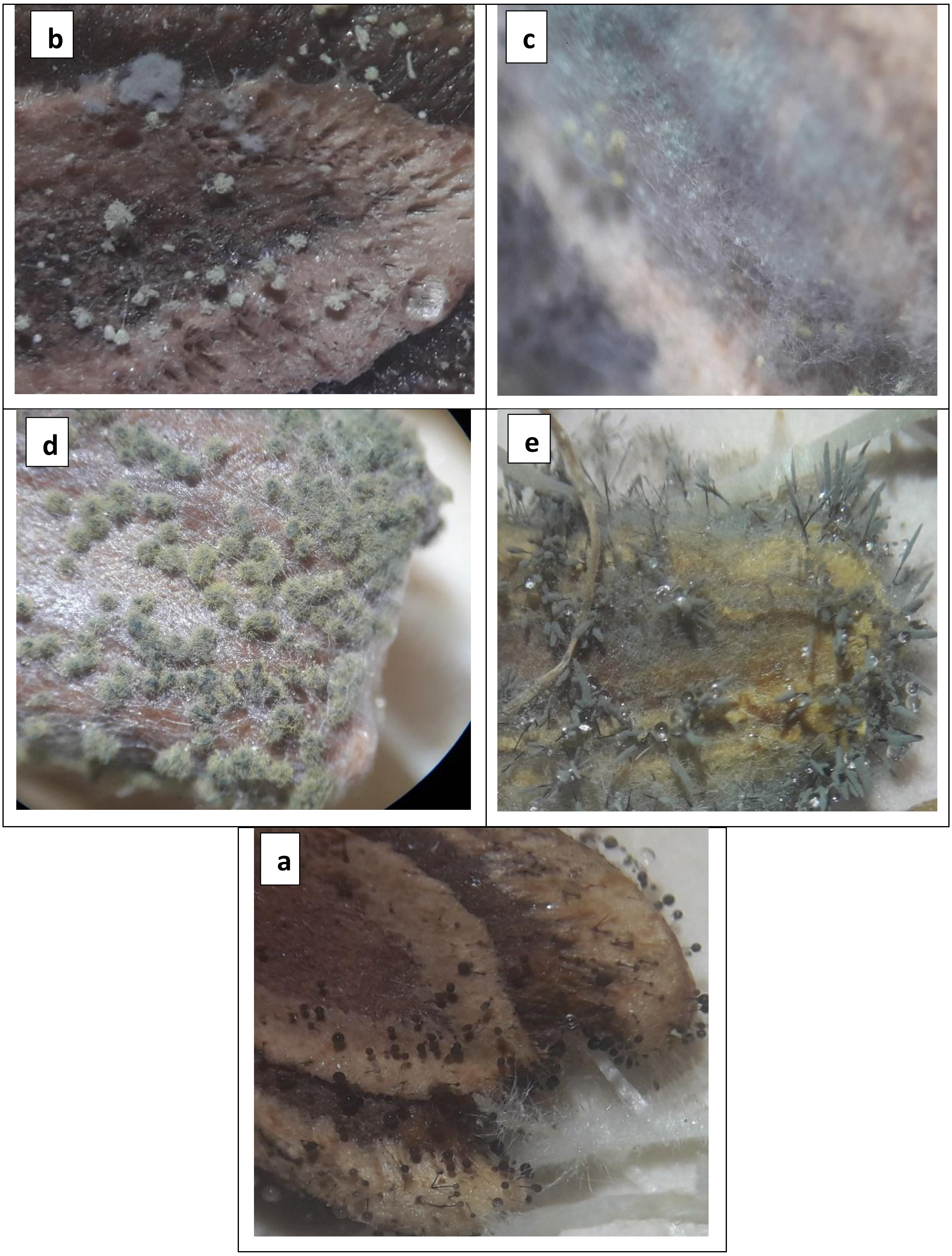

Figure 11 (a-e): Stereo microscopic view of pathogen on bottle gourd seed, (a) ***Aspergillus niger,*** **(b)** ***Aspergillus flavus, (c) Fusarium*** **sp.,** ***(d) Chaetomium*** **sp., (e) Unknown 1**

4.1.4 Effect of hot water treatment on different parameters of cucumber seed at different temperature

The effect of hot water treatment on different parameters of cucumber seed found to be differed significantly at different temperature. The parameters were % seed germination, % seed infection, % rotten seed and % dead seed.

In case of % seed germination, the highest seed germination (81.5%) was recorded in treatment T_4 where seeds were treated in 56°C temperature for 5 minutes which was statistically similar with T_3 (77.0%) where seeds were treated with 54°C and T_5 (76.0%) where seeds were treated with 58°C temperature for 5 minutes. The lowest seed germination (62.0%) was obtained in control where the seeds were treated with normal water at room temperature (Table 4).

In case of seed infection, the lowest infected seed (1.5%) was recorded in treatment T_6 where seeds were treated at 60°C temperature for 5 minutes which was statistically similar with T_4 (56 °C) and T_5 (58 °C) and the highest infected seed (30.0 %) was obtained in control where the seeds were treated with normal water at room temperature (Table 4).

In case of rotten seed, the lowest rotten seed (1.0%) was recorded at treatment T_5 (58 °C) and T_6 (60 °C) for 5 minutes which was statistically similar with T_4 (1.5%) where seeds were treated with 56°C temperature for 5 minutes. Temperature below 58°C increased the rotten seed. The highest rotten seed (8.0%) obtained in control where the seeds were treated with normal water at room temperature (Table 4).

In case of dead seed, the highest dead seed (24.5%) was recorded in treatment T_6 where seeds were treated at 60°C temperature for 5 minutes which was statistically similar with T_5 (20.0%) where seeds were treated with 58°C temperature for 5 minutes and the lowest dead seed (0.0%) was obtained in control (T_0) (Table 4).

Table 4. Effect of hot water treatment on different parameters of cucumber seed at different temperature

Treatment	Germinated seed (%)	Infected Seed (%)	Rotten Seed (%)	Dead Seed (%)
T_0	62.00 d	30.00 a	8.00 a	0.00 d
T_1	65.00 cd	25.50 b	3.50 b	6.00 c
T_2	71.00 bc	15.50 c	3.00 b	10.50 bc
T_3	77.00 ab	7.50 d	2.50 bc	13.00 b
T_4	81.50 a	3.00 e	1.50 cd	14.00 b
T_5	76.00 ab	3.00 e	1.00 d	20.00 a
T_6	73.00 b	1.50 e	1.00 d	24.50 a
CV (%)	5.82	7.04	13.15	12.73
$LSD_{(0.05)}$	6.01	3.07	1.47	5.31

T_0 = Control, T_1 = 50 ºC for 5 mins., T_2 = 52 ºC for 5 mins., T_3 = 54 ºC for 5 mins., T_4 = 56 ºC for 5 mins., $T_{5,}$ = 58 ºC for 5 mins., T_6 = 60 ºC for 5 mins.

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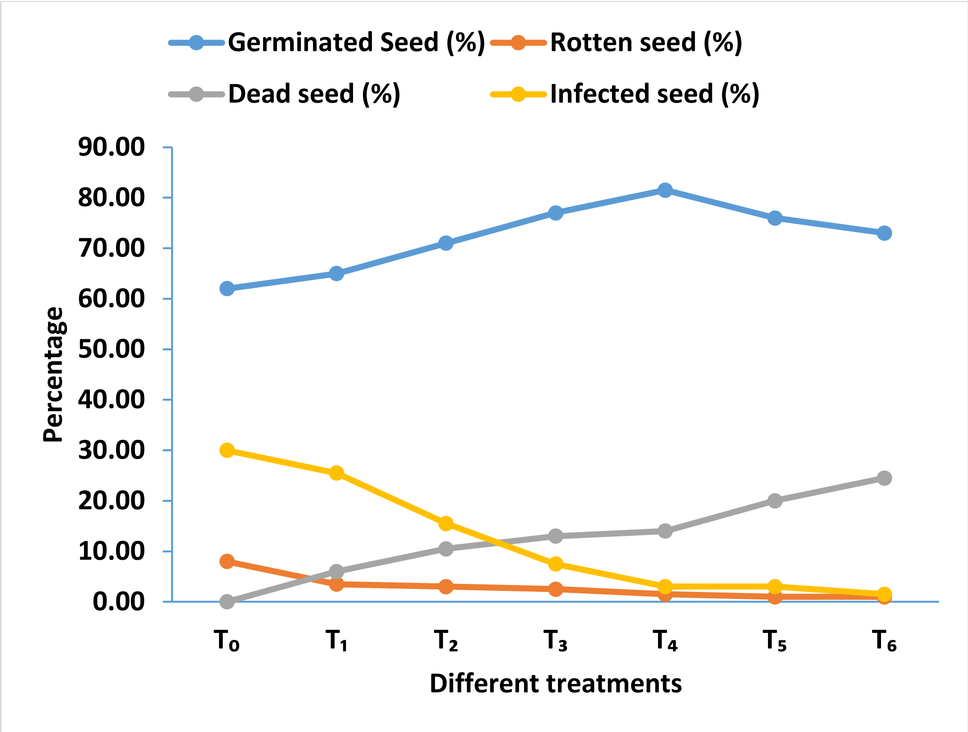


T_0=Control

T_1= 50 ºC for 5 mins

T_2 = 52 ºC for 5 mins

T_3 = 54 ºC for 5 mins

T_4 =56 ºC for 5 mins

$T_{5,}$ =58 ºC for 5 mins

T_6 =60 ºC for 5 mins

Graph 4. Effect of different hot water treatment on different parameter of cucumber seed

	
Figure 12. Seeds of cucumber from untreated control seed	**Figure 13.** Germinated seedlings of cucumber treated at 56 ºC for 5 minutes

