

INFLUENCE OF CHEMICALS AND CRUDE PLANT MATERIALS AS PRE-STORAGE TREATMENT ON SEED QUALITY OF ONION

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Abstract

Fresh onion seeds dried to 7.0% seed moisture content were stored with crude plant materials (red chili powder, neem leaf powder or lemon leaf powder each @ 20g/kg seed), and chemicals (common bleaching powder and mancozeb each @ 2g/kg of seed). The germination potential of onion seeds was found satisfactory in every treatment. Water uptake during imbibition was maximum in lemon leaf treated seed which indicates better germination as the imbibition of water is an essential part of germination. There was high correlation between EC measurements and germination indicated that conductivity readings have the potential to provide a rapid assessment of standardizing laboratory germination. In terms of seed-associated pathogens during storage, chemicals have shown better results in suppressing pathogens.

Keywords: Crude plant materials, Neem leaf, lemon leaf, Quality, Seed germination

Introduction

Seed vigor consists of those properties, which conclude the potential for rapid uniform emergence and development of normal seedlings under a wide range of field conditions. The main factors affecting seed storage capability are the content of high temperature, ambient relative humidity, and seed moisture (Abdul-Baki, 1980). The loss of viability in onion seed is very fast, normally within a year (Singh and Bhonde, 2003). The onion (*Allium cepa* L.) is a crop of major economic and dietary importance in all parts of the world. Compared with many other crops, the onion has a fairly complex life cycle involving several distinct development phases. It is generally known that onion seed is one of the shortest-lived seeds of the common vegetable crops (Angel *et al.*, 2014) rapidly losing viability after harvest unless special precautions are taken in its storage. It is therefore generally recommended that only fresh onion seed should be used for crop production (Riekels *et al.*, 1976) and only seeds of high germination percentage should be sold. The percentage and rate of germination of onion seeds also vary considerably among seed lots (Bedford & MacKay, 1973) and this leads to difficulties in establishing optimum plant populations in the field. It has long been known that the factors, which

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have the greatest influence on the longevity of seeds in storage are moisture, temperature, and oxygen partial pressure. It is usually agreed that the moisture content of the atmosphere is the most critical factor, with a rise in air moisture being more damaging than rising temperatures. However, it has been recommended that dry and very dry seeds should be humidified before germination to raise their moisture contents slowly in the initial stages (Powell & Matthews, 1979; Ellis *et al.*, 1985 a & b). Several other workers have also attempted to store the onion seeds under different storage conditions for different periods (Caneppele *et al.*, 1995; Pandey, 1996; Stumof *et al.*, 1997; Yanping *et al.*, 2000). Therefore, it becomes necessary to evaluate the physiological quality of commercially available onion (*Allium cepa* L.) seed stored for different periods for proper crop stand and performance in the field. In the present study, the experiment was conducted to find out possible way to improve the storability of onion seeds by using crude plant materials and chemicals as a pre-storage seed treatment.

Materials and Methods

Seed source and storage

Seeds of onion (*Allium cepa* L.) variety BARI Peaj-1 was obtained in April 2020 from the research field of Seed Technology Division, Bangladesh Agricultural Research Institute, Gazipur. After collection, seeds were cleaned and dried in the sunlight for 5-6 days to a moisture content of 7.0 % for safe storage. Seeds were then stored in the 500ml capacity rubber stopper glass bottles under ambient conditions in the laboratory till seed invigoration treatment. After cleaning and drying seeds were divided into six lots for Pre-storage seed invigoration treatments. After treatments seeds were stored in a 100 ml. capacity test tube, each containing 50 g seeds. The test tubes were then air-tied primarily with aluminum foil paper and covered again with polythene to control moisture absorption of the treated seed. Onion seeds were dry-dressed with three crude plant material, viz. neem leaf powder (*Azadirachta indica* L.) at 20g/kg of seed, red chili powder (*Capsicum frutescens* L) at 20g/kg of seed, lemon leaf powder (*Citrus limon* L.) at 20g/kg of seed, and two chemicals viz., mancozeb at 2 g/kg and bleaching powder at 2g/kg of seed in an airtight test tube at room temperature. After treatment, test tubes were shaken twice a day for up to 7 days for thorough mixing of crude plant materials and chemicals with the seeds, and the test tubes were kept in the laboratory under ambient conditions (25-30⁰ C).

