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HALOGENS, ALCOHOLS AND POTASSIUM PERMANGANATE EXTEND THE STORABILITY OF HOT PEPPER SEEDS (*CAPSICUM ANNUUM* L.) UNDER ACCELERATED AGEING CONDITIONS

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ABSTRACT

The feasibility of extending the storability of fresh hot pepper seeds, by modifying the storage atmosphere, using the respiration inhibitors and seed desiccants was examined. Halogens such as chlorine, bromine and iodine and methanol, ethanol and potassium permanganate were used for a period of 22 months under accelerated ageing conditions of high seed moisture and temperature. The various seed quality parameters – viability, vigour, speed of germination, seedling dry weight, and moisture content were evaluated. In the untreated control, decreased seed viability was observed within 10 months of storage. However, iodine and chlorine were proved effective in retaining high seed viability up to 90.7 and 88.0%, respectively, even after 22 months of storage. Chlorine treatment was able to retain the seed vigour (904.0), iodine (766.4), $KMnO_4$ (754.4) and methanol (566.7) whereas the value of vigour index in control was 72.0. Chlorine and iodine were on par in their ability to maintain the speed of seed germination (25.2 and 24.8, respectively), followed by $KMnO_4$ (20.2). Seedling dry weight was proved as a parameter not sensitive in estimating the seed quality since even after 22 months storage of seeds at atmospheres of chlorine, iodine, $KMnO_4$ and methanol did not show any differences. $KMnO_4$ sharply reduced the seed moisture content from 9.83 to 7.89% providing better storability over the control.

Key words: Accelerated ageing; *Capsicum*; germplasm conservation; halogens; modified storage atmosphere; pepper

INTRODUCTION

Ex situ conservation of genetic diversity in most of the sexually propagated crops is practised using seeds. For germplasm conservation in hot pepper, seeds are usually stored under refrigerated conditions. The basis of this is the temporary dormancy obtained by reducing the rate of respiration (Doijode 2001). The low-temperature storage is crippled by the high electricity charges, requirement of sophisticated refrigeration systems and risk from power failures.

Modified atmospheres prolong the postharvest life of perishables such as vegetables (Jacxsens et al. 2000; Daş et al. 2006), fruits (Jayas & Jeyamkondan 2002), meat (Jakobsen & Bertelsen 2000) and dairy (Floros et al. 2000). This method was applied for seeds also. Presence of halogens in storage atmosphere leads to reduced respiration rates in seeds (Rudrapal & Basu 1981), whereas alcohols affect seed desiccation. Potassium permanganate has oxidizing properties and can act as ethylene neutralizer or an antiseptic. It helped in germination of some legume seeds stored for 20 -44 years (Grauda et al., 2013). Hence, an attempt was made to examine the feasibility of modifying the storage atmosphere for freshly extracted seeds, to reduce respiration rates, leading to prolonged quality retention.

The seed quality of hot peppers generally declines after 8 months of storage under ambient conditions and with high seed moisture (Mathew 2011) and hence, if the experiment is laid for a shorter period, it will not be possible to draw the conclusive

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results. To solve this problem the duration of the experiments was extended to 22 months. Accelerated ageing is an experimentation system aimed to quicken results on the capability of different treatments to maintain the seed quality. In species forming orthodox seeds, that otherwise take many years to lose the viability and vigour, favourable conditions for accelerated ageing, such as higher humidity and high temperature, are employed to quicken the physiological activities, leading to faster deterioration. Thus, in our experiments, different treatments were compared under accelerated ageing conditions, imposed through high seed moisture (9.83%) and storage temperature of 32-36 °C. The aim of this work was experimental optimization of conditions enabling efficient germplasm conservation of hot pepper using chlorine, bromine and iodine as well as methanol, ethanol and potassium permanganate.