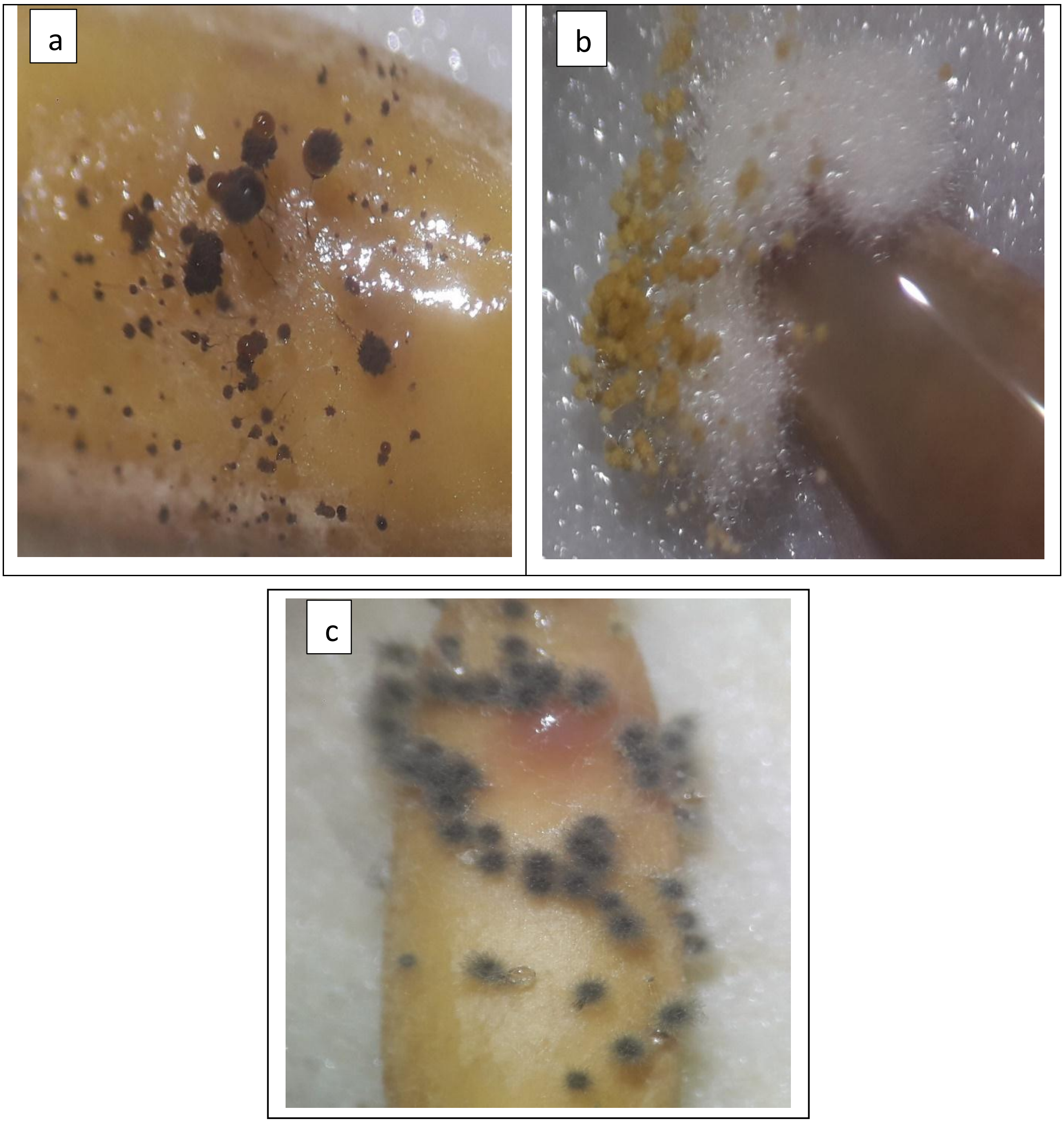


Figure 14 (a-c): Stereo microscopic view of pathogens on cucumber seed, (a) *Aspergillus niger*, (b) *Aspergillus flavus*, (b) *Chaetomium* sp.

4.1.5 Effect of hot water treatment on different parameters of eggplant seed at different temperature

The effect of hot water treatment on different parameters of eggplant seed found to be differed significantly at different temperature. The parameters were percent seed germination, percent seed infection, percent rotten seed and percent dead seed.

In case of percent seed germination, the highest seed germination (81.0%) was recorded in treatment T_5 where seeds were treated in 58°C temperature for 5 minutes which was statistically similar with treatment T_3 (78.0%, 54°C), T_4 (79.5%, 56°C) and T_5 (81.0%, 58°C) for 5 minutes. The lowest seed germination (54.00%) was obtained in control (T_0) (Table 5).

In case of seed infection, the lowest seed infection (0.0%) was recorded in treatment T_6 (60°C) for 5 minutes that was statistically similar with T_5 (0.5%,58°C) and the highest infected seed (34.0 %) was obtained in control treatment where the seeds were treated with normal water only(Table 5).

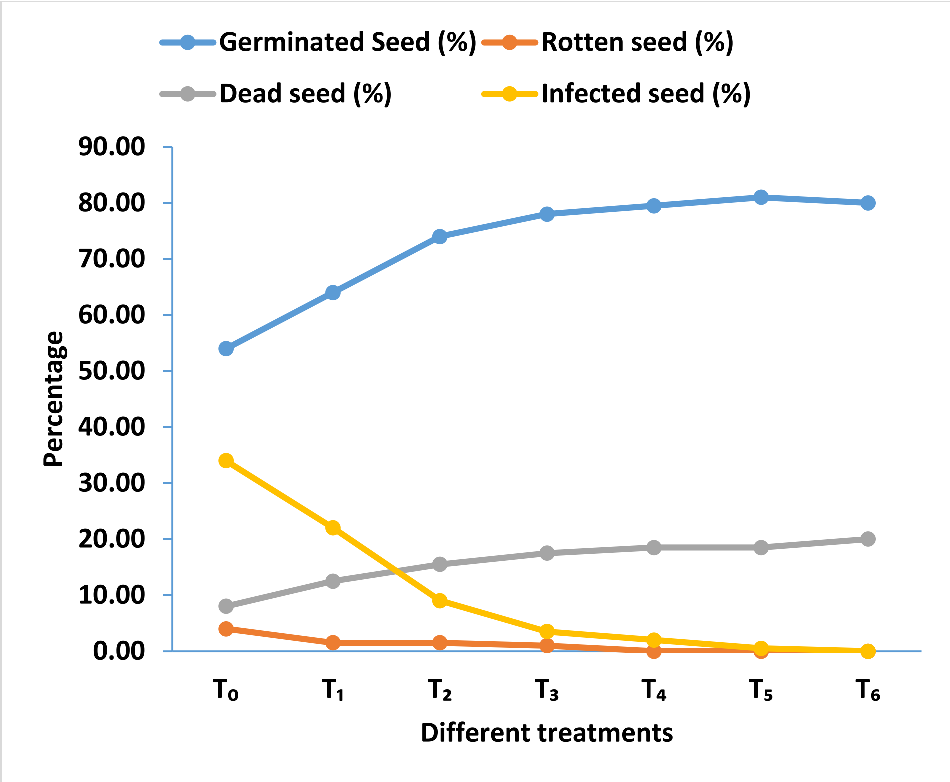
In case of rotten seed, the lowest rotten seed (0.0%) was recorded at treatment T_4 (56 °C), T_5 (58 °C) and T_6 (60 °C) for 5 minutes which was statistically similar with T_1 (50°C), T_2 (52 °C) and T_3 (54 °C). Temperature below 56 °C increased the rotten seed. The highest rotten seed (4.0%) obtained in control where the seeds were treated with normal water at room temperature (Table 5).

In case of dead seed, the highest dead seed (20.0%) was recorded at treatment T_6 where seeds were treated in 60°C temperature for 5 minutes which was statistically similar with T_3 (54°C), T_4 (56 °C) and T_5 (58 °C) for 5 minutes and the lowest dead seed (8.0%) was obtained in control where the seeds were treated with normal water at room temperature (Table 5).

Table 5. Effect of hot water treatment on different parameters of eggplant seed at different temperature

Treatment	Germinated seed (%)	Infected Seed (%)	Rotten Seed (%)	Dead Seed (%)
T_0	54.00 d	34.00 a	4.00 a	8.00 d
T_1	64.00 c	22.00 b	1.50 b	12.50 c
T_2	74.00 b	9.00 c	1.50 b	15.50 bc
T_3	78.00 a	3.50 d	1.00 b	17.50 ab
T_4	79.50 a	2.00 de	0.00 b	18.50 ab
T_5	81.00 a	0.50 e	0.00 b	18.50 ab
T_6	80.00 a	0.00 e	0.00 b	20.00 a
CV (%)	3.68	11.38	7.36	14.03
LSD $_{(0.05)}$	3.94	2.44	1.63	3.25

T_0 = Control, T_1 = 50 ºC for 5 mins., T_2 = 52 ºC for 5 mins.,
T_3 = 54 ºC for 5 mins., T_4 = 56 ºC for 5 mins., $T_{5,}$ = 58 ºC for 5 mins.,
T_6 = 60 ºC for 5 mins.



