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Research Article
Effect of Hydrogen Peroxide Treatment on Health and Quality of Chilli Seed

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Abstract

Background and Objectives: Delay seed germination, Seedling vigor and mortality due to seed borne pathogens are causing increasingly the economic losses in chilli. The experiment was conducted to determine the effect of H_2O_2 on seed health, germination and vigor of chilli seeds. **Materials and Methods:** The experiment was carried out in the laboratory of the Department of Plant Pathology, Patuakhali Science and Technology University, Patuakhali, Bangladesh. There were 4 treatments viz. water soaked seeds, seed treated with 1% H_2O_2, seed treated with 2% H_2O_2 and seed treated with 3% H_2O_2. The experiment was conducted by Completely Randomized Design (CRD) with three replications. Data were analyzed using one-way ANOVA with the Web Agri Stat Package 2.0 (WASP). Means were compared by the Duncan' s multiple tests and statistical significance was determined at 5% level. **Results:** Six fungal species were isolated from treated and untreated chilli seeds. Isolated fungi were *Aspergillus flavus*, *Rhizopus stolonifer*, *Colletotrichum capsici*, *Curvularia lunata*, *Alternaria alternata* and *Fusarium moniliforme*. The highest 50.74% mycelial growth inhibition of *Aspergillus flavus* was obtained by 3% H_2O_2 treatment. For 1% hydrogen peroxide treated seeds the germination percentages were 55.47, 60.53 and 84.80% at 7, 10 and 15 Days After Sowing (DAS), respectively. The maximum root length 4.767 cm was found in seedling raised from seeds treated with 1% H_2O_2. Significantly higher shoot length (2.769 cm) was obtained from the seedlings raised from 1% H_2O_2 treated seeds. The highest 640.7% vigor index was recorded in seedlings obtained from 1% H_2O_2 treated seeds. **Conclusion:** The treatment of chilli seeds with hydrogen peroxide, regardless of concentration, significantly reduced seed infestation with seed borne and improved health of chilli seeds. Among the concentrations of H_2O_2, 1% H_2O_2 is more effective in increasing seed germination percentage, vigor index and percent inhibition of mycelial growth.

Key words: H_2O_2, mycelial radial growth, germination percentage, vigor index, chilli seed

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Delayed and erratic germination of chilli seeds because of seed infection by seed borne pathogens is one of the reasons of low yield of chilli. In Bangladesh due to seed borne diseases at least 10% annual crop loss occurred out of 16% loss[1]. Fungal diseases play a vital role in reducing the germination of chilli[2]. Yield losses occur due to seed borne diseases where seeds perform as passive carriers. For crop establishment, yield and productivity the healthy seeds play a vital role. Seed testing is needed to attain this[3]. In recent years seedling mortality due to anthracnose of chilli caused by *Colletotrichum capsici* is causing increasingly the economic losses in chilli[4]. The use of chemical to control of seed borne pathogens is responsible for the increase in the productivity and quality of the crop but it is inappropriate and nondiscriminatory use has put human and animal health at risk, as well as contaminating the environment[5].

The results of some experiments revealed about having the antimicrobial properties of hydrogen peroxide against plant pathogens[6]. Some studies showed about the efficacy of H_2O_2 treatment to control of seed-borne pathogens before sowing[7]. Hydrogen peroxide exerts antimicrobial activity on wide range of microorganisms, indicating their consistency in interfering with the infection process of the pathogens. Both chemicals and in particular hydrogen peroxides are readily available, easy to handle and worth considering for disease management[8]. It has been known for long that H_2O_2 treatment of seeds as oxidants can breaking the primary seed dormancy[9]. In Hydrogen peroxide, the peroxide attacks various organic compounds. Generally it damages the genetic material and cell membranes of living cells. Peroxide in sufficient concentration kill bacteria, bacterial endospores, yeast and spores of fungi. It can also kill small airborne particles of fungi and the contaminants associated with human skin. Hydrogen peroxide thus acts against all commonly-encountered airborne contaminants. On the contrary, antibiotics generally act only against bacterial contamination and fungicides act only against fungi[10]. There are few studies on the control of seed-borne pathogens by the treatment of seeds with H_2O_2 before sowing[11]. It has been reported that hydrogen peroxide can improve the germination of zinnia seeds[12], as well as Eastern gamagrass (*Tripsacum dactyloides*)[13], rice (*Oryza sativa*)[14], maize (*Zea mays*)[15], watermelon (*Citrullus lanatus*)[16] and muscadine (*Vitis rotundifolia*)[17]. H_2O_2 promoted the germination of seeds by the oxidation of germination inhibitors present in the pericarp[18]. The present study was undertaken to determine the effect of H_2O_2 on the germination and vigor of chilli seeds and to evaluate the efficacy of H_2O_2 in controlling seed borne fungi of chilli.