MATERIALS AND METHODS

The entire experiments were carried out at the Division of Plant Genetic Resources, Indian Institute of Horticultural Research, Bangalore, India. Freshly extracted seeds of hot pepper cv. MI-2 were dried to a moisture content of 9.83%. Seeds were stored for 22 months in glass desiccators with 330 cm^3 volume in the presence of various halogens (chlorine, bromine and iodine), alcohols (ethanol and methanol) or $KMnO_4$. The desiccators with halogens contained 10 g of seeds and desiccators with others chemicals, 2 g of seeds. The ambient storage temperature was 32-36 °C. The edges of the desiccators were sealed with vaseline to avoid gaseous exchange.

Potassium iodide in powder form, fuming bromine water in liquid form and commercially available bleaching powder were used as sources for iodine, bromine and chlorine. To each desiccator, 0.1 g of bleaching powder or one drop of 100% bromine water (0.015% v/v – volume of bromine water/volume of storage atmosphere within the desiccator) or 0.1 g potassium iodide powder (1% w/w – weight of potassium iodide powder/weight of seeds) was added. Methanol and ethanol were added in the concentrated liquid form at 0.06% v/v (volume of alcohol/volume of storage atmosphere within the desiccator, approximately 4 drops in each desiccator) and $KMnO_4$ powder was added at 2 g per desiccator (20% w/w – weight of potassium permanganate/weight of seeds). Seed quality evaluation was carried out every 6 months. The following tests were performed.

Seed viability was evaluated following the roll towel germination test as recommended by ISTA (1985). Fifty seeds from each treatment were sown in 5 × 10 fashion on wet crepe kraft germination paper and incubated in a germinator at 40 °C and 100% relative humidity. Numbers of germinated seeds were recorded until the 10th day.

For assessing the **seed vigour**, protocol given by Abdul-Baki and Anderson (1972) was followed. Vigour index was calculated as the mean germination percentage and mean seedling length (including roots) on the 10^{th} day of germination test. S**peed of germination** was calculated using the formula [(number of seeds germinated on the first day of initiation of germination/1) + (number of seeds germinated on the second day of initiation of germination/2) + (number of seeds germinated on the third day of initiation of germination/3) +...+ (number of seeds germinated on the last day of germination/number of the last day on which the germination was observed)], which was proposed by Maguire (1962) and Doijode (2001).

As an indirect measure of seed vigour, **dry weight** of five seedlings (dried at 60 °C in air oven for 72 h), after 10 days of sowing was also assessed. **Seed moisture** content was estimated at the high constant temperature protocol given by ISTA (1985), using tightly capped glass vials. Around 250 seeds were transferred to each vial and the weight of vial with the seeds was estimated. Vials with their caps open were incubated in air electric oven at 130 °C for 1 h and after the incubation, the capped vials were quickly weighed. The loss in weight was used to calculate the seed moisture content.

The first set of experiments has been completed during 2008-2010 and the entire set of experiments was repeated during 2009-2011. The experiments were conducted under factorial completely randomized design and in both set of experiments, treatments were replicated 5 times. In all the seed tests, 100 seeds each were used and the means from both experiment sets were used for the statistical analyses. The results were subjected to analysis of variance followed by Tukey's test for mean separation.

RESULTS AND DISCUSSION

Seed viability

A gradual decline was observed under all treatments except those under iodine and chlorine environments (Fig. 1). Though bromine is reported to be a good agent for seed storage in crops other than hot pepper (Dharmalingam 1982; Mandal & Basu 1986; Vyakaranahal 1998), in our experiments on hot pepper seeds it was detrimental probably due to too high concentration used (although it was not experimentally confirmed), leading to the complete loss of viability within 16 months of storage. The same explanation can be applied to ethanol. Under accelerated conditions in control, the viability was significantly decreased to 16.7% within the initial 10 months. Under iodine storage, there was no significant loss in viability throughout the experiment; even after 22 months of storage, 90.7% of seeds were viable. Using chlorine, seed viability has fallen from 96.6 to 88.0% only. These results provide much stronger evidence to the possible use of iodine and chlorine for long-term seed germplasm conservation, in line with the previous reports by Sengupta et al. (2005) and Wilson Jr. (1995), respectively. Using $KMnO_4$, there was no significant loss in viability (97.3 to 96.8% during the initial 10 months of storage). After that, 81.3% seed viability was maintained. Under methanol storage, there was no significant loss of viability for the initial 16 months (96.6 to 94.7%). However, a quick fall to 76.3% was observed later. Under control conditions, after 10 months of storage, a significant and continuous fall in the seed viability was recorded. This process was continued during the 16th and 22nd months of storage. After 22 months, only 52.8% of the seeds were viable.