T_0=Control

T_1= 50 ºC for 5 mins

T_2 = 52 ºC for 5 mins

T_3 = 54 ºC for 5 mins

T_4 =56 ºC for 5 mins

$T_{5,}$ =58 ºC for 5 mins

T_6 =60 ºC for 5 mins

Graph 5. Effect of different hot water treatment on different parameter of eggplant seed

	
Figure 15. Germinated seedlings of eggplant from untreated control	**Figure 16.** Germinated seedlings of eggplant treated at 58 ºC for 5 minutes

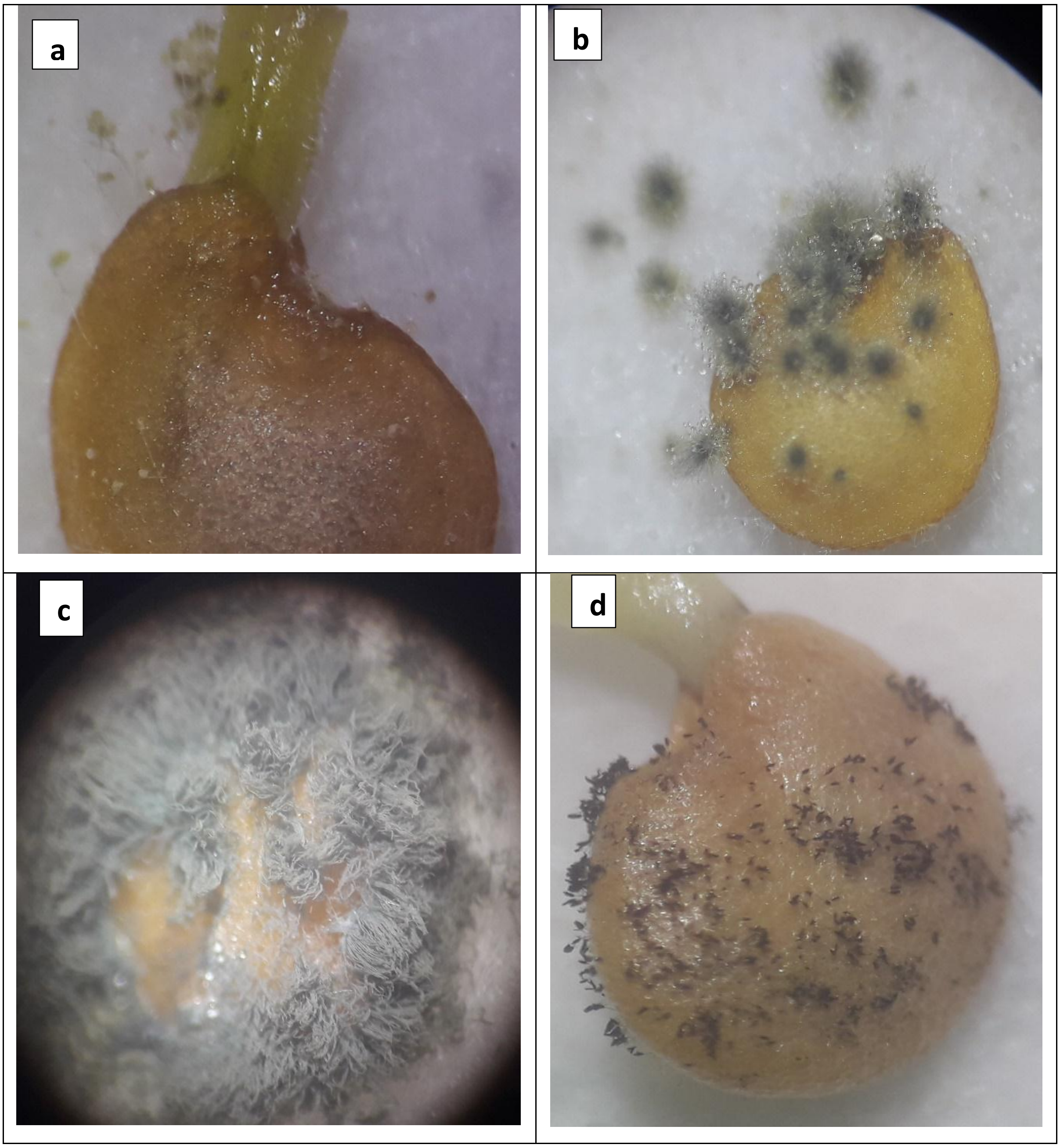


Figure 17. (a-d): Stereo microscopic view of pathogens on eggplant seed, (a) *Aspergillus flavus*, (b) *Chaetomium* sp., (c) *Penicillium* sp., (d) Unknown 1

4.1.6 Effect of hot water treatment on different parameters of chilli seed at different temperature

The effect of hot water treatment on different parameters of chilli seed found to be differed significantly at different temperature. The parameters were % seed germination, % seed infection, % rotten seed and % dead seed.

In case of % seed germination, the highest seed germination (87.0%) was recorded in treatment T_3 (54°C) for 5 minutes and the lowest seed germination (66.0%) was obtained in control where the seeds were treated with normal water at room temperature (Table 6).

In case of seed infection, the lowest infected seed (1.5%) was recorded in treatment T_6 (60°C) for 5 minutes which was statistically similar with T_4 (3.0%) and T_5 (2.0%). The highest infected seed (26.0 %) was obtained in control where the seeds were treated with normal water at room temperature (Table 6).

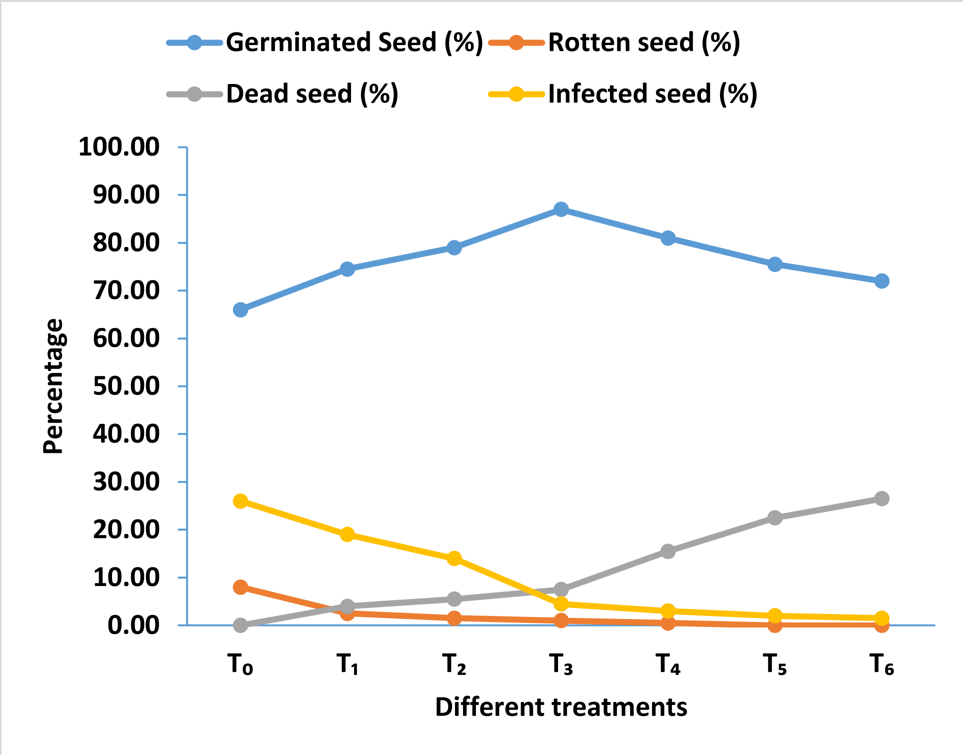
In case of rotten seed, the lowest rotten seed (0.0%) was recorded at treatment T_5 (58 °C) and T_6 (60 °C) for 5 minutes which was statistically similar with T_3 (1.0%, 54 °C) and T_4 (0.5%, 56 °C). The highest rotten seed (8.0%) was obtained in control where the seeds were treated with normal water at room temperature (Table 6).

In case of dead seed, the highest dead seed (26.5%) was recorded in treatment T_6 where seeds were treated in 60°C temperature for 5 minutes and the lowest dead seed (0.0%) was obtained in control where the seeds were treated with normal water at room temperature (Table 6).

Table 6. Effect of hot water treatment on different parameters of chilli seed at different temperature

Treatment	Germinated seed (%)	Infected Seed (%)	Rotten Seed (%)	Dead Seed (%)
T_0	66.00 e	26.00 a	8.00 a	0.00 e
T_1	74.50 d	19.00 b	2.50 b	4.00 d
T_2	79.00 bc	14.00 c	1.50 bc	5.50 d
T_3	87.00 a	4.50 d	1.00 cd	7.50 d
T_4	81.00 b	3.00d e	0.50 cd	15.50 c
T_5	75.50 cd	2.00 e	0.00 d	22.50 b
T_6	72.00 d	1.50 e	0.00 d	26.50 a
CV (%)	3.63	8.46	10.80	13.03
LSD $_{(0.05)}$	4.08	2.17	1.57	3.94

T_0 = Control, T_1 = 50 ºC for 5 mins., T_2 = 52 ºC for 5 mins.,
T_3 = 54 ºC for 5 mins., T_4 = 56 ºC for 5 mins., $T_{5,}$ = 58 ºC for 5 mins.,
T_6 = 60 ºC for 5 mins.



T_0=Control

T_1= 50 ºC for 5 mins

T_2 = 52 ºC for 5 mins

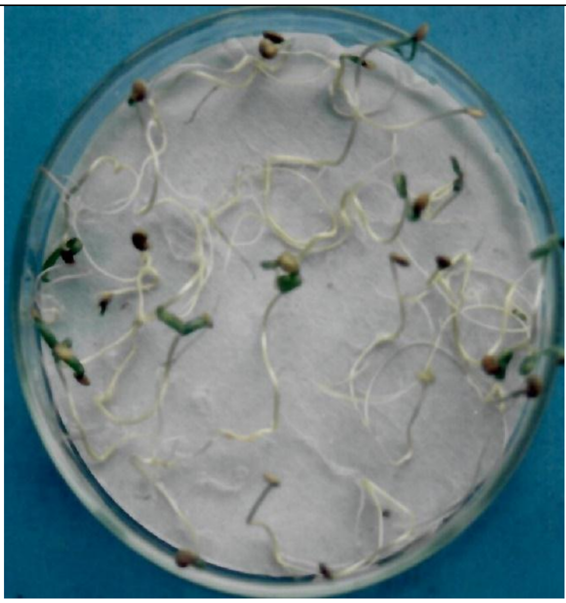
T_3 = 54 ºC for 5 mins

T_4 =56 ºC for 5 mins

$T_{5,}$ =58 ºC for 5 mins

T_6 =60 ºC for 5 mins

Graph 6. Effect of different hot water treatment on different parameter of chilli seed

	
Figure 18. Germinated seedlings of chilli from untreated control seed	**Figure 19.** Germinated seedlings of chilli treated at 54 ºC for 5 minutes

