EFFICIENCY OF HOT WATER SEED TREATMENT DEVICE FOR CONTROLLING PHOMOPSIS FRUIT ROT OF EGGPLANT

A THESIS

BY

MOHAMMAD TOFAJJAL HOSSAIN

Examination Roll No. 03 Ag. P.Path. JJ-22 M

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MASTER OF SCIENCE (M.S.)

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EFFICIENCY OF HOT WATER SEED TREATMENT DEVICE FOR CONTROLLING PHOMOPSIS FRUIT ROT OF EGGPLANT

ABSTRACT

Effect of different temperatures with varying times were evaluated against *Phomopsis vexans* of eggplant using Vegetable Seed Treating Plant in IPM Laboratory and Net house of the Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh during June to December 2003. Seed treatment at 55°C temperature for 15 minutes was found most effective against seed borne pathogen *Phomopsis vexans* in the laboratory experiments followed by seed treatment at 53°C temperature for 15 minutes. *Phomopsis vexans* was completely controlled at 55°C temperature treated for 15 minutes having the highest seed germination (87.0%). Seed treatment at higher temperature than 55°C controlled *Phomopsis vexans* but reduced the seed germination gradually with the increase of temperatures. Net house experiment for raising seedlings treating with 53°C and 55°C temperatures for 15 minutes were yielded similar results against *Phomopsis vexans* as observed in the *in vitro* experiments with some extent. Incidence of *Phomopsis vexans* in the raised seedlings (damping off and seedling blight) was nil while seed treated with 55°C temperature for 15 minutes. The performance of 53°C temperature treated for 15 minutes was not satisfactory against *Phomopsis vexans* in net house.

INTRODUCTION

Eggplant/Brinjal (*Solanum melongena* L.) is an important solanaceous vegetable crop worldwide and also here in Bangladesh. It is also known as eggplant, brinjal, aubergine, melongena and guina squash in different countries of the globe. It is widely grown throughout Bangladesh in all seasons (Rabi & Kharif) and is always available in the market.

That the phomopsis blight and fruit rot (*Phomopsis vexans*) of eggplant is a serious disease in Bangladesh has been established (Meah, 2002). The disease was first reported from Gujrat in 1914 and since then from many parts of India. Occurrence of the disease in Bangladesh was first reported by Fakir (1983). The disease has become a major constraint in intensive cultivation of eggplant because of mainly to aggressive nature of the pathogen, *Phomopsis vexans*. About 15 - 20% in general and 30- 50% in severe case of fruit rots of eggplant are caused seriously due to the *Phomopsis vexans* (Das, 1998; Khan 1999). About 80 million taka is lost by the phomopsis fruit rot (Ittafaq, 5th February, 2003, Page 19, Col-1).

The pathogen can grow over a temperature range between 15 to 30^{0}C and the best growth was found at 25^{0}C (Divinagracia, 1969; Harada *et al.*, 1973; Chinenye, 1974; Ahmed, 1987; Sugha *et al.*, 2002) who found 25^{0}C as the best temperature to obtain maximum mycelial growth of *Phomopsis vexans*. The mycelial growth is inhibited at 35^{0}C and this is supported by the finding of Divinagracia (1969).

It is a serious disease which may cause damping off symptoms at seedling stage. When the leaves are infected, blight occurs. In case of fruit infection circular, spots with concentric rings are produced ultimately fruits become mummified and rotten (Meah *et al.*, 2003).

Infected seeds fail to germinate, the young seedlings emerging from the infected seeds may die and often the growing plant escaping early infection, succumb to death or get partly affected due to this disease.

It is unquestionable that proper disease control measures can substantially improve the quality of eggplant and significantly increase the yield. Seed treatment with hot water might be the cheapest and safest method of direct plant disease control.

The control of plant diseases through the use of chemicals is discouraged for health hazard and environmental pollution and obvious development of tolerance to pathogen as well as for involvement of high cost. To eradicate the pathogen from seeds by hot water treatment is essential as a part of integrated pest management. Hot water seed treatment has drawn a very urgent attention in Bangladesh for obtaining good plant seeds, it can be useful in reducing the amount of pesticides required to manage a disease. Hot water treatment of eggplant seeds was found effective in warding off the Phomopsis Blight or Fruit rot infection by *Phomopsis vexans* (Raychoudhuri, 1967) and the method has been

recognized as an acceptable and standard practice for elimination of Phomopsis infection in eggplant seeds (Roychoudhuri and Lele, 1966).

Treatment of eggplant seeds by Hot Water Seed Treating Device is to kill disease causing organisms (pathogens) carried within or on the seed has been shown to prevent plant disease epidemics (epiphytotics). The eggplant farmer of 21 districts in Bangladesh repeatedly treated the vegetable seeds by Hot Water Treating Device for disinfecting and eradicating seed infecting pathogens. The use and expectation of seed treatments are greater today due to the impact of environmental regulation that have either banned or restricted the use of older fungicides.

A physical treatment of hot water treatment has been used since 1920's, has been little used in practice (Neergaard, 1977). Then a modern Hot Water Treatment system was developed by Dr. Arnold Hara, UH Hilo Entomology in 1999. A "hands on" Hot Water Treatment demonstration was conducted in all major cut flower and foliage producing islands. Hot Water system shows a reduction in pesticide use by 80-90%, a reduction in labour requirements and reduction in export rejection rates (Hara, 2000). The Hot Water Treatment also increases the vase life of certain products. In our country, Bangladesh, Hot Water seed treating plant was first used by Prof. Dr. Bahadur Meah in vegetables (The Daily Star, August 3, 2003). The Hot Water Seed Treating Plant so developed in the IPM lab has been found very effective in eliminating the seed infection by pathogenic fungi including *Phomopsis vexans*, increasing seed

germination and reducing nursery diseases (Meah, 2003). An International Integrated Pest Management Laboratory established at Bangladesh Agricultural University (BAU), Mymensingh has estimated about 300 million taka that is possible to save by using the developed Hot Water Seed Treating Device in the eggplant field only (The Daily Star, August 3, 2003). Seed treatment with Hot Water Treating Device could be the successful way to control *Phomopsis vexans*. No research work has been done with Hot Water Seed Treating Device for controlling *Phomopsis vexans* in Bangladesh.

Adoption of non-chemical Hot Water Treatment by shippers will eliminate the use of post-harvest insecticidal dips and promote the efficient and sustainable agricultural practices that protect, sustain and enhance water and soil resources and ultimately achieve greater harmony between agriculture and environment.

The present piece of research work was undertaken with the following objectives:

1. To determine the efficacy of the Device for hot water treatment of eggplant seeds in eliminating seed borne *Phomopsis vexans*.

2. To determine the effective temperature with minimum time to control seed borne *Phomopsis* vexans of eggplant using Vegetable Seed Treating Plant.

REVIEW OF LITERATURE

That the Phomopsis blight and fruit rot (*Phomopsis vexans*) of eggplant is a serious disease in Bangladesh has been established (Meah, 2002). Its infestation as seedling blight, leaf spot and fruit rot was reported (Rangaswamy, 1979). The most destructive phase of the disease is the fruit rot. Study shows that 15-20% in general, 30-50% in severe case eggplant yield losses due to Phomopsis Fruit rot caused by *Phomopsis vexans* (Das, 1998, Khan *et al.*, 2002), about 80 million taka is lost by the Phomopsis fruit rot (Ittefaq, 5th February, 2003, Page 19, Col-1). In India, the fruit rot phase of the disease causes heavy damage in the field and during transit (Singh, 1992).

Evidence of research work regarding Phomopsis Fruit rot of eggplant as a seed-borne disease is very dearth. The possibility of controlling the diseases through seed treatment with hot water is being explored in some countries of the world. But in Bangladesh, with pathological point of view firstly seed treatment with hot water is done by the help of innovative Hot Water Seed Treatment Plant, (The Daily Star, August 3, 2003). So, information available on the present work is also limited. An attempt has, therefore, been made to review briefly the literatures on the seed treatment of different crops with Hot Water Seed Treating Device.

✓2.1 Seed treatment with hot water and its importance:

Marquenie *et al.* (2003) stated that dipping in hot water (40–48^{0}C), ultraviolet light (uv–c, 1.00 J/cm^2) and short flashes of intense white light were used to control *Botrytis cinerea* in stored strawberries. Hot Water decreased firmness, while low rates of uv–c, inhibited fungal growth without affecting firmness. The combinations of heat (40^{0}C, 3 minutes) plus uv–c and heat (3 or 5 minutes) plus light flashes (120 seconds) resulted in more effects (compared to single treatments) while using less intensive treatments.