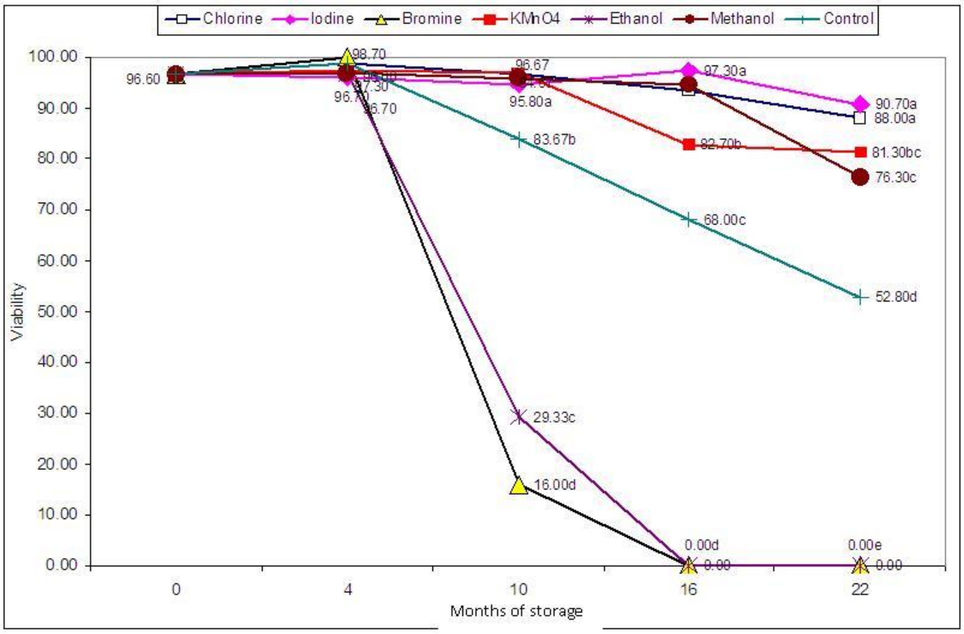

Fig. 1. Viability of hot pepper seeds cv. MI2 in percentages, stored under ambient conditions in the presence of different chemicals. Means associated with similar letters do not differ significantly at p = 0.05. In each treatment in both the experiment sets, 100 seeds each were used. The values presented in the graph are mean values over both experiment sets

All the applied treatments were on par till 4 months of storage. After 10 months, all treatments, which received optimum concentration of chemicals, were superior over the control. After 16 months, chlorine, iodine and methanol were found to be significantly superior over $KMnO_4$ and control and after 22 months, iodine was proved to be the best for seed viability retention (90.7%) followed by chlorine (88.0%), $KMnO_4$ (81.3%) and methanol (76.3%). Halogens influence the temporary entry into dormancy due to reduced respiration, thus maintaining high seed quality during storage (Mandal & Basu 1986). This is the first report on the effectiveness of methanol and potassium permanganate on the longer retention of seed viability.

Seed vigour

During storage, a gradual fall in seed vigour was observed in all treatments (Fig. 2). Within 16 months of storage, vigour of seeds treated with bromine and ethanol was lost completely. Starting from the 10th month of storage, treatments that received chlorine, iodine, $KMnO_4$ and methanol were significantly superior over control. After 22 months, chlorine was most effective for retaining the seed vigour (904.0) followed by iodine (766.4), $KMnO_4$ (754.4) and methanol (566.7). Similarly, Rudrapal and Basu (1981) and Vidyadhar and Singh (2000) observed that halogenation of mustard and maize seeds with iodine and chlorine during storage ensures higher seed vigour, and this will lead to enhanced yield (Farooq et al. 2008). Methanol acts in two ways: reduction of the seed moisture and respiration. Figure 5 shows that methanol reduced seed moisture content, although less than $KMnO_4$ and bromine. Previously, Kumar et al. (1999) reported that methanol can retain the cotton seed vigour during storage, leading to higher productivity.