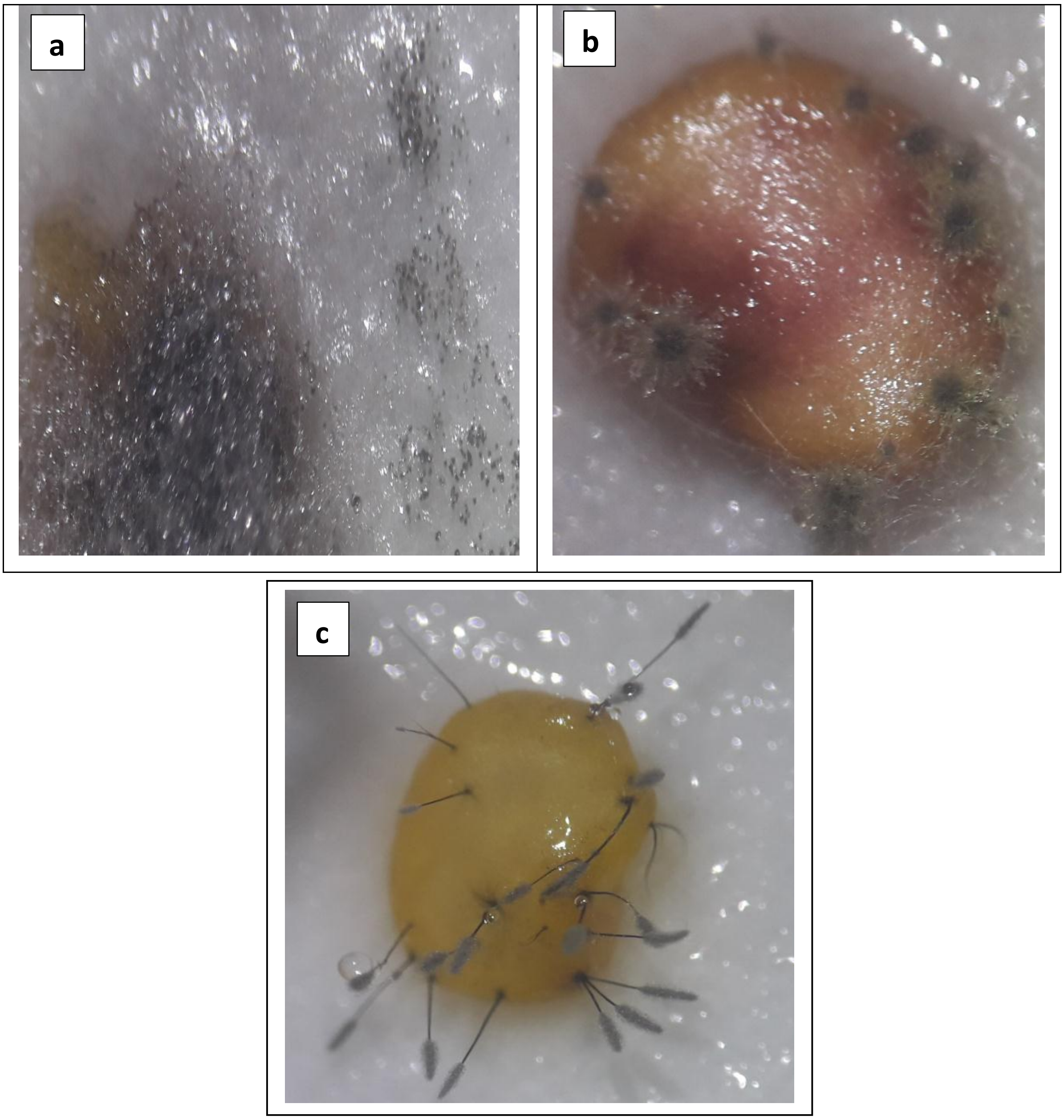


Figure 20. (a-c): Stereo microscopic view of pathogens on chilli seed,

(a) *Aspergillus niger*, (b) *Chaetomium* sp., (c) Unknown 1

4.1.7 Effect of hot water treatment on different parameters of tomato seed at different temperature

The effect of hot water treatment on different parameters of tomato seed found to be differed significantly at different temperature. The parameters were % seed germination, % seed infection, % rotten seed and % dead seed.

In case of % seed germination, the highest seed germination (85.0%) was recorded at treatment T_3 where seeds were treated in 54°C temperature for 5 minutes. The lowest seed germination (58.0%) was obtained in control where the seeds were treated with normal water at room temperature (Table 7).

In case of seed infection, the lowest infected seed (1.0%) was recorded at treatment T_6 where seeds were treated in 60°C temperature for 5 minutes which was statistically similar with T_5 (3.0%) where seeds were treated with 58°C temperature for 5 minutes. The highest infected seed (34.0 %) was obtained in control where the seeds were treated with normal water at room temperature (Table 7).

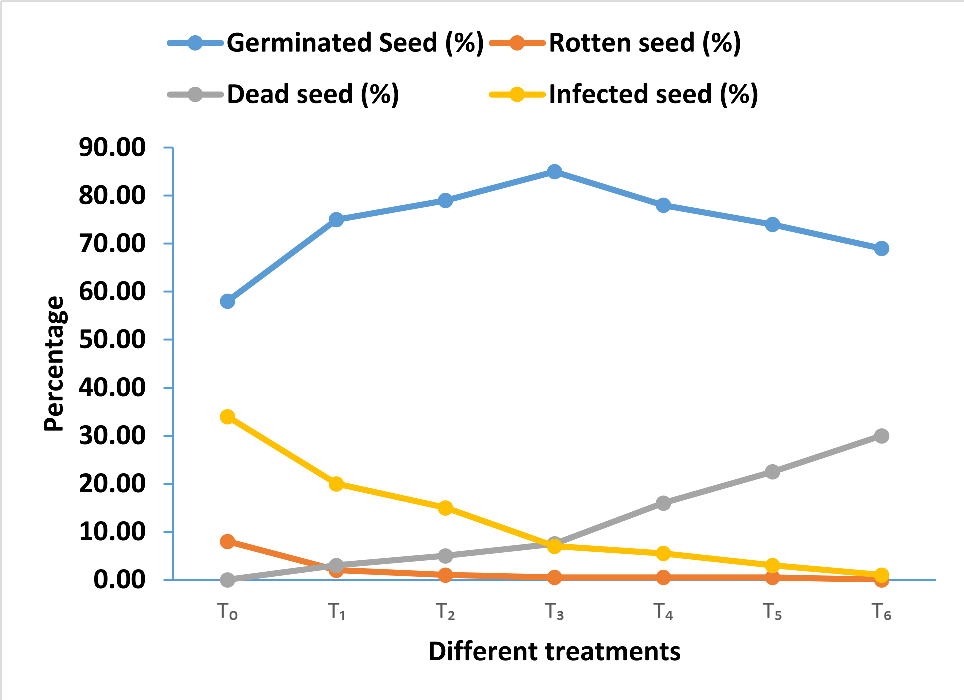
In case of rotten seed, the lowest rotten seed (0.0%) was recorded at treatment T_6 (60 °C) for 5 minutes which was statistically similar with T_2 (52° C), T_3(54 °C), T_4 (56 °C) and T_5 (58 °C). The highest rotten seed (8.0%) obtained in control (T_0) (Table 7).

In case of dead seed, the highest dead seed (30.0%) was recorded at treatment T_6 where seeds were treated in 60°C temperature for 5 minutes and the lowest dead seed (0.0%) was obtained in control where the seeds were treated with normal water at room temperature (Table 7).

Table 7. Effect of hot water treatment on different parameters of tomato seed at different temperature

Treatment	Germinated Seed (%)	Infected Seed (%)	Rotten Seed (%)	Dead Seed (%)
T_0	58.00 f	34.00 a	8.00 a	0.00 f
T_1	75.00 cd	20.00 b	2.00 b	3.00 e
T_2	79.00 b	15.00 c	1.00 bc	5.00 de
T_3	85.00 a	7.00 d	0.50 c	7.50 d
T_4	78.00 bc	5.50 de	0.50 c	16.00 c
T_5	74.00 d	3.00 ef	0.50 c	22.50 b
T_6	69.00 e	1.00 f	0.00 c	30.00 a
CV (%)	3.34	8.47	16.00	10.06
LSD $_{(0.05)}$	3.63	2.77	1.47	2.83

T_0 = Control, T_1 = 50 ºC for 5 mins., T_2 = 52 ºC for 5 mins., T_3 = 54 ºC for 5 mins., T_4 = 56 ºC for 5 mins., $T_{5,}$ = 58 ºC for 5 mins., T_6 = 60 ºC for 5 mins.



T_0=Control

T_1= 50 ºC for 5 mins

T_2 = 52 ºC for 5 mins

T_3 = 54 ºC for 5 mins

T_4 =56 ºC for 5 mins

$T_{5,}$ =58 ºC for 5 mins

T_6 =60 ºC for 5 mins

Graph 7. Effect of different hot water treatment on different parameter of tomato seed

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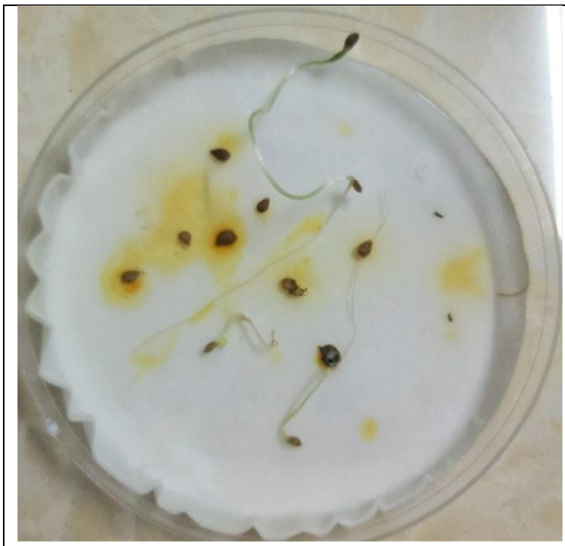


Figure 21. Germinated seedlings of tomato from untreated control seed	**Figure 22.** Germinated seedlings of tomato treated at 54 ºC for 5 minutes