Gaur (2003) reported that twenty one fungicides combined with hot water treatment were evaluated in the field against seed borne inoculum of *Ascochyta rabiei* in chickpea cv. Four-hours seed dip in 0.2% thiabendazole solution significantly controlled seed-borne infection of *A. rabiei* with no deleterious effect on germination (88.6%). This treatment gave minimum number of diseased plant (2- 9%) at flowering stages. He reported that 53^0 for 15 minutes as a hot water treatment with chlorothalonil, carbendazim, benomyl, calixin-M, and tridemorph (each at 0.2%) were the best treatment for control of seed-borne infection but seed treatment with calixin-M & hot water had adverse effects on germination (63.6 & 41.9% respectively).

Nega *et al.* (2003) stated that five important vegetable crops (carrot, cabbage, celery, parsley lamb's lettuce) and their most important seed-borne pathogens (*Alternaria* spp, *Phoma* spp,

Septoria spp, *Xanthomonas* spp, *Peronspora valerianellae*) have been investigated in laboratory with hot water treatments of 40°C & 50 to 55^0 for 10 to 30 min, in some cases to 60 min and found no infected seeds from those vegetables. Seed-borne pathogens could be reduced without significant losses of germination by hot water treatments at 50^0C for 20 to 30 min up to 53^0C for 10 to 30 min.

Fallik *et al.* (2002) reported that the effectiveness of a short pre-storage hot water rinsing and brushing on resistance to decay development and chilling injury was studied on pink tomato cv. 189 fruit that were kept for 15 days at 5 or 12^0C plus three days at 22^0C. He suggested the alternative method of a very short (15 S) HWRB (Hot Water Rinsing & Brushing) at 52^0C for desirable tomatoes. This treatment extended storability well over three weeks at 5°C by minimizing CI (Chilling Injury) and enhancing resistance against pathogen during storage.

Sadek *et al.* (2001) stated that hot water treatment at 10°C for 10 minutes with potassium permanganate (1%) or copper sulphate (1%) application effectively controlled the pathogen in infected seeds of tomato, tobacco, cowpea, bean and pepper. By this treatment irregular necrotic spots were overcome finely.

Gesunde (2001) stated that the method of hot water treatment was applied to five important vegetables (carrots, cabbage, celery, parsley and lamb's Lettuce) and here, to the most important pathogens (*Alternaria* spp, *Phoma* spp, *Septoria* spp, *Peronospora valerianellae, Xanthomonas* spp) and found out a good effective

result at 50^{0}C for 30 minutes. Germination was not affected under these conditions and crop yields were sometimes increased by the hot water treatment.

Muniz (2001) stated that the dry heat treatment on the control of seed transmitted pathogens and its effects on the viability of tomato seeds treated at 70^{0}C for 12 days eradicated fungi associated with tomato seeds. But in hot water treatment at 50^{0}C for 30 minutes under laboratory research the associated fungi in tomato seeds were eradicated.

Winter *et al.* (2001) stated that the incidence of common bunt (*Tilletia caries*) in winter wheat was strongly reduced by a seed treatment with skim milk powder and warm water. The combined seed treatment with warm water at 45^{0}C for 2 hours and skim milk powder (160 g/litre water) controlled well the seed-borne infection of *Tilletia caries* (common bunt), *Gerlachia nivalis* (Snow mould), *Fusarium graminearum* and *Septoria nodorum* (damping off) in winter wheat.

Satvinder and Kaur (2000) stated that some physical techniques (dry heat, hot water, solar heat, washing, radiation, microwave treatment, ultrasonic waves and forced air circulation) for the management of plant disease including post harvest disease were very successful.

Forrer *et al.* (2000) stated that the epidemic late blight of potato caused by *Phytophthora infestans* could be overcome by hot water seed tuber treatment at 43^{0}C for 1 h or 55^{0}C for 10 min. for effective growth and yield of potato.

Karunaratne (1999) reported that the effect of hot water treatments (different temperature-time combination) of tomatoes, cucumbers and *Momordica charantia* (55^0C for 1 min.), *Capsicum annum* (Chilli) cv. and carrots (50^0C for 1 min.) *Phaseolus vulgaris* (50^0C for 30S) and okras (52^0C for 30S) on the self life of each commodity at room temperature (27 ± 3^0C) and relative humidity (65±5%) were determined, found out no disease symptoms.

Hermansen *et al.* (1999) stated that the effect of hot water treatments of carrot seeds on seed-borne fungi, germination emergence and yield were studied from at 44^0C to 59°C for 20 min. for controlling seed-borne pathogen of *Alternaria dauci.* Hot water treatment of carrot seeds at 44, 49 and 54^0C generally improved germination of infected seeds and reduced the incidence of *Alternaria dauci.* Hot water treatment of seed and seed treatments with the biological control agents had no effect on carrot yield and storage quality but reduced the incidence of the saprophyte *Ulocladium atrum* on the seeds. The germination percentages found in the laboratory correlated well with the emergence percentages in the growth chamber. It concluded that hot water treatment as an alternative to fungicides was used to eradicate seed-borne pathogens in carrots in organic farming system.

Fallik *et al.* (1999) stated that hot water treatment qualified sweet pepper in storage condition after treating with 55±1°C for 12±2 S. This treatment significantly improved the general appearance of the fruits, reduced decay incidence and maintained

fruit firmness. The respiration rate of rinsed and cleaned fruits was significantly lower than that of untreated fruits during storage and shelf-life simulation.

Lurie *et al.* (1998) stated that a pre-storage dry heat treatment and a hot water dip were done at 30^{0}C for 48 to 72 h and 50 to 53°C for 2 to 3 min respectively for reducing storage rots on capsicum bell peppers and tomatoes. Under these conditions *in vitro* germination and growth of *Alternaria alternata* and *Botrytis cinerea* were weakened or prevented.

Ranganna *et al.* (1998) stated that hot water treatment at 57.5^{0}C for 20-30 min. for controlling storage pathogen like *Fusarium solani, Erwinia carotovora* was effectively done for potatoes tubers.

Hanks *et al.* (1997) stated that chlorphyrifos as a pre-planting treatment to protect Narcissus bulbs from attack by *Merodon equestris* was applied in a hot water treatment tank. Before HWT, bulbs were stored at ambient temperatures at 18^{0}C for 2 weeks or at 30^{0}C for 1 week to reduce the phytotoxic effects of the HWT (Hot Water Treatment) and chlorphyrifos.

Agrios (1997) stated that hot water treatment of certain seeds, bulbs and nursery stock is used to kill any pathogens with which they are infected or which may be present inside seed coats, bulb scales etc. or which may be present in external surfaces or wounds. In some diseases, seed treatment with hot water was for many years the only means of control, as in the loose smut of cereals in which

the fungus over-winters as mycelium inside the seed where it cannot be reached by chemicals. Similarly treatment of bulbs and nursery stock with hot water frees them from nematodes that may be present with them such as *Ditylenchus dipsaci* in bulbs of various ornamentals and *Radolpholus similis* in citrus root stock. The effectiveness of the method is based on the fact that dormant plant organs can withstand higher temperatures than those their respective pathogens can survive for a given time. The temperature of the hot water used and the duration of the treatment varies with the different host pathogen combination. Thus in the loose smut of wheat the seed is kept in hot water at 5^0C for 11 min. and bulbs treated for *Ditylenchus dispsaci* are kept at 43^0C for 3 hours.

Shahda *et al.* (1995) stated that hot water treatment at 55^0C for 20 min. was effective for isolating seed-borne pathogen (*Atternaria alternata, Fusarium semitectum, Fusarium sambucinum. Alternaria chlamydospora, Cephalosporium* sp and *Fusarium oxysporum*) from sweet melon and vegetable marrow. Pathogenicity of these pathogens showed highly pathogenic on their respective hosts. The optimum temperature for its growth ranged from 25 to 30^0C and the optimum P^H was 6.0. If the seeds were treated under HWT, they would not treat under fungicides like etridiazole 30 h, carbendazim and thiophanate methyl at the rate of 4 g/kg seed. So hot water treatment at 55^0C/20 min. was next to fungicides.

Padma *et al.* (1993) stated that germination was improved in *A. nilotica* through acid (concentrated sulfuric acid) scarification for

one hour and hot water treatment for 30 minutes. $K_2Cr_2O_7$ at 0.6% concentration, acid scarification for 6 minutes and hot water treatment for 15 minutes improved germination in *A. auriculiformis*. In *A. albida* germination was improved by incubating seeds at a high temperature (50^0C) for 45 days, by acid scarification for 15 minutes and by hot water soaking for 5 minutes.