Speed of germination

Over time of storage, a gradual loss in the germination speed of pepper seeds was observed in all treatments (Fig. 3). Up to 4 months, there was no significant fall in the speed of germination, except for ethanol and bromine treatments. Subsequently, a significant fall in the speed of germination has started in all the treatments, with the fastest in the control. After 16 and 22 months, chlorine, iodine, $KMnO_4$ and methanol were most effective for this parameter. After 22 months of storage, the speed of germination of seeds in the control treatment had fallen from 39.7 to 6.4.

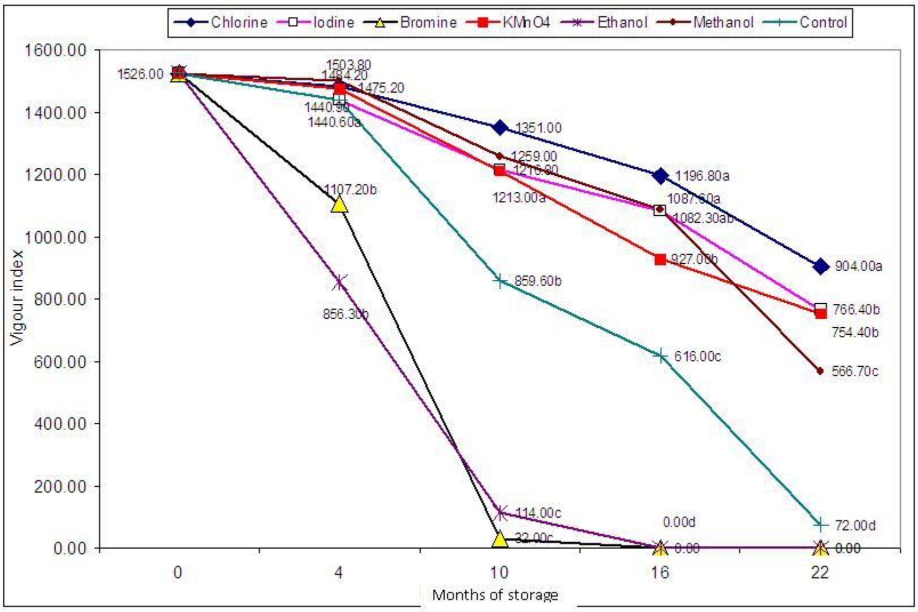

Fig. 2. Vigour index of hot pepper seeds cv. MI2, stored under ambient conditions in the presence of different chemicals. For explanation of the data, see Fig. 1