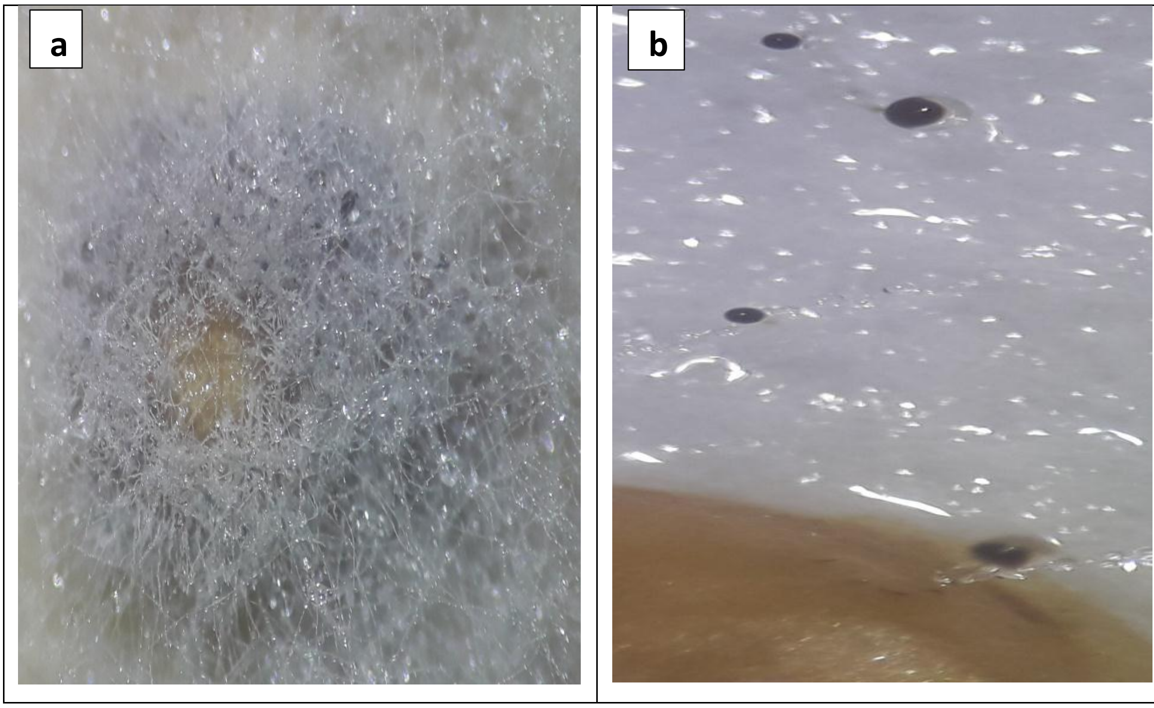


Figure 23. (a-b): Stereo microscopic view of pathogen on tomato seed, (a) *Fusarium* sp., (b) *Aspergillus niger*

4.1.8 Effect of hot water treatment on different parameters of lady´s finger seed at different temperature

The effect of hot water treatment on different parameters of lady´s finger seed found to be differed significantly at different temperature. The parameters were % seed germination, % seed infection, % rotten seed and % dead seed.

In case of % seed germination, the highest seed germination (80.0%) was recorded at treatment T_4 where seeds were treated in 56°C temperature for 5 minutes which was statistically similar with T_5 (75.5%, 58°C) for 5 minutes. The lowest seed germination (50.0%) was obtained in control (T_0) (Table 8).

In case of seed infection, the lowest infected seed (2.0%) was recorded at treatment T_6 where seeds were treated in 60°C temperature for 5 minutes which was statistically similar with T_4 (56 °C) and T_5 (58 °C) and the highest infected seed (38.0 %) was obtained in control (T_4) (Table 8).

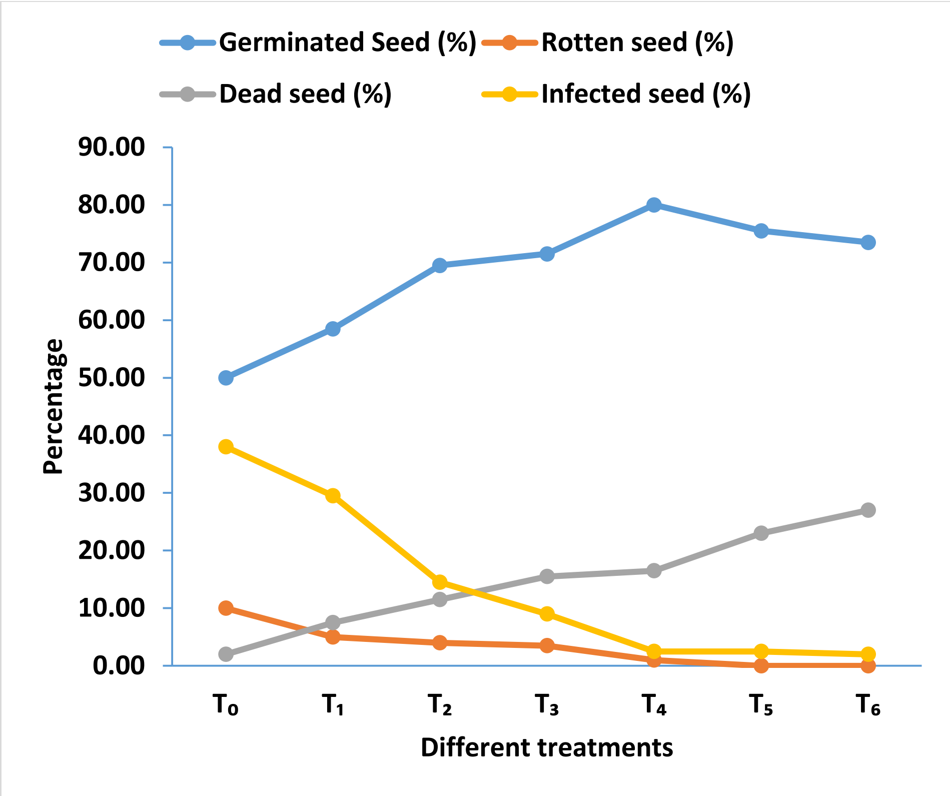
In case of rotten seed, the lowest rotten seed (0.0%) was recorded at treatment T_5 (58 °C) and T_6 (60 °C) for 5 minutes which was statistically similar with T_4 (1.0%) where seeds were treated with 56°C temperature for 5 minutes. Temperature below 58°C increased the rotten seed. The highest rotten seed (10.0%) obtained in control where the seeds were treated with normal water at room temperature (Table 8).

In case of dead seed, the highest dead seed (27.0%) was recorded in treatment T_6 where seeds were treated at 60°C temperature for 5 minutes which was statistically similar with T_5 (23.0%, 58°C) for 5 minutes and the lowest dead seed (2.0%) was obtained in control where the seeds were treated with normal water at room temperature (Table 8).

Table 8. Effect of hot water treatment on different parameters of lady´s finger seed at different temperature

Treatment	Germinated seed (%)	Infected Seed (%)	Rotten Seed (%)	Dead Seed (%)
T_0	50.00 e	38.00 a	10.00 a	2.00 e
T_1	58.50 d	29.50 b	5.00 b	7.50 d
T_2	69.50 c	14.50 c	4.00 b	11.50 cd
T_3	71.50 bc	9.00 d	3.50 b	15.50 bc
T_4	80.00 a	2.50 e	1.00 c	16.50 b
T_5	75.50 ab	2.50 e	0.00 c	23.00 a
T_6	73.50 bc	2.00 e	0.00 c	27.00 a
CV (%)	4.94	9.72	16.42	11.18
LSD $_{(0.05)}$	4.96	4.09	2.29	4.58

T_0 = Control, T_1 = 50 ºC for 5 mins., T_2 = 52 ºC for 5 mins.,
T_3 = 54 ºC for 5 mins., T_4 = 56 ºC for 5 mins., $T_{5,}$ = 58 ºC for 5 mins.,
T_6 = 60 ºC for 5 mins.



T_0=Control

T_1= 50 ºC for 5 mins

T_2 = 52 ºC for 5 mins

T_3 = 54 ºC for 5 mins

T_4 =56 ºC for 5 mins

$T_{5,}$ =58 ºC for 5 mins

T_6 =60 ºC for 5 mins

Graph 8. Effect of different hot water treatment on different parameter of lady´s finger seed

	
Figure 24. Germinated seedlings of lady´s finger from untreated control seed	**Figure 25.** Germinated seedlings of lady´s finger treated at 56 ºC for 5 minutes

4.1.9 Effect of hot water treatment on different parameters of radish seed at different temperature

The effect of hot water treatment on different parameters of radish seed found to be differed significantly at different temperature. The parameters were percent seed germination, percent seed infection, percent rotten seed and percent dead seed.

In case of % seed germination, the highest seed germination (80.5%) was recorded in treatment T_4 (56°C) and T_5 (58°C) for 5 minutes which was statistically similar with T_3 (79.0%) where seeds were treated with 54°C and T_6 (78.0%) where seeds were treated with 60°C temperature for 5 minutes. The lowest seed germination (68.0%) was obtained in control where the seeds were treated with normal water at room temperature (Table 9).

In case of seed infection, the lowest infected seed (0.0%) was recorded in treatment T_6 where seeds were treated in 60°C temperature for 5 minutes which was statistically similar with T_4 (2.5%, 56°C) and T_5 (0.5%, 58°C) for 5 minutes. The highest infected seed (26.0 %) was obtained in control (T_4) (Table 9).