Tenente *et al.* (1993) stated that some nematodes were detected and treatments were made aimed at the eradication of these parasites. Hot water treatment with different regimes of temperature and period of exposure. *Aphelenchoides* sp. and *A. besseyi* were eradicated 100% from rice seeds at 57°C for 15 minutes. Wheat and Arachis seeds infested with *Ditylenchus* sp and *Aphelenchoides* sp. were treated at 54^0C for 15 minutes with 100% control also.

Bunchner *et al.* (1991) stated that as an alternative to chemical dipping, hot water treatment was effective for pest and disease control. Having taken nursery plants of chandler with different temperatures for three years 48.8°C for 5 min. or 49.4°C for 7 min. were considered acceptable for good quality plants under hot water treatment.

Napoles *et al.* (1991) found that hot water treatment at 50°C for 1 h. gave good disease control of *Xanthomona campestris* pv. *campestris* in cabbage seeds.

Roy (1988) tested radiation (150 krad), hot water (55°C/ 6 min.) and guazatine (1-5%) singly and in combination for their ability to control *G. candidum* infection in tomatoes. 80% disease control was done singly by hot water seed treatment.

Muller (1987) found that standard industrial seed cleaning methods removed 78% of the contamination and that polishing (rubbing and grading) of seeds before pelleting removed larval clinging to the seeds surface, further reducing contamination to insignificant levels. Additional hot water treatment produced reliably clean seeds. The optimal conditions were 57^0C for 20 min.

Chen *et al.* (1986) observed that hot water treatment at 55°C for 10 min or 45°C for 50 min killed nematodes inside the tubers and made them resistant. He also stated that rice seeds can be controlled from nematodes by hot water treatment under 61°C for 10 min.

Sangar *et al.* (1985) stated that treatment of dormant diseased tubers of potatoes at 53°C for 10 to 15 min was effective for controlling mycoplasma-induced disease. A mycoplasmal disease called "Marginal flavescence" (MF) caused heavy reduction in Potato yield besides inducing hairy sprouts in the infected tubers, was treated by hot water seed treating device finely.

Singh (1983) reported that the method involved soaking of eggplant seeds in water at 20-30°C for 4-6 hr. then dipping in water at 49°C for 2 min, followed by drying before planting. This method may be used only in situations where temperature controlled water baths are available. There are chances of reduction in germination if there is an increase in either temperature or duration of soaking of the seed. Because of the inherent problems in the method and in general the fact that only smaller quantities of seed can be treated, the technique had limited application.

Singh and Chakrabarti (1982) studied the effect of seed treatment of phomopsis infected seed with 7–chemicals and hot water at 51°C for 15 minutes. They found that the treatment of brinjal seeds before sowing with the chemicals like Captan, Difolatan, Benlate, Thiram, Bavistin, Calixin and Panolil did not have significantly effect on emergence and stand of the brinjal seedlings in nursery beds. In the spray treatments minimum disease incidence and maximum seed yields were obtained from Difolatan and Captan treatments, Calixin was Phytotoxic to the crop even at lower recommended concentration. He reported that the losses caused to the seed crop were much more than on vegetable crops.

Prabhu and Prasada (1970) reported that seed treatment of eggplant with hot water effectively controlled seed-borne inoculum. Seed treatment increased germination of seeds. After soaking seeds in water the seeds were treated by hot water treatment at 52 – 54°C for 10 min.

Raychoudhuri (1967) reported that hot water treatment of brinjal seeds at 50°C for 30 minutes helped in warding of the phomopsis blight or fruit rot infection by *Phomopsis vexans*.

Raychoudhuri and Lele (1966) reported that phomopsis blight of brinjal called 'fruit rot' could be controlled by hot water treatment of seeds. It was eradicative aimed at destroying disease causing fungi and bacteria which carried with the seeds. Hot water treatment (treating seeds at 50-52°C for 15-30 minutes) was an acceptable and standard practice and was recommended for chilli, Brinjal, Brassicas and Cole crops.

2.2 Symptoms, incidence and severity of *Phomopsis fruit* rot of eggplant.

Singh (1992) have reported that the disease is present in one or other from the seedling stage of the plants to its maturity. In seed bed, it appears as damping off. After transplanting the leaves coming in contact with soil may get infected and show clearly defined circular, gray to brown spots with light colored centre. The old spots show numerous black pycnidia. The affected leaves turn brown and ultimately die. The fruit is attacked while on the plant.

Kumar *et al.* (1986) stated that *Phomopsis vexans* caused fruit rot which appeared as minute, circular, water soaked, sunken, grayish spots with brownish halo and have a bright coloured centre which latter enlarged to produce concentric rings and brownish zones. Spots increased in size and formed large rotten areas, which developed pycnidia causing most of the rotten fruit surface leading to blackening of the affected area.

Ashrafuzzaman (1986) has stated that, due to this disease, damping off takes place at seeding stage. Leaf may be attacked at any time. Generally first symptoms appear on the lower leaves, spots are clear, circular and greyish.

According to Walker (1952) the first phase of *Phomopsis* blight is a blight of young seedlings. The stem is girdled slightly above the soil line. The plant topples over and dies. The stem lesion is dark brown, becoming gray in the centre as the pycnidia develop.

2.3 Seed borne nature and its impact

Phomopsis vexans is an important fungus isolated from surface sterilized brinjal seed. It was stated to be seed borne by Walker (1952) in USA. In India Panwar and Patel (1957) studied the disease, but did not state whether it was seed borne or not. They reported that the causal organisms of the disease remained viable for about 14 months in soil debris and in the seed from infected fruits, which were poor in germination.

Khan (1999) studied the seed-borne nature of *Phomopsis vexans* and reported from the analyzed 22 seed samples of eggplant cultivars collected from 8 growing areas of Bangladesh. The infection of seeds by *Phomopsis vexans* ranged from 0.5 to 7%.

Pan *et al.* (1995) studied the seed-borne nature of *Phomopsis vexans* (Sacc and Syd) Harter and it was shown that *Phomopsis vexans* was present on seed coat and on the cotyledons of aubergine seeds collected from disease fruit in West Bengal, India.

Karuna *et al.* (1994) studied on seed-borne inoculum of *Phomopsis vexans* (Sacc and Syd) Harter and the effect of infection on seed quality in egg plant (*Solamum melongena* L.) and found that seed borne infection of aubergine varieties BR-7, PBR-5, MHB-1 and Pant rituraj was 29%, 36%, 35% and 56% respectively. Seed infection caused various degrees of seed discoloration.

Singh (1992) has stated that the fungus is seed borne and also subsists between crop seasons on infected plant debris in the field. The organism is apparently disseminated as water borne pycnidiospores. The spores exuded out through the ostiole and can easily be thrown away by rain drop splashes.

MATERIALS AND METHODS

3.1 Experimental site

The laboratory experiments were conducted in Integrated Pest Management (IPM) laboratory and the pot experiment was done in net house of the Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh.

3.2 Experiment period

Laboratory and pot experiments were conducted during January to May, 2004.

3.3 Materials

Eggplant Seeds

Seeds of eggplant cultivar Dohazari were collected from Dohazari, Chittagong and these were used in the experiments.

Hot Water Seed Treating Device

Hot water seed treating device, developed at the IPM Lab, BAU was used in the experiments. It is made up of a tank of stainless steel sheet of capacity 2 litres, a timer, thermostat bulb, switch; and a heater coil. Here, timer is used for controlling or regulating time, thermostat bulb is used for regulating temperature or selecting the desired temperature. The machine is devised so that time and Temperature can be controlled (Plate 1).

Plate 1. Vegetable Seed Treatment Plant

3.4. Methods

3.4.1 Heat application in the Device

The vegetable seed treating plant made up of locally available materials works automatically controlling temperature with time. Firstly seeds were soaked in normal water for 3–4 hr. in a cotton fabric bag. Then, pouring 2 litre water in the device below the red marking the device was connected with electricity. With the help of heater coil the device was heated. With the time & thermostat bulb the device was regulated to the desired temperatures such as 53, 55, 57, 59 & 61^{0}C. With the thermometer the desired temperature was denoted.

3.4.2. Fixing temperature and time

Thermostat bulb was regulated to fix the desired temperature. Generally, it took 10 min by the machine to reach the desired temperature. After the desired temperature obtained, the seeds were dipped in water for a fixed period of time. Time was controlled by stop watch.