MATERIALS AND METHODS

The experiment was carried out in the laboratory of the Department of Plant Pathology, Patuakhali Science and Technology University, Bangladesh. The experiment was conducted by Completely Randomized Design with three replications.

Isolation and identification of seed borne fungi: Isolation ofseed-borne fungi was done using recommended techniques by the ISTA[19] namely, Standard moist Blotter (SB) methods. A total number of 100 seeds from each treatment were used.

Different fungal colonies grown on chilli seeds were observed under stereo-binocular microscope (Carl Zeiss Axio vision). All the fungi were identified using keys and manuals[7,20-22]. In case of confusion, temporary mounts were prepared and examined under compound microscope (Carl Zeiss) for identification of the associated fungi. Prevalence of fungi was expressed in percentage based on total number of seeds plated. The % incidence of fungi of particular species within a genus of fungi was calculated[23]:

$$\text{Incidence (\%)} = \frac{\text{No. of seeds in which particular fungus appeared}}{\text{Total no. of seeds studied}} \times 100$$

***In vitro* evaluation of different conc. of H_2O_2:** Performance of 3 different conc. of H_2O_2 (1, 2 and 3% H_2O_2) were used to evaluate *in vitro* against seed borne fungi, by Poisoned food technique[22]. Each treatment was replicated thrice. When medium in the untreated control plates was fully covered with mycelial growth of the test fungus, radial mycelial growth was measured in all the treatment plates. Percent inhibition of mycelial growth (%) in treated plates was calculated by applying the formula given by Sundar *et al.*[24]:

$$\text{Inhibition (\%)} = \frac{X - Y}{X} \times 100$$

Where:
X = Radial growth (mm) of control plates
Y = Radial growth (mm) of fungicide treated plates

Germination and vigor test: One hundred and fifty seeds of each treatment were counted. Plastic tray was filled with fine

sand moistened with distilled water. Fifty seeds were sown in each tray and considered as a replication and there were three such replications of each treatment. After 7, 10 and 15 days of sowing each seedling was evaluated in accordance with the general principles laid down in ISTA rules[19]. The Germination Index (GI) was calculated by using the formula as suggested by the AOSA[25]:

$$\text{Germination index} = \frac{\text{No. of germination seed}}{\text{Days of first count}} + \ldots\ldots\ldots + \frac{\text{No. of germination seed}}{\text{Days of first count}}$$

For Vigor Test root and shoot length of randomly taken 10 seedlings per replicate were measured after 15 days. The seedling vigor was determined following the formula[25]:

$$\text{Vigor index} = \text{Mean root length} + \text{Mean shoot length} \times \text{Germination (\%)}$$

Statistical analysis: Data were analyzed using one-way ANOVA with the Web Agri Stat Package 2.0 (WASP). Means were compared by the Duncan' s multiple tests and statistical significance was determined at 5% level using WASP.

RESULTS AND DISCUSSION

Prevalence of seed borne fungi: A total of 6 different fungi belonging to 6 genera were isolated from chilli seeds. *Aspergillus flavus*, *Rhizopus stolonifer*, *Colletotrichum capsici*, *Curvularia lunata*, *Alternaria alternata* and *Fusarium monilliforme* were found to be associated with chilli seeds. Among them the most predominant fungi was *Aspergillus flavus* followed by *Colletotrichum capsici*, *Curvularia lunata*, *Fusarium moniliforme*, *Alternaria alternata* and *Rhizopus stolonifer* (Fig. 1). Alam *et al*.[2], Chigoziri and Ekefan[26] and Solanke *et al*.[27] also reported about the association of seed borne fungi.

***In vitro* effect of hydrogen peroxide treatment on radial mycelial growth of seed borne fungi:** The inhibition (%) of the growth of the fungus with different percent concentration of hydrogen peroxide over control was calculated and presented in (Table 1).