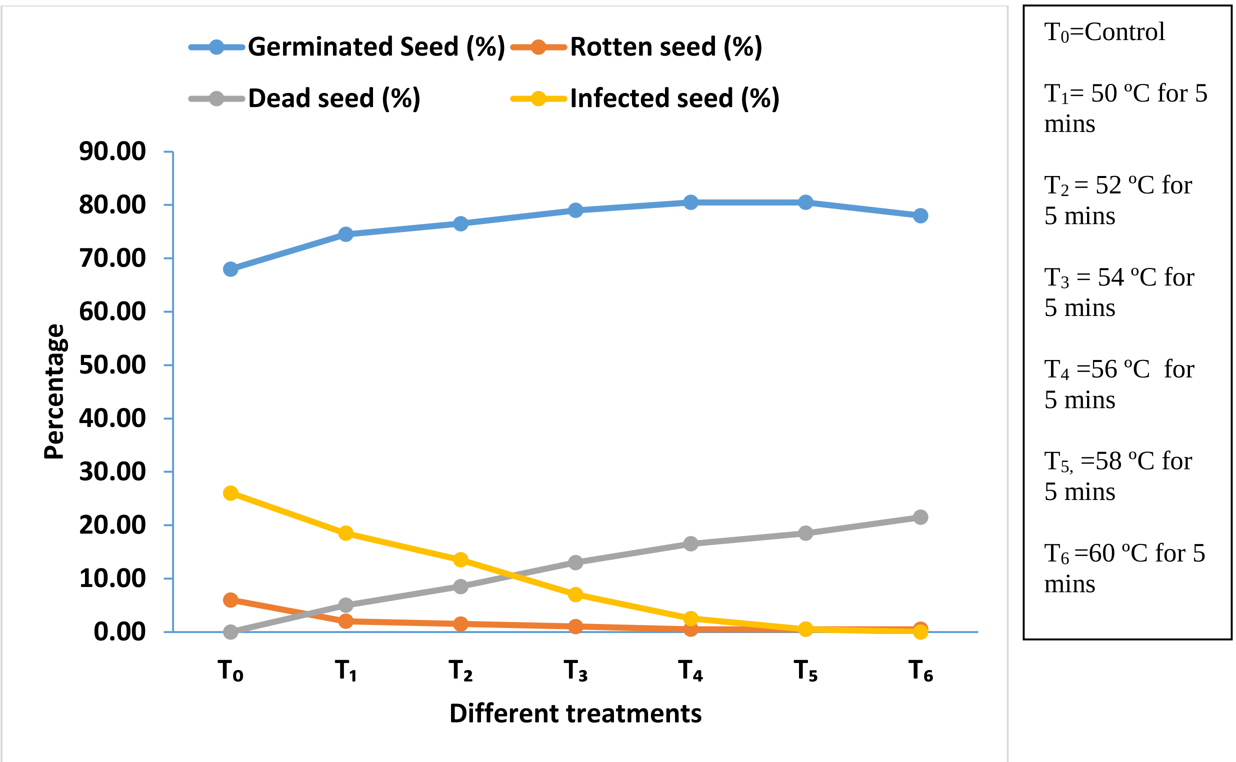
In case of rotten seed, the lowest rotten seed (0.5%) was recorded in treatment T_4 (56° C), T_5 (58 °C) and T_6 (60 °C) for 5 minutes. Temperature below 56°C increased the rotten seed. The highest rotten seed (6.0%) obtained in control (T_4) (Table 9).

In case of dead seed, the highest dead seed (21.5%) was recorded in treatment T_6 where seeds were treated in 60°C temperature for 5 minutes and the lowest dead seed (0.0%) was obtained in control where the seeds were treated with normal water at room temperature (Table 9).

Table 9. Effect of hot water treatment on different parameters of radish seed at different temperature

Treatment	Germinated seed (%)	Infected Seed (%)	Rotten Seed (%)	Dead Seed (%)
T_0	68.00 d	26.00 a	6.00 a	0.00 f
T_1	74.50 c	18.50 b	2.00 b	5.00 e
T_2	76.50 bc	13.50 c	1.50 b	8.50 d
T_3	79.00 ab	7.00 d	1.00 b	13.00 c
T_4	80.50 a	2.50 e	0.50 b	16.50 b
T_5	80.50 a	0.50 e	0.50 b	18.50 b
T_6	78.00 ac	0.00 e	0.50 b	21.50 a
CV (%)	3.12	11.13	12.36	9.04
LSD $_{(0.05)}$	3.52	4.44	1.57	2.79

T_0 = Control, T_1 = 50 ºC for 5 mins., T_2 = 52 ºC for 5 mins.,
T_3 = 54 ºC for 5 mins., T_4 = 56 ºC for 5 mins., $T_{5,}$ = 58 ºC for 5 mins.,
T_6 = 60 ºC for 5 mins.



Graph 9. Effect of different hot water treatment on different parameter of radish seed

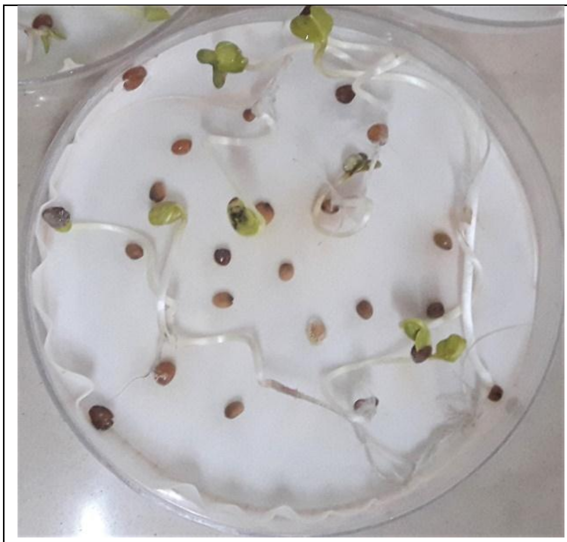
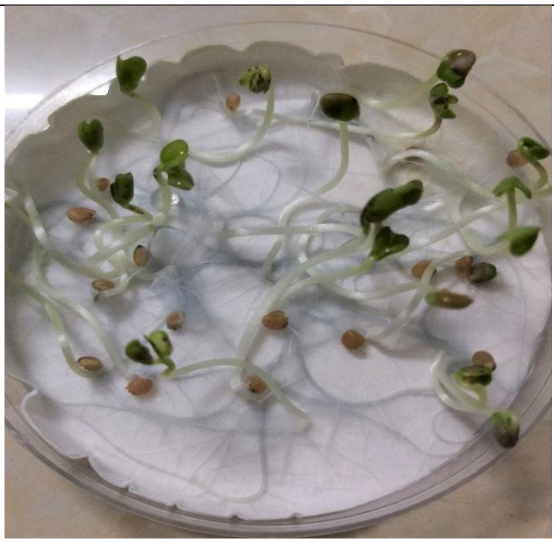
	

Figure 26. Germinated seedlings of radish from untreated control seed	**Figure 27.** Germinated seedlings of radish treated at 58 ºC for 5 minutes

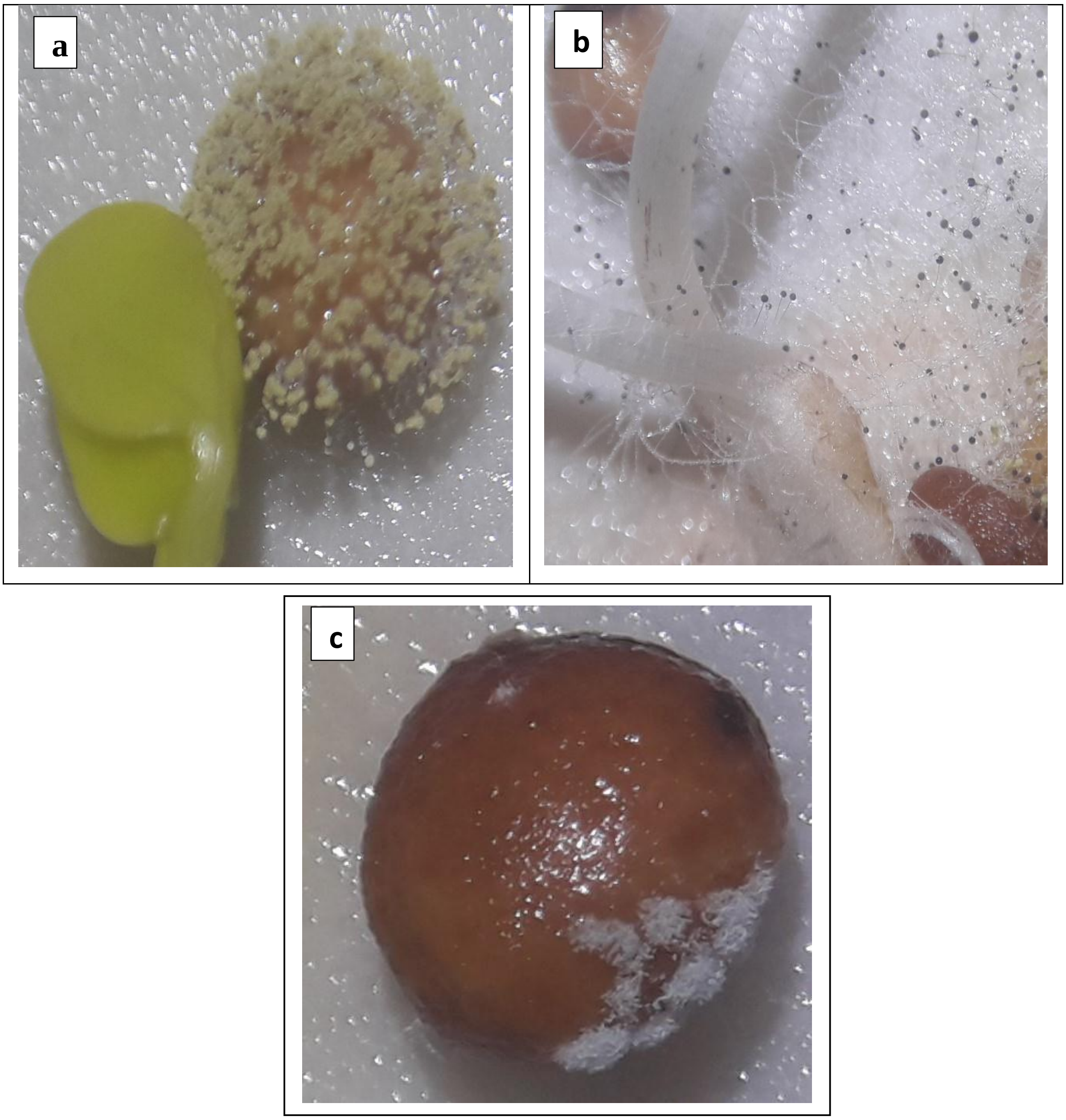


Figure 28. (a-c): Stereo microscopic view of pathogen on radish seed, (a) *Aspergillus flavus,* (b) *Aspergillus niger,* (c) *Fusarium* sp.

4.1.10 Effect of hot water treatment on different parameters of country bean seed at different temperature

The effect of hot water treatment on different parameters of country bean seed found to be differed significantly at different temperature. The parameters were percent seed germination, percent seed infection, percent rotten seed and percent dead seed.

In case of percent seed germination, the highest seed germination (80.5%) was recorded in treatment T_4 where seeds were treated at 56°C temperature for 5 minutes which was statistically similar with T_3 (79.0%) where seeds were treated with 54°C temperature for 5 minutes. The lowest seed germination (48.0%) was obtained in control where the seeds were treated with normal water at room temperature (Table 10).