3.4.3. Treating seeds in the machine

Once the desired temperature was achieved, the pre-soaked seeds in a cotton bag were dipped in the hot water for a fixed period of time. During the dipping, the bag with the seeds was frequently stirred for uniform exposure of seeds to hot water and also to maintain uniform heat all over the tank. At the end of the treatment, seeds were taken out of the tank, drained off and spread on a piece of brown paper and shade dried. Then the seeds were plated for test.

3.4.4 Analysis of the treated seeds: Blotter Techniques

After washing the plastic petridish, it was wiped out by the spirit. Then two filter papers were soaked in water and set in the petridish. 200 seeds were taken from working sample for each treatment. 50 seeds are plated after hot water treatment. The petridishes were incubated at room temperature for one week. After one week, germination, abnormal germination, dead seeds, rotten seeds and pathogens (*Phomopsis vexans*) are recorded from each petridish and observations continued up to 14 days. Blotter technique was employed following the procedure of ISTA (2002).

In blotter tests, seeds those plated were classified into 5(five) catcgories:

a. Category I: Germinated seeds
b. Category II: Abnormal germinated seeds
c. Category III: Dead seeds
d. Category IV: Rotten seeds
e. Category V: Pathogen borne seeds.

a. **Germinated seeds**

Germination in a laboratory test is defined as the emergence and development from the seed embryo of those essential structures which indicate the ability to develop into a normal plant under favourable conditions. When the seedlings produced in a laboratory, germination tests are evaluated as the percentage germination from any abnormal seedlings. According to ISTA (International Seed Testing Association), Germinated seeds are those which show the capacity for continued development into normal plants and are grown in good quality under favourable conditions.

A well developed root system including a primary root, a well-developed and intact hypocotyls without damage to the conducting tissues, an intact plumule with a well-developed green leaf within or emerging through the coleoptiles or an intact epicotyls with a normal plumule bud are shown in germinated seeds.

b. **Abnormal germinated seeds**

According to ISTA (International 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 epicotye, hypocotye or root; seedling without a primary root are shown, are called abnormal germinated seeds.

c. **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 fro-dormancy are classified as fresh ungerminated seeds and must be reported them as dead seeds.

d. **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 epicotyle, hypocotyl or root. Seedlings with any of the essential structures so diseased or decayed that normal development is prevented, called rotten seeds.

e. **Pathogen borne-seeds**

The seeds which carried pathogen inside or outside the seed with any part of seed are called pathogen borne-seeds. Seeds infected by *Phomopsis vexans* were identified observing α conidia Plate-2 on prepared slide under compound microscope.

3.5 Experimental detail

3.5.1 Treatment

The experiments in the lab were conducted in three phases. In the first phase, seeds were treated at a series of temperature regime under a fixed time period. In the second phase, best results of the first phase were repeated under a series of time period while in the 3rd phase the best temperature and time were tested.

First Phase: Five temperature regime- 53, 55, 57, 59 and 61^{0}C

Time of dipping seeds: 15 min

Second Phase: Temperature regime: 53 and 55^{0}C

Time of dipping seeds: 3, 5, 8, 10, 12, 15, 18 and 20 min

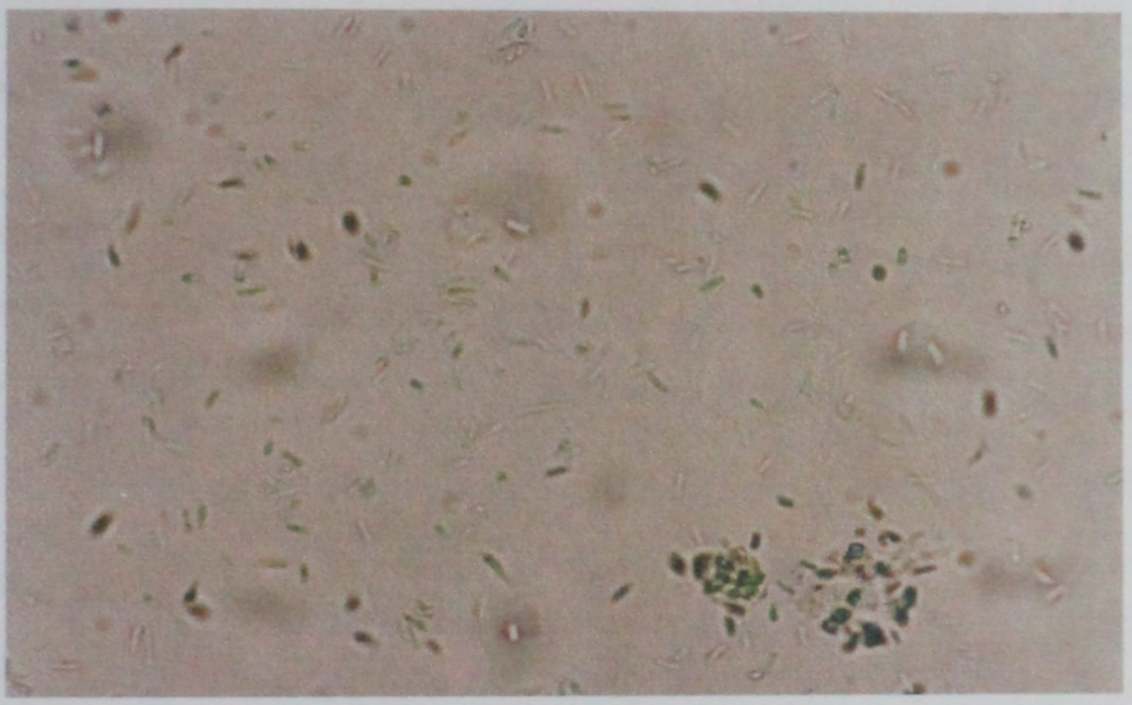

Plate 2. Alpha conidia of *Phomopsis vexans*

For each temperature and time combination, 200 seeds were used and four replications were maintained. In each case, untreated seeds were plated which served as control.

In the Net house, seeds treated at 53° and 55°C for 15 min were sown in earthen pots (filled up with sterilized soil mix in the ratios of soil: cow dung: sand 2: 1: 1).

3.5.2 Data collection

After 7 days of plating/sowing, the data on 5 parameters were recorded. The parameters were germinated seeds, abnormal germination, dead seed, rotten seed and the no. of *Phomopsis vexans* in the seeds and all data were expressed in percentage following the formula given below.

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

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

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

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

$$\% \textit{ Phomopsis vexans} = \frac{\text{Number of colonies of Phomopsis vexans in the seed}}{\text{Number of total seeds in petridish}} \times 100$$

3.6 Experimental Design

The laboratory and net house experiments were laid out in a Completely Randomized Design (CRD) with 4 replications. 200 seeds were set up in the 4 petridishes. Each petridish took 50 seeds for determining the germination, abnormal germination, deed seed, rotten seed and *Phomopsis vexans* percentage.

3.7 Statistical Analysis of Data

All the recorded data were analyzed with the Analysis of Variance Technique and differences among treatment means were compared with Duncan's Multiple Range Test (DMRT) using a statistical computer pakages (MSTAT). Arc sine transformation was done on data regarding germination percentage and square rot transformation was done on data regarding abnormal germination, dead seed, rotten seed and *Phomopsis vexans* percentage prior to conducting analysis of variance (Gomez and Gomez, 1983).

CHAPTER IV

RESULTS

4.1 Effect of different temperatures against *Phomopsis vexans* of eggplant seeds treated using Vegetable Seed Treating Plant for 15 minutes.

The effects of different temperatures against *Phomopsis vexans* of eggplant seeds were summarized in the Table 1. Different temperatures showed significantly different effect on the incidence of *Phomopsis vexans* over control (Plate 3). In case of seed germination, the highest per cent germination (87.0%) was recorded in 55°C temperature. The second highest germination (84.0%) was observed in 53°C temperature but the value was statistically similar to 55°C temperature. The germination values decreased with the increase of temperature from 55°C to above and at 61°C the germination value was the lowest (69.5%). The effect of temperatures on other parameters viz. abnormal seed germination, dead seed, rotten seed and presence of *Phomopsis vexans* were more or less similar to that of percent germination. The lowest abnormal germination (8.0%) and dead seed (4.0%) were found in case of 55°C temperature which was statistically similar to 53°C temperature. The percent rotten seed and incidence of *Phomopsis vexans* were nil in case of 55°C temperature and above but the per cent germination decreased with the increase of temperature. In case of 53°C temperature a few *Phomopsis vexans* and rotten seed were observed but values were significantly indifferent with the effect of 55°C temperature.

Table 1. Effect of temperature with fixed time against *Phomopsis vexans in vitro* using Hot Water Seed Treating Device.