At 3% H_2O_2 treatment the highest mycelial growth inhibition of *Aspergillus flavus* was obtained. The growth of *Rhizopus stolonifer* was poorly inhibited by H_2O_2 treatment compare to other isolated fungi. The highest inhibition of colony growth of *Colletotrichum capsici* was observed by the treatment of 3% H_2O_2 followed by 2 and 1% H_2O_2. The mycelial growth of *Curvularia lunata* was highly inhibited by 1% H_2O_2 compare to 2% H_2O_2 and 3% H_2O_2. In case of *Alternaria alternata*. The highest inhibition of colony growth was obtained at 3% H_2O_2 concentration. The mycelial growth of *Fusarium moniliforme* was highly inhibited by 2% H_2O_2. The results of this study indicated that all the treatments reduced the seed borne fungi over the control. Among them, 3% H_2O_2 showed the best performance in terms of reducing percent seed-borne infection followed by 1% H_2O_2. Similar findings also observed by Kotchoni *et al*.[8]. They showed that

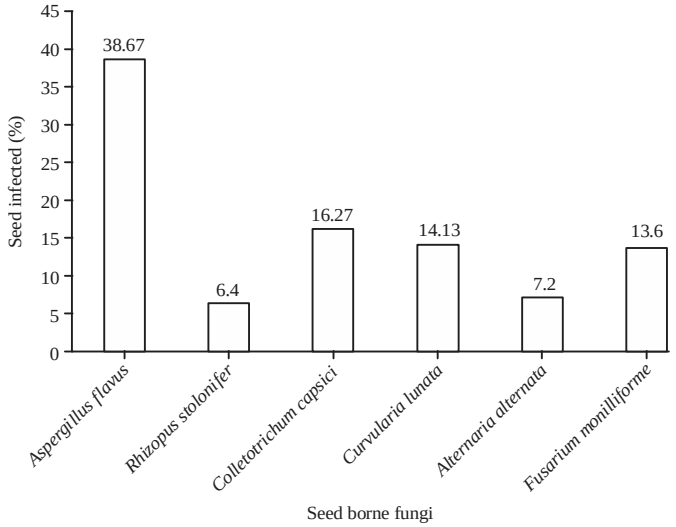

Fig. 1: Percent fungi obtained from different sources of chili seeds

Table 1: *In vitro* effect of Hydrogen peroxide on inhibition of mycelial growth of seed borne fungi

	Inhibition (%)					
Treatments	*Aspergillus flavus*	*Rhizopus stolonifer*	*Colletotrichum capsici*	*Curvularia lunata*	*Alternaria alternata*	*Fusarium moniliforme*
1% H_2O_2	39.25[b]	1.86[ab]	61.48[b]	55.92[c]	61.48[c]	72.59[b]
2% H_2O_2	49.63[a]	3.33[a]	62.97[b]	65.92[b]	75.19[b]	78.14[a]
3% H_2O_2	50.74[a]	3.33[a]	72.59[a]	72.97[a]	78.89[a]	81.86[a]
Control	00.00[c]	00.00[b]	00.00[c]	00.00[d]	00.00[d]	00.00[c]
LSD (0.05)	0.476	0.2147	0.3994	0.3094	0.2917	0.3522

Means followed by same letter in a column did not differ significantly at 5% level by LSD

Table 2: Mean effect of seed treatment on germination of chilli seed at different days after sowing

	Germination (%)		
Treatments	7 DAS	10 DAS	15 DAS
Water soaked	25.33[b]	48.53[b]	60.40[b]
1% H_2O_2	55.47[a]	60.53[a]	84.80[a]
2% H_2O_2	18.93[c]	21.33[c]	22.40[c]
3% H_2O_2	4.26[d]	5.60[d]	5.86[d]
LSD (0.05)	2.380	4.328	2.470

Means followed by same letter in a column did not differ significantly at 5% level by LSD

Table 3: Mean effect of seed treatment on root and shoot length of chilli seedling

Treatments	Root length (cm)	Shoot length (cm)	Vigor index (%)
Water soaked	2.401[c]	2.276[b]	279.4[b]
1% H_2O_2	4.767[a]	2.769[a]	640.7[a]
2% H_2O_2	2.885[b]	1.812[c]	105.4[c]
3% H_2O_2	2.213[c]	1.740[c]	23.20[d]
LSD (0.05)	0.1966	0.1650	20.81

Means followed by same letter in a column did not differ significantly at 5% level by LSD

H_2O_2 inhibited pathogen growth in seeds and seedlings. Similar results were also obtained by Szopinska[28]. He stated that, treatment with hydrogen peroxide, regardless of concentration, positively affected the health of the seeds, significantly increasing (at 5% level of significance) the percentage of seeds free from fungi.