In case of seed infection, the lowest infected seed (1.5%) was recorded in treatment T_6 where seeds were treated at 60°C temperature for 5 minutes which was statistically similar with T_4 (3.5%) where seeds were treated with 56°C and T_5 (3.0%) where seeds were treated with 58 °C and the highest infected seed (44.0%) was obtained in control (T_4) (Table 10).

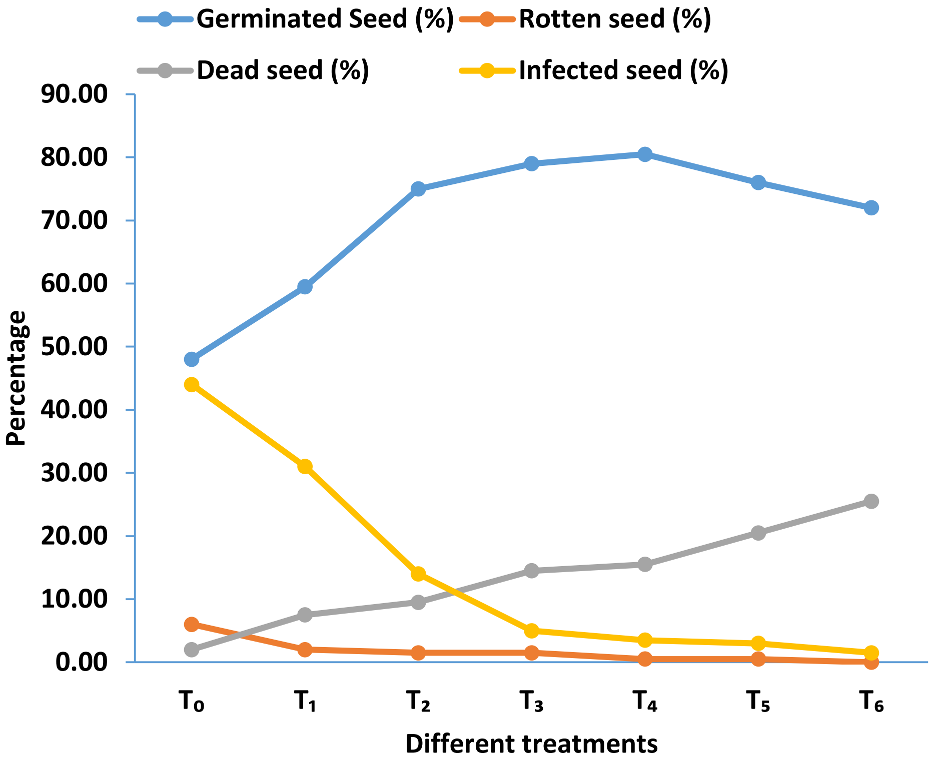
In case of rotten seed, the lowest rotten seed (0.0%) was recorded in treatment T_6 where seeds were treated at 60°C temperature for 5 minutes which was statistically similar to that of treatment T_1 to T_5 (50 to 58°C). Temperature below this (50-60°C) increased the rotten seeds. The highest rotten seed (6.0%) obtained in control where the seeds were treated with normal water at room temperature (Table 10).

In case of dead seed, the highest dead seed (25.5%) was recorded in treatment T_6 (60°C) for 5 minutes and the lowest dead seed (2.0%) was obtained in control where the seeds were treated with normal water at room temperature (Table 10).

Table 10. Effect of hot water treatment on different parameters of country bean seed at different temperature

Treatment	Germinated seed (%)	Infected Seed (%)	Rotten Seed (%)	Dead Seed (%)
T_0	48.00 d	44.00 a	6.00 a	2.00 e
T_1	59.50 c	31.00 b	2.00 b	7.50 d
T_2	75.00 ab	14.00 c	1.50 b	9.50 d
T_3	79.00 a	5.00 d	1.50 b	14.50 c
T_4	80.50 a	3.50 de	0.50 b	15.50 c
T_5	76.00 ab	3.00 de	0.50 b	20.50 b
T_6	72.00 b	1.50 e	0.00 b	25.50 a
CV (%)	6.58	15.99	9.79	12.85
LSD $_{(0.05)}$	6.77	3.42	2.30	4.56

T_0 = Control, T_1 = 50 ºC for 5 mins., T_2 = 52 ºC for 5 mins.,
T_3 = 54 ºC for 5 mins., T_4 = 56 ºC for 5 mins., $T_{5,}$ = 58 ºC for 5 mins.,
T_6 = 60 ºC for 5 mins.



T_0=Control

T_1= 50 ºC for 5 mins

T_2 = 52 ºC for 5 mins

T_3 = 54 ºC for 5 mins

T_4 =56 ºC for 5 mins

$T_{5,}$ =58 ºC for 5 mins

T_6 =60 ºC for 5 mins

Graph 10. Effect of different hot water treatment on different parameter of country bean seed

	
Figure 29. Germinated seedlings of country bean from untreated control seed	**Figure 30.** Germinated seedlings of country bean treated at 56 ºC for 5 minutes

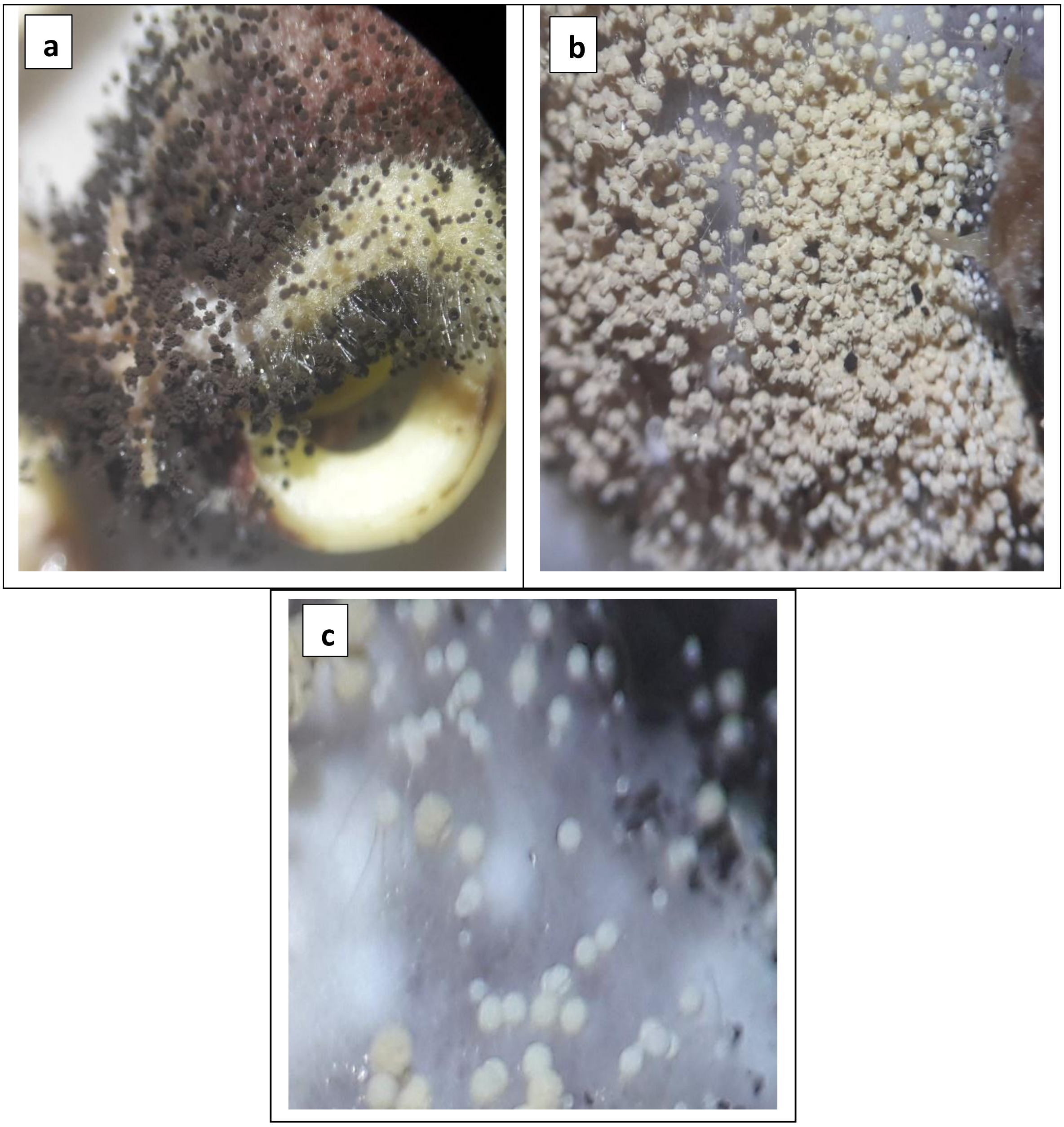


Figure 31. (a-c): Stereo microscopic view of pathogen on country bean seed, (a) *Aspergillus niger,* (b) *Aspergillus flavus,* (c) *Fusarium* spp.

DISCUSSION

5.1.1 Effect of hot water treatment of bitter gourd seeds

Infected country bitter gourd seeds treated in hot water at different temperature range of 50°C to 60°C for 5 minutes were subjected to investigation of the effect of the treatment on the incidence of pathogen. Hot water seed treatment had significant effect in controlling pathogen and increasing seed germination over control. *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* sp. and *Chaetomium* sp. were found in bitter gourd seed. The incidence of pathogen gradually decreased with the increase of water temperature. Simultaneously, the seed germination gradually increased with the increase of temperature and raised to the highest (78.5%) at 54°C. If the temperature increased beyond 54°C, incidence of pathogen remained statistically identical but the seed germination decreased gradually. Having thick seed coat sometimes proper absorption of water becomes difficult in case of bitter gourd.