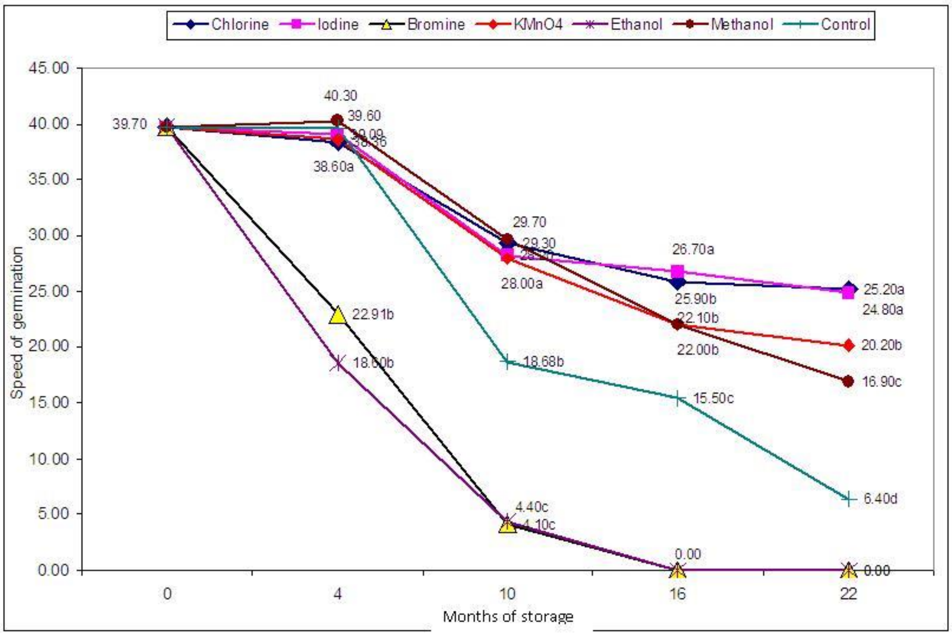

Fig. 3. Speed of germination of hot pepper seeds cv. MI2, stored under ambient conditions in the presence of different chemicals. For explanation of the data, see Fig. 1

Seedling dry weight

A comparatively slower fall in the dry weight of seedlings was observed when seeds were stored using chlorine, iodine, methanol and $KMnO_4$ (Fig. 4). In these treatments, there was no significant fall in the dry weight of seedlings even after 16 months of storage. Under control, significant loss in dry weight was observed during the 10th month of storage. During 16 and 22 months of storage, all the above-mentioned chemicals were on par in their capability to retain the dry weight of seedlings. As was detailed in the first experiment, dry weight of seedlings was proven to be a less sensitive seed quality parameter since under chlorine, iodine, $KMnO_4$ and methanol storages, this failed to show any significant difference even after 22 months of storage.

Seed moisture content

In all the chemical treatments, a general fall in seed moisture content was observed during 22 months of storage (Fig. 5). A noticeable fall has occurred only in the seeds stored using $KMnO_4$ (from 9.83 to 7.89%) and bromine (8.36%). In control, the decrease in seed moisture content was lowest (9.38%). After 22 months of storage, $KMnO_4$ has maintained a high level of viability (81.3%), vigour (754.4), speed of germination (20.2) and seedling dry weight (8.01 mg). It is evident from this study that the mode of action of $KMnO_4$ may be connected with the remarkable fall in the seed moisture content. The reduced seed moisture content during storage is proven to reduce the respiration rates and thus the catabolic processes within the seed (Benech-Arnold & Sanchez 2004). Doijode (1995) observed that viability of onion seeds could be retained comparatively longer by storing with seed desiccant silica gel.

Chlorine and iodine have the potential to retain the seed quality (Mandal & Basu 1986; Bhattacharya & Basu 1990). This points to the fact that halogen treatment could be an alternative for deep freezing and should be regarded as the best methodology since it demands for lesser input cost including electric charges (Dharmalingam 1982). Bromine and ethanol were probably used here at a very high concentration.