In the present investigation, 1, 2 and 3% H_2O_2 were tested against six fungi viz., *Aspergillus flavus*, *Rhizopus stolonifer*, *Colletotrichum capsici*, *Curvularia lunata*, *Alternaria alternata* and *Fusarium moniliforme* isolated from chili seeds by Poison food technique. The results revealed that all concentration inhibited the mycelial growth of all fungi tested in comparison with control having large colony diameter. It is may be due to antimicrobial properties of hydrogen peroxide[6]. Among them, 1% H_2O_2 was superior in inhibition of mycelia growth of all fungi tested.

Effect of seed treatment on germination of chili seeds collected from different sources: There was significant difference (at 5% level of significance) among different conc. of Hydrogen peroxide in respect of germination % at different DAS (Table 2). The germination percentages were 55.47, 60.53 and 84.80% at 7, 10 and 15 DAS, respectively were obtained from 1% hydrogen peroxide treated seeds. The minimum germination percentages were 4.26, 5.60 and 5.86% at 7, 10 and 15 DAS, respectively were found at 3% Hydrogen peroxide treated seeds. This might be due to the reason of higher oxidative reactivity of hydrogen peroxide.

In this experiment higher percentage of germination was found in chili seeds when treated with 1% H_2O_2. Similar results were obtained by Szopinska[28]. He reported the negative influence of higher concentration of hydrogen peroxide in germination of seed.

Vigor index: The root length, shoot length and vigor index was significantly influenced (at 5% level of significance) by different concentrations of hydrogen peroxide (Table 3). The maximum root length (4.767 cm) was found in seedling raised from seeds treated with 1% H_2O_2. Lower root length (2.401 cm) was recorded in seedlings raised from water soaked seeds which is statistically similar to 3% H_2O_2 treated seeds. Significantly higher shoot length was obtained from the seedlings raised from 1% H_2O_2 treated seeds. Significant lower value of shoot length 1.812 and 1.704 cm were obtained from seedlings raised from the seeds treated with 2 and 3% H_2O_2, respectively. The highest 640.7% vigor index was recorded in seedlings obtained from 1% H_2O_2 treated seeds and significantly the lowest vigor index 23.20% was recorded from seedlings obtained from 3% H_2O_2 treated seeds.

Higher vigor index also found in seeds treated with 1% H_2O_2 compared to other concentrations of hydrogen peroxide over the untreated control seeds of each seed source. This could have resulted from the ability of the chemical to eliminate most of the seed-borne pathogens from the seed. Similar findings also reported by Ogawa and Iwabuchi[18] and Narimanov[29]. Ogawa and Iwabuchi[18] discussed the possibility that endogenously generated H_2O_2 functions as a promoter of zinnia seed germination by oxidizing germination inhibitors. Narimanov[29] observed that short seed treatment in H_2O_2 solution promoted the early appearance of sprouts and accelerated the development of barley, maize, haricot, melon, vegetable marrow, garden radish and carrot.

CONCLUSION

It can be safely concluded that the hydrogen peroxide seed treatment is highly effective, economical and easily applicable as it can reduce the seed-borne mycoflora, improve seed germination and vigor. Based on the findings of the present study, the treatment of chili seeds with hydrogen peroxide, regardless of concentration of H_2O_2, significantly reduced seed infestation with seed borne fungi in all types of seed sources and improved health of chilli seeds. Among the concentrations of H_2O_2, 1% H_2O_2 is more effective in increasing seed germination percentage, vigor index and percent inhibition of mycelial growth.

SIGNIFICANCE STATEMENT

This study discovers the efficacy of H_2O_2 against different mycoflora associated with chilli seeds and enhancement of seed germination and vigor. This study will help the researcher to uncover the mystery of H_2O_2 efficiency.

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