Treatment (0C) 15 min.	Seed germination %	Abnormal seed germination %	Dead seed %	Rotten seed %	*Phomopsis vexans*
53	84 ab (66.46)	9.5 b (3.070)	5 c (2.225)	1.5 b (1.232)	1 b (1.000)
55	87 a (68.93)	8.0 b (2.818)	4 c (1.965)	0.0 b (0.7000)	0 b (0.7000)
57	80 bc (63.48)	12 ab (3.443)	5 c (2.225)	1 b (0.8775)	0 b (0.7000)
59	75 cd (60.02)	11.5 ab (3.372)	12 b (3.455)	0.5 b (0.707)	0 b (0.7000)
61	69.5 d (56.60)	12 ab (3.463)	11 b (3.277)	0.5 b (0.707)	0 a (0.7000)
Control	59.5 e (50.48)	14.5 a (3.807)	21 a (4.572)	5 a (2.225)	8 a (2.818)

Values in a column with same letter(s) do not differ significantly

Note: Figures in the parenthesis are the transformed values.

Plate 3. The incidence of *Phomopsis vexans* on seed germination, abnormal seed germination, dead seed and rotten seed treated with different temperatures for 15 min.

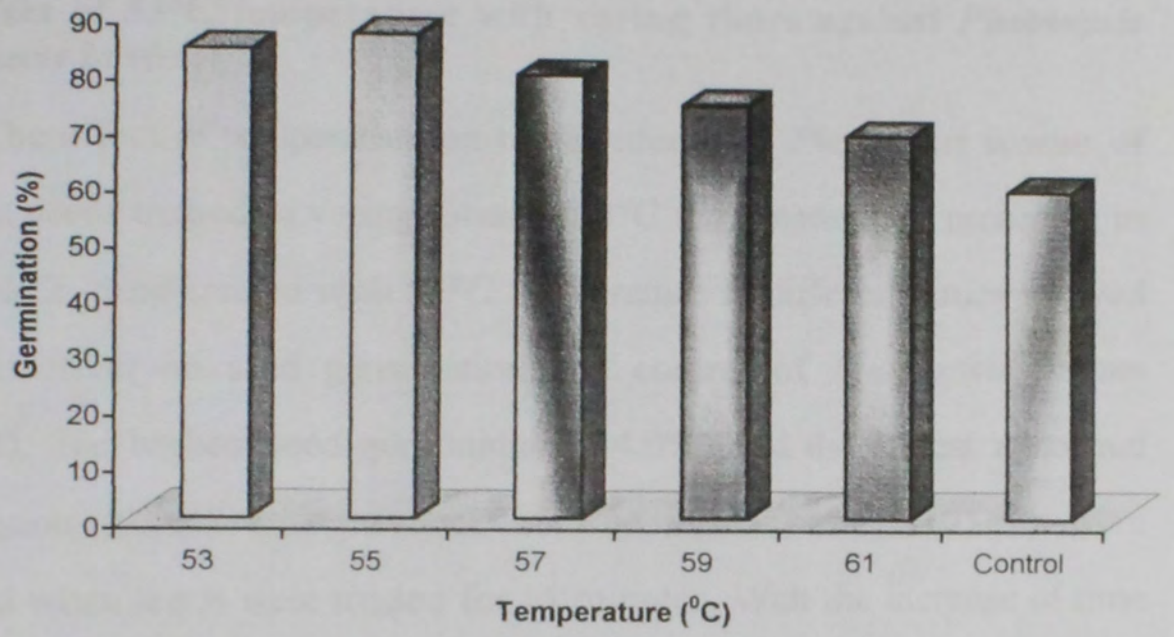

Fig.1. Showing germination of egg plant seeds treated with different temperatures for 15 min.

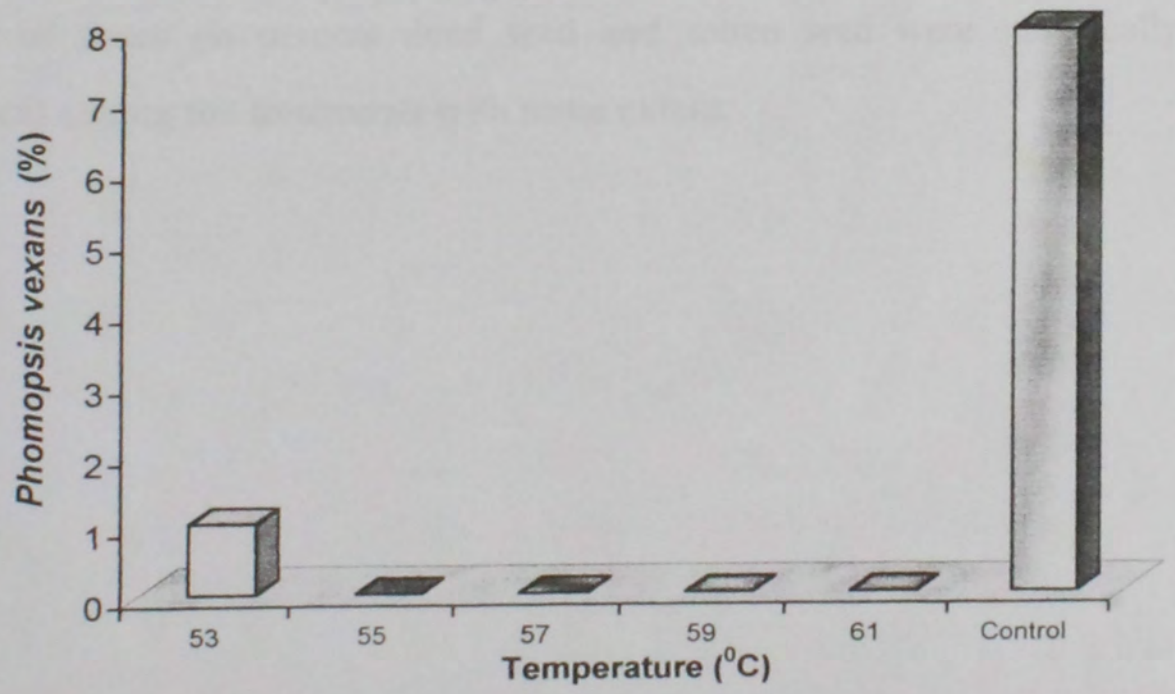

Fig. 2. Showing control of *Phomopsis vexans* of egg plant seeds treated with different temperatures for 15 min.

4.2 Effect of 53°C temperature with varing times against *Phomopsis vexans in vitro*

The effect of temperature on the incidence of *Phomopsis vexans* of eggplant seeds treated in varing times at 53°C temperature are presented in the Table 2. Seed treated with 53°C temperature at different times showed different effect on seed germination and control of *Phomopsis vexans* (Plate 4). The highest seed germination (84.0%) and the lowest abnormal germination (9.0%) and presence of *Phomopsis vexans* (0.0%) were recorded when seeds were treated for 15 minutes. With the increase of time from 15 minutes to above the *Phomopsis vexans* was controlled completely as in 15 minutes but the seed germination was decreased sharply. The effect of times on percent dead seed and rotten seed were statistically identical among the treatments with some extent.

Table 2. Effect of 53^{0}C temperature with varying time against *Phomopsis vexans in vitro* using Hot Water Seed Treating Device.

Treatment 53^{0}C) with varying time min.	Seed germination %	Abnormal seed germination %	Dead seed %	Rotten seed %	*Phomopsis vexans*
3	81 bc (64.17)	12 ab (3.453)	4.5 d (2.078)	3.5 b (1.852)	4 b (2.000)
5	81 bc (64.20)	11.5 ab (3.362)	5 d (2.225)	2.5 bc (1.558)	3 bc (1.705)
8	81.5 abc (64.55)	12 ab (3.443)	4 d (2.000)	2.5 bc (1.558)	2.5 bcd (1.558)
10	79 cd (62.74)	14 a (3.735)	5 d (2.225)	2 bc (1.380)	2 cd (1.410)
12	82.5 ab (65.28)	10.5 ab (3.227)	5.5 d (2.320)	1.5 c (1.232)	1.5 cde (1.232)
15	84 a (66.44)	9 b (2.962)	6 d (2.432)	1.5 c (1.055)	0.0 e (0.7000)
18	78 d (62.04)	12 ab (3.438)	9 c (2.995)	1.5 c (1.055)	1.5 de (1.055)
20	77 d (61.37)	9 b (2.987)	13.5 b (3.665)	1.5 c (1.055)	0.0 e (0.707)
Control	58 e (49.60)	10.5 ab (3.215)	22.5 a (4.735)	9 a (2.995)	9 a (2.975)