5.1.2 Effect of hot water treatment of pumpkin seeds

The best result in case of seed germination of pumpkin 80.0% was found in 54ºC temperature for 5 minutes and reduced the infection of *Aspergillus niger*. Several studies have reported on the effectiveness of hot water treatments against bacterial diseases (Linders, 2000; Nega *et al.*, 2003; Schmitt *et al.*, 2006). Hot water treatments for control of *Xanthomonas campestris* pv. *campestris* (Xcc) have been reported at 50°C for 10–60 min (Shekawat *et al.*, 1982; Sha *et al.*, 1985; Babadoost *et al.*, 1996; Nega *et al.*, 2003). Generally physical seed treatment with hot water demonstrated good antibacterial effect against Xcc on rape seeds in the current study and treatment at 50°C for 30 min controlled Xcc on seeds and increased germination. Under greenhouse conditions the same hot water treatment increased rape seedling emergence, effectively reduced black rot incidence and was comparable to the healthy control. However, as the treatment temperature increased to 53 °C and with an increase in treatment time to 20 min or 30 min, Xcc populations were significantly reduced but rape seed germination was adversely affected. Ivey and Miller (2005) reported that even though hot water treatment is effective in decreasing pathogen levels on vegetables, sometimes reduction in seed germination rates has been recorded, and Nega *et al.* (2003) recommended that for high treatment temperatures a shorter duration is needed especially with sensitive crops such as brassicas.

5.1.3 Effect of hot water treatment of bottle gourd seeds

Infected bottle gourd seeds treated in hot water at different temperature range of 50°C to 60°C for 5 minutes. Hot water seed treatment played a vital role in controlling pathogen and increasing seed germination over control. The best result in case of seed germination was 82.5% where seeds were treated with 56°C. Above this temperature seed infection was decreased and seed germination also decreased.

5.1.4 Effect of hot water treatment of cucumber seeds

The performed experiment with six treatments showed that the treated seeds resulted better germination percentage than untreated. The best result in case of seed germination 81.5% was found for the treatment soaking seeds in warm water (56ºC) for 5 minutes. *Aspergillus niger*, *Aspergillus flavus,* and *Chaetomium* sp were recorded in cucumber seed. If the temperature increased beyond 56°C, incidence of pathogen remained statistically identical but the seed germination decreased gradually. Each crop cultivar requires a critical soaking duration and it should be less than the safe limit (Harris *et al*., 2000).

5.1.5 Effect of hot water treatment of brinjal seeds

Hot water treatment of eggplant seeds at 58°C for 5 mins yielded seed germination 81.0% and reduced seed borne infection of pathogen (*Aspergillus flavus*, *Chaetomium* sp., and *Penicillium* sp.,). Infection of pathogen recorded nill (0.00%) at treatment 60°C but at this temperature germination was decreased. 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°C for 15 mins. Prabhu and Prasada (1970) reported seed treatment at 52-54°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°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°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°C for 15 mins completely controlled *Phomopsis vexsans* and increased seed germination by 53.8% over control.

5.1.6 Effect of hot water treatment of chilli seeds

Hot water treatment of chilli seeds at 54°C for 5 mins yielded seed germination 87% and reduced seed borne infection.

Hot water treatments of chilli seed prior to storage has been observed to affect the seed quality parameters significantly (Musazura and Bertling, 2013). The increase of temperature range from 45-53°C decreased seed viability with no significant effect on seed vigour. Under present investigation also, the seed germination reduced when the temperature was raised higher than 53-55°C. However, the seed germination was more when the seed were treated with a lower range of temperature, i.e. 50-52°C. Miller and Ivey (2004) had reported that pepper seeds are more sensitive to hot water treatment. The soaking of pepper seeds in hot water at appropriate temperature improved their germination rate. Ramamoorthy *et al.*, (1989) had reported similar effects of hot water seed treatment on improved seed and percentage of germination in other plant species like *Pinus pinnata*. Pasiecznik *et al.*, (1998) also reported that treated seeds of Prosopis species by boiling water increased germination. The decrease in the number of days to germination in treated seeds by hot water was attributed to the increasing of permeability of seed coat to water. According to Mwase and Mvula (2011) soaking in boiled water made the seed coats permeable to water and the seeds imbibed and swell as the water cools. Various studies have reported that immersion in hot water was the best method for breaking the dormancy of hard coat seeds (Azad *et al.,* 2010, 2011).

5.1.7 Effect of hot water treatment of tomato seeds

Hot water treatment of tomato seeds at 54°C for 5 mins yielded seed germination 85% and reduced seed borne infection. Tomato seeds seemed to be delicate to hot water treatment. Seed treatment with hot water at 50°C for 30 min reduced the bacterial population to 3.1 cfu/ml compared to the untreated inoculated control (6.0 cfu/ml) and found significantly higher germination percentage (84%) was recorded after seed treatments with acetone extracts of *Agapanthus caulescens* (15 mg/ml) and hot water at 50°C for 30 min. In the greenhouse trials, acetone extracts of *A. caulescens* (15 mg/ml), *Paenibacillus sp*. and hot water at 50°C for 30 min significantly increased seedling emergence and reduced black rot incidence and severity on rape leaves.

5.1.8 Effect of hot water treatment of lady´s finger seeds

The best result in case of lady´s finger seed germination was 80% where seeds were treated with 56°C. According to Ngure *et al.*, (2008), the treatments assessed were immersion of Okra pods in hot water dips at 40 °C for 7 min, 50°C for 1 min, room temperature (15-20 °C) for 10 min and control (no hot water treatment). After treatment pods were stored in refrigerators at 4, 8.5 and 13°C or room temperature conditions to simulate the most common farmer's method of storage. Treatments were replicated six times and the experiment repeated twice. Pods treated in hot water at 50°C for 1 min and stored at room temperature had the least decay and weight loss.

5.1.9 Effect of hot water treatment of radish seeds

Hot water treatment of radish seeds at 56°C for 5 minutes yielded seed germination 80.5% and reduced pathogen infection. 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 reduced germination of sensitive crops. According to Nega *et al.*, (2003) in most cases efficacy of hot water treatments against Alternaria species (*A. dauci, A. radicina, A. alsernata, A. brassicicola*) was high (efficacy > 95%). Treatment was also very efficient against Phoma species (*Ph. lingam, Ph. valerianella*) (80-95%). The reduction of *Ph. valerianella* on the seed of lamb's lettuce correlated in the first test year with the reduction of the disease in the field. The number of spores in the pycnidia of *S. apiicola* and *S. petroselini* was significantly reduced by hot water treatment. This correlates with the reduction in disease incidence and yield increase. The hot water treatment reduced the number of oospores of *P. valerianellae* in trials on weakly infected seed, but was ineffective on highly infected seed. For *Xanthomonas campestris* on carrot and cabbage, laboratory trials yielded good effects at 50°C for 30 min.

5.1.10 Effect of hot water treatment of country bean seeds

Dipping country bean seeds in hot water at 56°C for 5 mins yielded seed germination 80.5% and the infection of pathogen became lower. *Aspergillus niger* was recorded in country bean seeds. As the seed coat of country bean is thick and hard. The seed wall 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.

CHAPTER V

SUMMERY AND CONCLUSION

The experiment was conducted to standardize of hot water treatment compared to chemical, botanical and bio-pesticides in controlling major seed borne pathogens of vegetables during the period from September 2017 to March 2018, 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 minutes against seed borne microflora were explored to standardize the temperature and time in controlling seed borne pathogens of bitter gourd, pumpkin, bottle gourd, cucumber, eggplant, chilli, tomato, lady´s fingers, radish and country bean seeds. The treatments were T_0 = Control, T_1 = 50ºC for 5 minutes, T_2 = 52ºC for 5 minutes, T_3 = 54ºC for 5 minutes, T_4 =56ºC for 5 minutes, T_5 = 58ºC for 5 minutes and T_6 = 60ºC for 5 minutes. Data were collected on percent germination, percent infected seed, percent rotten seed and percent dead seed. The experiments were conducted following Completely Randomized Design (CRD) and analyzed by computer package MSTAT-C. The analysis of variance was performed and means were compared by Least Significant Difference (LSD) test at 5% level of probability for interpretation of results (Gomez and Gomez, 1984).

Significant difference was found in all studied parameters of bitter gourd, pumpkin, bottle gourd, cucumber, brinjal, chilli, tomato, lady´s fingers, radish and country bean seeds dipped in hot water at different temperatures.

Hot water treatment of bitter gourd seeds and pumpkin seeds were found effective at 54ºC for 5 min to reduce seed borne infection with the highest seed germination respectively 78.5% and 80.0%. For bottle gourd, cucumber and country bean seeds 56ºC for 5 min was found effective with maximum seed germination 82.5%, 81.50% and 80.5% respectively to reduce infection. In case of brinjal seeds 58ºC for 5 min was found effective with maximum seed germination (81.0%) to reduce infection. For chilli and tomato seeds maximum seed germination 87.0% and 85.0% was obtained at temperature for 5 min. For radish seeds 56ºC and 58ºC temperature for 5 min was found effective against the pathogen with maximum seed germination 80.0% and 80.5% was obtained at 56ºC for 5 min.

In conclusion the researcher found that the highest seed germination of maximum studied vegetables were recorded in 54°C to 58°C and the lowest was obtained at T_0 (control). The lowest rotten seeds (0.0%) of maximum vegetables recorded in treatment 60°C and the highest rotten seeds obtained at T_0 (in control). The highest dead seed of studied vegetables was recorded in 60°C and the lowest (0.0%) was obtained in treatment T_0 (control). The lowest infected seed of studied (0.0%) was recorded in treatment 60°C and the highest was obtained in T_0 (control).

The area under vegetable cultivation in Bangladesh is small, but the importance of vegetables cannot be overemphasized. Bangladesh is facing a chronic food shortage which is now approaching 2 million g per year. For the last few decades, the government has been trying in vain to become self-sufficient in food production.

The management of seed-borne infection might play a vital role on their production of vegetables. In safe agriculture, a concept of pollution free, environmental friendly and safe 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. In this outlook, use of hot water treatment in seed may become easy, less costly technology in controlling seed borne pathogens. In future, conventionally produced seed will not be allowed for organic farming. Therefore, effective non-chemical methods are needed to control seed borne diseases have been investigated in laboratory, model and field trials. If we able to substitute the hazardous chemicals for seed treatment by eco-friendly hot water, we will able to keep safe our food chain and safe our environment. Further researchers are desired to re-check the efficacy of hot water treatment of bitter gourd, pumpkin, bottle gourd, cucumber, brinjal, chilli, tomato, lady´s fingers, radish and country bean seeds against seed borne pathogens and to take step to transfer the technology to the farmers.

CHAPTER VI

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