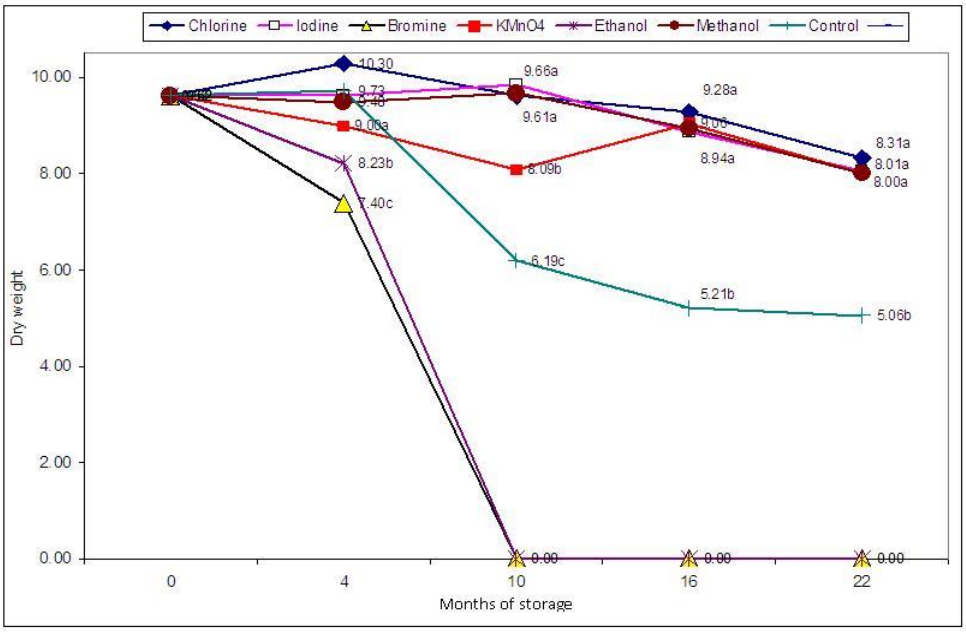

Fig. 4. Dry weight (in mg) of five randomly selected hot pepper seedlings cv. MI2, measured after 10 days of sowing (mg) obtained from the seeds stored under ambient conditions in the presence of different chemicals. For explanation of the data, see Fig. 1

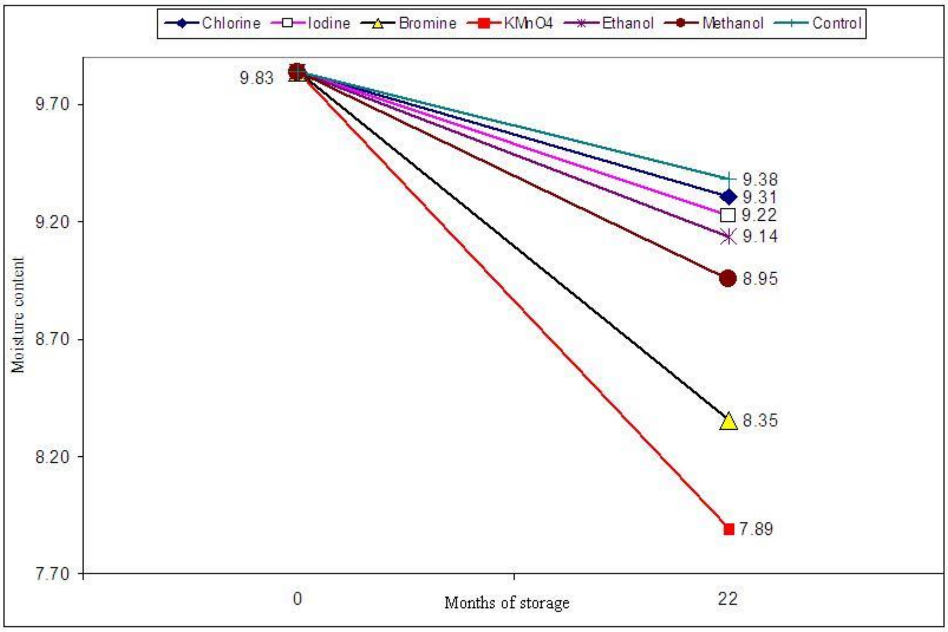

Fig. 5. Moisture content (in %) of hot pepper seeds cv. MI2, stored under ambient conditions in the presence of different chemicals

CONCLUSION

A simple and cost-effective method for medium-term seed germplasm conservation of hot pepper was established through modified atmospheric storage with chlorine, iodine, alcohols or $KMnO_4$. Under accelerated ageing conditions of high temperature and seed moisture content, seed quality parameters such as viability and vigour of seeds, speed of germination, dry weight and moisture content were studied for 22 months. Iodine and chlorine were found best to retain the seed viability, followed by $KMnO_4$. Seed vigour was the highest when seeds were stored with chlorine, followed by iodine and $KMnO_4$. Chlorine and iodine were on par in maintaining the speed of germination of seeds. $KMnO_4$ has reduced seed moisture, thus leading to reduced respiration-dependent catabolic processes those lead to seed deterioration. Among the studied experimental treatments, chlorine and iodine at 1% w/w (weight of powder/weight of seed) are recommended for seed germplasm conservation.

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