Values in a column with same letter(s) do not differ significantly

Note: Figures in the parenthesis are the transformed values

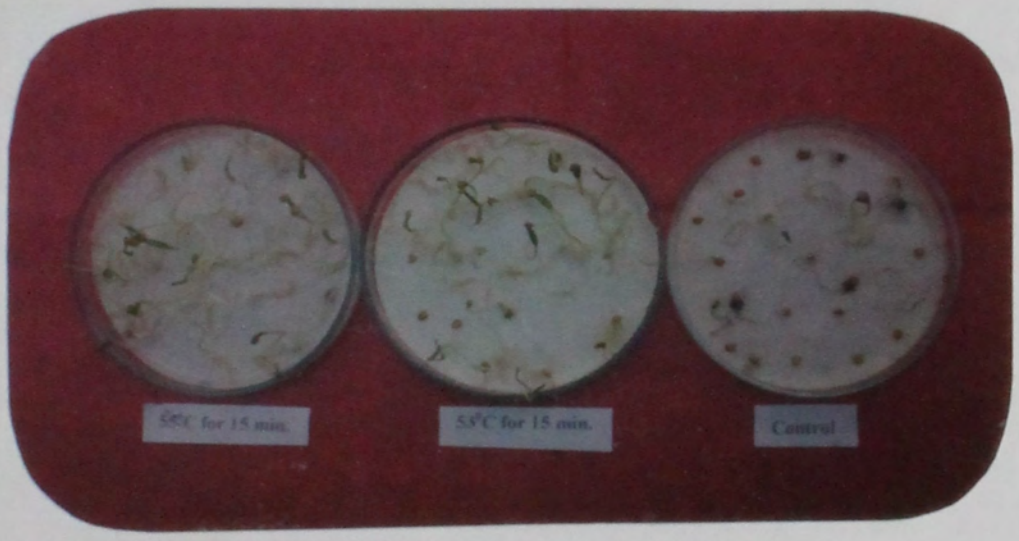

Plate 4. Showing the performance of seed germination at 53°C and 55°C temperatures treated for 15 min.

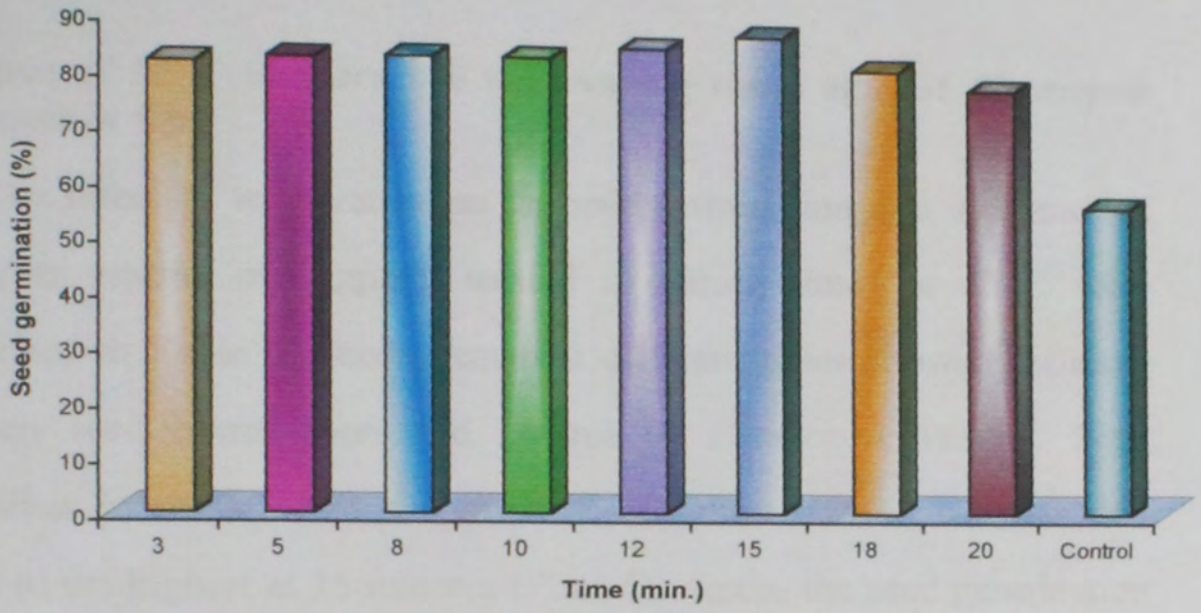

Fig.3. Showing germination of egg plant seeds treated with different times for 53^{0}C temperature

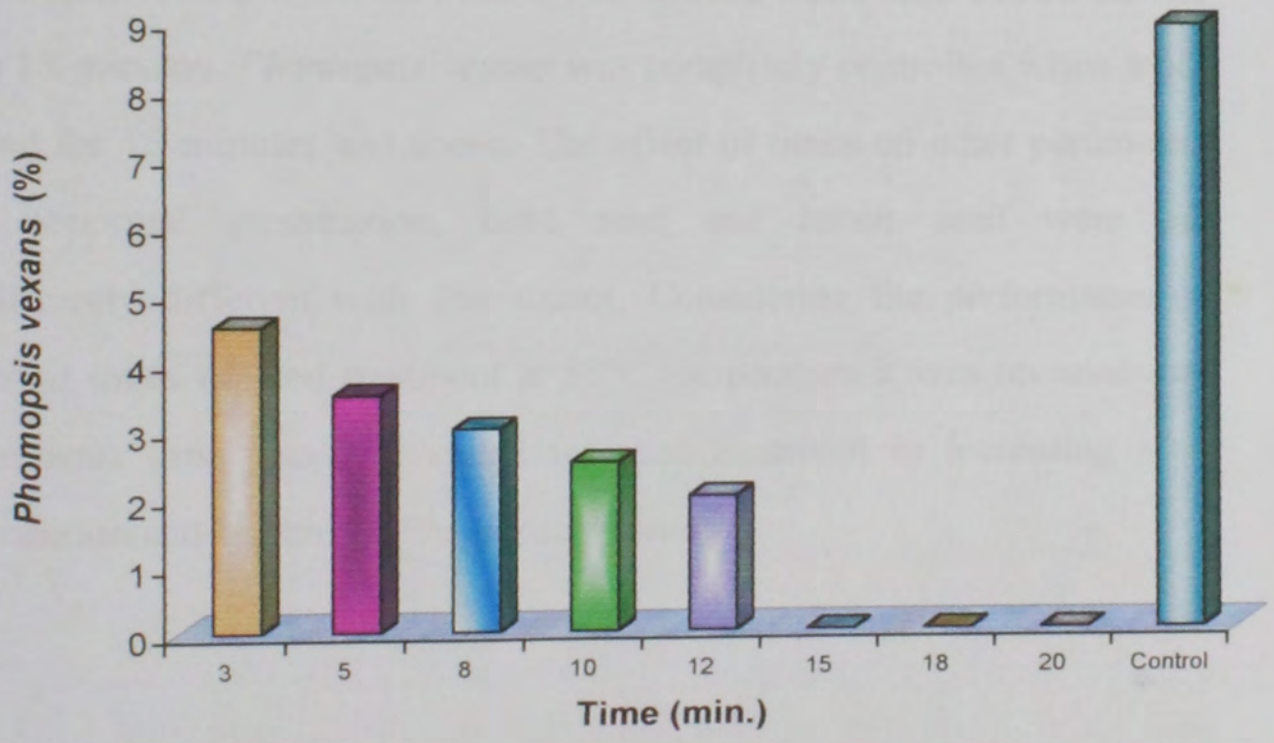

Fig. 4. Showing effect of 55^{0}C temperature against *Phomopsis vexans* of egg plant seeds treated at different times. with different times for 55^{0}C temperature

4.3 Effect of 55°C temperature with varing times against *Phomopsis vexans in vitro*

The effect of temperature on the seed germination and incidence of *Phomopsis vexans* of eggplant treated in varing times at 55°C were summarized in Table 3. Seeds treated at different times showed different effect on seed germination and control of *Phomopsis vexans*. Seed germination increased with the increase of times from 3 minutes and reached to the highest at 15 minutes (Plate 5). Again, the seed germination decreased with the increase of treating times from 15 minutes to above. The incidence of *Phomopsis vexans* was noticed while seed treated for less than 15 minutes. *Phomopsis vexans* was completely controlled when seeds treated for 15 minutes and above. The effect of times on other parameters viz. abnormal germination, dead seed and rotten seed were not significantly different with few extent. Considering the performance of different times of seed treatment at 55°C temperature it was revealed that 15 minutes time was best suited for seed treatment in increasing seed germination and control of *Phomopsis vexans*.

Table 3. Effect of 55^{0}C temperature with varying time against *Phomopsis vexans in vitro* using Hot Water Seed Treating Device.

Treatment 55^{0}C) with varying time min.	Seed germination %	Abnormal seed germination %	Dead seed %	Rotten seed %	*Phomopsis vexans*
3	81.5 c (64.55)	10 a (3.153)	3 e (1.558)	6 b (2.432)	4.5 b (2.112)
5	82.5 bc (65.28)	10 a (3.145)	3 de (1.705)	4.5 bc (2.112)	3.5 bc (1.852)
8	83 bc (65.66)	9 a (2.975)	3.5 de (1.852)	4.5 bc (2.112)	3 bc (1.705)
10	83 bc (65.70)	10.5 a (3.203)	3 de (1.705)	3.5 cd (1.852)	2.5 cd (1.558)
12	85 b (67.27)	9 a (2.975)	4 de (1.965)	2 de (1.380)	2 cd (1.410)
15	87.5 a (69.34)	7 ab (2.610)	4.5 d (2.112)	1.5 e (1.055)	0 de (0.70)
18	81.5 c (64.54)	5 bc (2.225)	12.5 c (3.525)	1.5 e (1.055)	0 de (0.70)
20	78 d (62.04)	3.5 c (1.852)	17.5 b (4.172)	1.5 e (1.055)	0 de (0.70)
Control	56.5 e (48.74)	8.5 a (2.900)	25 a (4.993)	10 a (3.153)	9 a (2.975)

Values in a column with same letter(s) do not differ significantly

Note: Figures in the parenthesis are the transformed values.

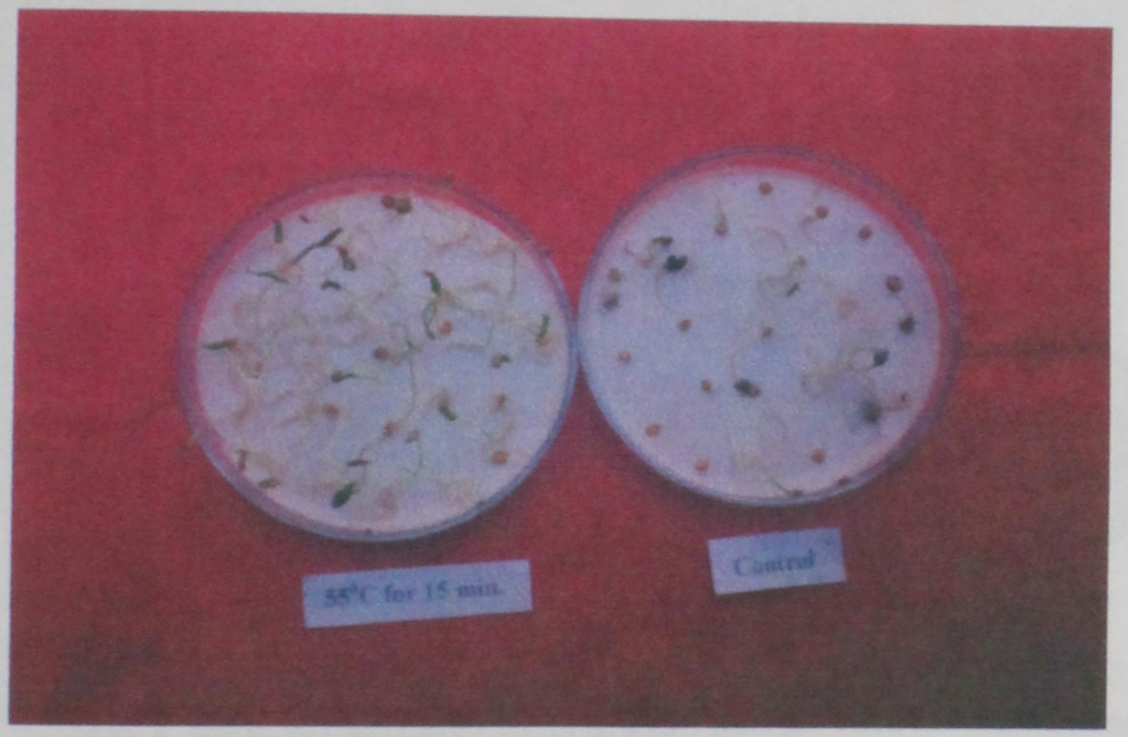

Plate 5. Showing the effect of 55°C temperature in controlling *Phomopsis vexans* treated for 15 min.

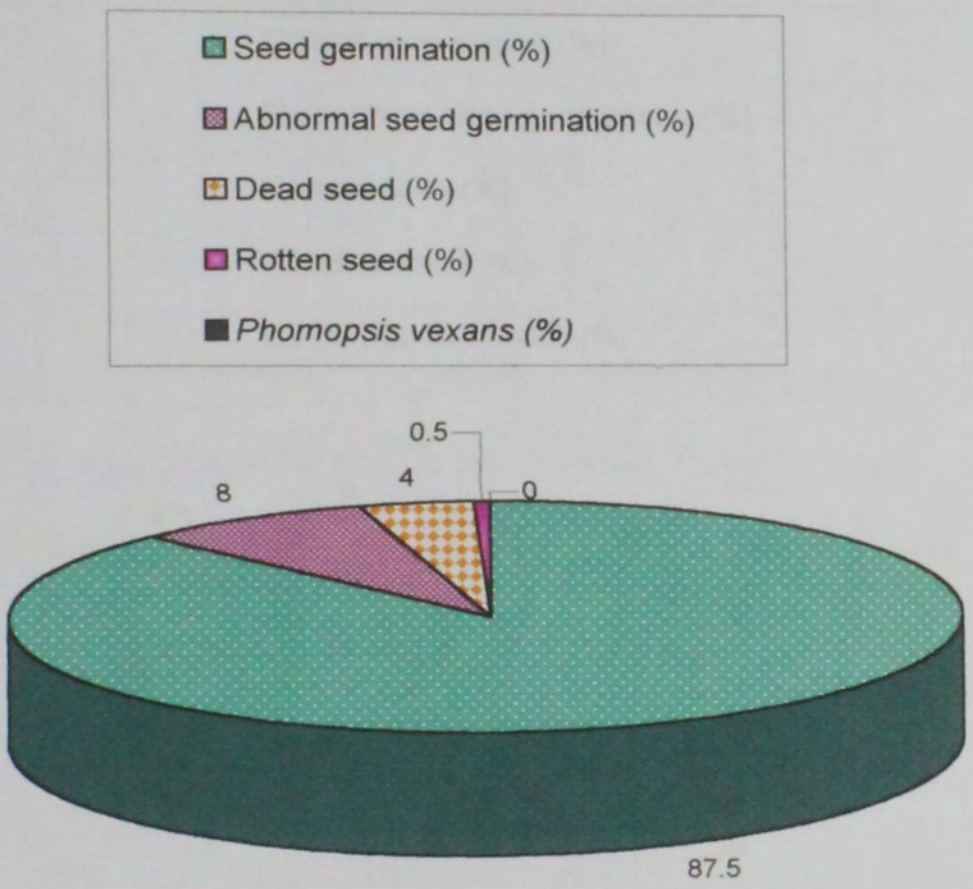

Fig. 5. Showing the percentage of seed germination, abnormal seed germination, dead seed, rotten seed and *Phomopsis vexans* of egg plant seeds treated with 55^0c temperature for15 min.

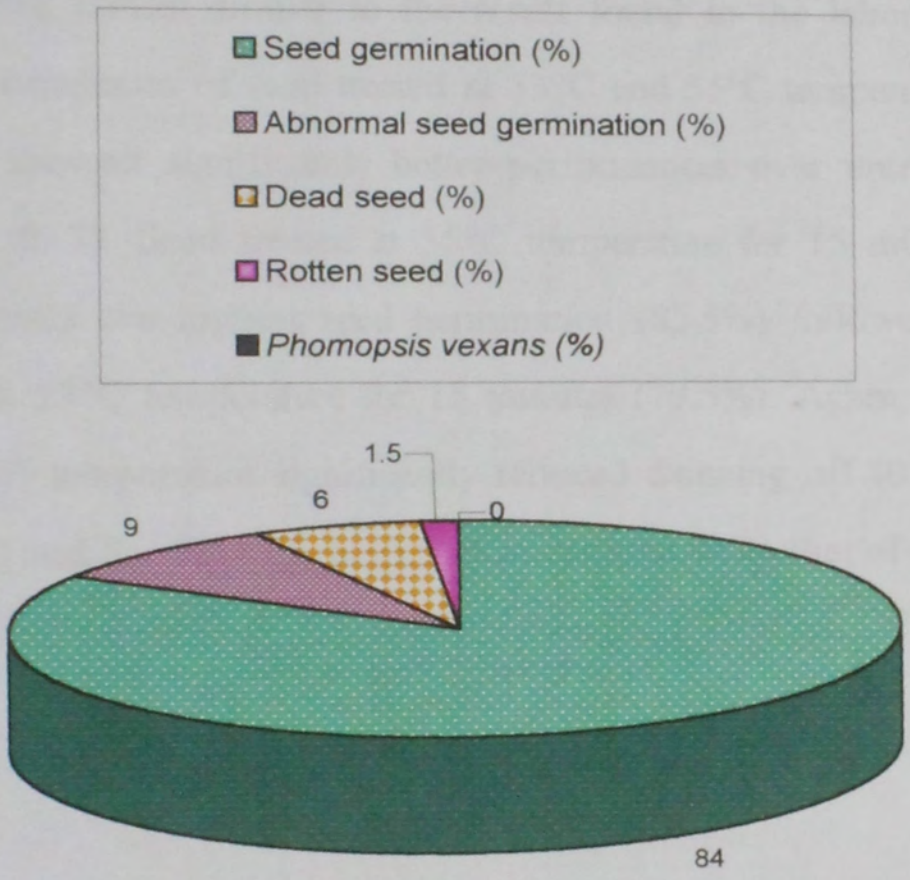

Fig. 6. Showing the percentage of seed germination, abnormal seed germination, dead seed, rotten seed and *Phomopsis vexans* of egg plant seeds treated with 53^0c temperature for 15 min.

4.4 Effect of temperature on the incidence of *Phomopsis vexans* of eggplant seed in net house

The effect of temperature on incidence of *Phomopsis vexans* found in net house were almost similar to the result found in the laboratory (Table 4). Both categories of seed treated at 53°C and 55°C temperatures for 15 minutes showed significantly better performances over untreated control (Plate 6 & 7). Seed treated at 55°C temperature for 15 minutes showed significantly the highest seed germination (82.5%) followed by treated seed with 53°C temperature for 15 minutes (79.5%). Again, seed treated with 55°C temperature significantly reduced damping off (0.0%), Tip over (1.25%) and Seedling blight (0.0%) in comparison to that of 53°C temperature.

Table 4. Effect of temperature on the incidence of *Phomopsis vexans* of eggplant seed in net house.

Treatment	Germination (%)	Damping off (%)	Tip over (%)	Seedling blight (%)
Untreated seed (control)	57.25 c (7.51)	6.50 a (2.54)	9.5 a (3.15)	7.50 a (2.82)
Seed treated at 53°C for 15 min.	79.50 b (8.90)	2.00 b (1.51)	4.5 b (1.98)	4.00 b (2.05)
Seed treated at 55°C for 15 min.	82.50 a (9.08)	0.00 c (0.70)	1.25 c (1.06)	0.0 c (0.70)

Values in a column with same letter(s) do not differ significantly

Note: Figures in the parenthesis are the transformed values.

Plate 6. Seedlings of eggplant raised in net house treated with 55°C temperature for 15 min.

Plate 7. Seedlings of eggplant raised in net house from untreated control seeds.

DISCUSSION

Hot water treatment of farmer seeds improved seed germination and reduced nursery diseases. Control of *Phomopsis vexans by* Hot Water Treatment of eggplant seeds were found effective by the previous worker (Raychoudhuri, 1967). The method of Hot Water Seed Treatment has been recognized as an acceptable and standard practice for elimination of *Phomopsis vexans* infection of eggplant seeds (Roychoudhuri and Lele, 1966).

In the present experiment, seed treatment with 53°C and 55°C temperatures for 15 minutes were found to be effective in controlling *Phomopsis vexans* and increasing seed germination in the laboratory in comparison to 57°, 59°, 61° and control. These results agreed with the findings of Prabhu and Prasada (1970) and Meah (2003) with some extent. Meah found 53°C for 15 minutes as the best suited temperature for seed treatment of eggplant for controlling *Phomopsis vexans*. Prabhu and Prasada (1970) found 52-54°C temperatures for 10 minutes suitable temperatures to increase seed germination and inoculum reduction. Our *in vitro* results little bit differed with the findings of Raychoudhuri and Lele (1966). They found

50-52°C temperatures were suitable. But their time ranges were 15-30 minutes.

When seeds were treated for varying times viz. 3, 5, 8, 10, 12, 15, 18 and 20 minutes at 53°C and 55°C temperatures ,it was observed that 15 minutes time for seed treatment was suitable in controlling *Phomopsis vexans* and increase of seed germination. If the seeds were treated for less than 15 minutes *Phomopsis vexans* remained with the seeds and caused germination failure. This was for the cause that the pathogen may tolerate the temperature (53°C, 55°C) for below 15 minutes. When temperature was existed for 15 minutes and above the pathogen could not tolerate the temperature and ultimately died.

Again, when the temperature increased from 55°C temperature to above the pathogen *Phomopsis vexans* controlled completely but seed germination reduced sharply. It happened that was the increased temperature became intolerable both for the pathogen and the embryo of seeds. Even when the temperature was existed in 55°C temperature but time was increased above 15 minutes, the seed embryo could not tolerate the temperature and thus the seed germination were reduced.

The results of net house experiment were more or less similar to the *in vitro* experiments. Raising of seedling attributed significantly better performance when seeds were treated at 55°C temperature for 15 minutes than 53°C but for better than control. Seed germination was the highest (82.5%) and incidence of *Phomopsis vexans* (damping off, seedling blight) was nil in case of seed treatment with 55°C temperature for 15 minutes. Few incidence of tip over of seedlings were also observed in case of 55°C temperature but it might be the presence of tip over causing seed borne pathogen like *Fusarium* spp. other than *Phomopsis vexans*.

The results of net house experiments were little bit differed with the results of Meah, 2003. They selected 53°C for 15 minutes as the suitable temperature for seed treatment against *Phomopsis vexans*. But they did not include 55°C in their practices.

SUMMARY AND CONCLUSION

Different temperatures 53°, 55°, 57°, 59° and 61°C for different times 3, 5, 8, 10, 12, 15, 18 and 20 minutes, respectively were evaluated for their effect on the prevalence of *Phomopsis vexans*, seed germination, dead seed, rotten seed and seedling diseases of eggplant.

In laboratory investigation, 55°C temperature for 15 minutes revealed significantly the best effective treatment for control of *Phomopsis vexans* and increasing seed germination of eggplant seed. Cent percent control of *Phomopsis vexans* and the highest seed germination (87.0%) was found in this treatment. The second highest seed germination (84.0%) was recorded at 53°C temperature treated for 15 minutes which was statistically identical with that of 55°C temperature. A few *Phomopsis vexans* was remained in this case but it was also statistically similar to that of 55°C temperature. The higher temperature than 55°C controlled *Phomopsis vexans* effectively but seed germination was gradually decreased with the increase of temperatures. The results of abnormal seed germination, dead seed and rotten seed were not so differed among the treatments other than control.

Evaluation of different times for seed treatment at 53°C and 55°C temperatures showed different results among the treatments. At times less than 15 minutes for both 53°C and 55°C temperatures showed lower

germination. Seed treated for 15 minutes for both the temperatures proved to be the best suited treatment against *Phomopsis vexans* as well as increasing seed germination. Increase of time above 15 minutes controlled *Phomopsis vexans* completely but the seed germination decreased gradually with the increase of time for treatment. Net house experiment for raising of seedlings using the 53°C and 55°C temperatures treated for 15 minutes produced similar results against *Phomopsis vexans* as observed in the *in vitro* experiment.

Incidence of *Phomopsis vexans* in raised seedlings (damping off and seedling blight) were nil while seed treated with 55°C temperature for 15 minutes. Seed germination was also recorded the highest (82.5%) at 55°C temperature. The performance of 53°C temperature was not satisfactory against *Phomopsis vexans* in net house experiment.

From the overall study, it is concluded that seeds treated with 55°C for 15 minutes is effective and standard measure for controlling *Phomopsis vexans* of egg plant. It must be concluded that the Vegetable Seed Treating Plant used in the experiment is a potential device for seed treatment in controlling seed borne *Phomopsis vexans* of eggplant seed. The Device efficiently hold up the desired temperature for its thermostative activities. However, further studies are needed to standardize the required temperature for controlling other seed borne microflora to extend the wide use of Vegetable Seed Treating Plant for different vegetable crops.

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