Determination of seed moisture content

Three replicate samples, each of 1000 seeds were taken and weighed (M₂), evenly spaced in 90 mm glass Petri dishes and placed in an oven at 103±2°C for 17 ± 1 h, cooled in a silica gel container for 15–30 min, after which they were reweighed (M₃). The moisture content was expressed as a percentage of their wet weight in accordance with the International Rules for Seed Testing (ISTA, 1985) and calculated as:

$$\text{Percentage moisture content (\% MC.)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

Where, M₁ is the weight of the dish, M₂ is the weight of the dish and its contents before drying and M₃ is the weight of the dish and its contents after drying.

Water uptake during the imbibition period

Seed samples were counted, weighed (W_2), and then imbibed in Petri dishes on double sheets of filter paper moistened with 10 ml of distilled water in ambient conditions at $25\pm 2^\circ\text{C}$. Dishes were removed from the series at intervals, and the seeds were drained and surface water removed by blotting between sheets of paper towel. The seeds were then immediately weighed (W_1), and the percentages of water taken up were recorded and calculated as:

$$\text{Percentage water uptake} = \frac{W_1 - W_2}{W_2} \times 100$$

Where,

W_1 is the weight of the seeds after imbibition and

W_2 is the weight of seeds before imbibition.

Germination test

The germination potential of the onion seeds was estimated (ISTA, 1985). Germination percentages, using four replicates of 100 seeds, were determined by placing the seed samples in 120 mm Petri dishes on filter papers (Whatman No. 1) moistened with 3 ml of distilled water. Seeds were distributed evenly within each dish. Petri dishes were covered with their lids and then placed in a germination room, maintained at $20\pm 2^\circ\text{C}$ temperature. Each Petri dish was watered daily with an amount of distilled water according to its requirement. The first counting of germination was recorded on the 6th day and the final counting was recorded on the 12th day. The germination percentage was recorded by the following formula

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds placed for germination}} \times 100$$

The following recognizable abnormalities of onion seedlings were recorded as abnormal seedling, as listed in Section 5.8.2 (ISTA, 1966).

Ia - no primary root

Ib - primary root short, stunted and weak or spindly

IVc - poorly developed leaf-like cotyledon without a definite bend or "knee"

Va - decayed cotyledon

Ve - decayed primary root

Vg - completely decayed seedling (an additional category to those in Section 5.8.1)

VIa - short and weak, or spindly, or watery seedling

Electrolyte leakage

Solute leakage of treated seeds was estimated by placing triplicates of 2 g seeds each in 20 ml of distilled water for 12 h at 25°C . Electrical conductivity of the medium was measured with a conductivity meter (Model EC-407L, NeoMet., USA).

Tetrazolium viability test

1g of 2,3, 5-triphenyl tetrazolium chloride (TTC) was dissolved in 100 ml of distilled water to make a 1% solution of the tetrazolium salt. The test was conducted with

two replicates of 100 seeds soaked in distilled water for 18 - 20 h. Each seed was cut longitudinally without completely separating the two halves. The seeds were submerged in 1% TTC solution for at least 8 h at 25°C in darkness, after which the staining patterns were recorded.

Seed health

A total of six different treatments (viz. T₁= lemon leaf powder, T₂= neem leaf powder, T₃= red chili powder, T₄= mixture of T₁, T₂ & T₃, T₅= bleaching powder, T₆= Mancozeb) along with control were applied to assess the quality of onion seeds in storage. The prevalence of seed-associated pathogens was identified in two different ways; a) growing treated seeds in PDA plates, b) growing untreated seeds in PDA plates prepared with treatment elements. Pathogens were observed after 72 hours of incubation at 25°C. The prevalence of pathogens was documented from different treatments.

Results and Discussion

Moisture content of seeds after storage

The initial moisture of the seed was 7.0%. Seeds were stored after harvesting for up to the next growing season for 8 months.

Replicate samples of the onion seeds of different treatments were withdrawn from the test tube in which they had been stored. These were weighed, and their water contents were determined on a dry-weight basis (ISTA, 1985). The lowest moisture content (7.24%) was recorded when the seed was treated with lemon leaf powder which was probably due to the high absorption ability of lemon leaf powder from the air and the highest moisture content (9.17%) was recorded when the seed treated with Mancozeb which was statistically identical to the moisture content of seeds treated with red chili powder (9.13%). The moisture content of the other seed treatments varied from 7.43-7.97% (Table 1).

Moisture content and water uptake during imbibition

Replicate samples of onion seeds were tested for their moisture contents and the amounts of water required for full imbibition were determined. The results presented in Table 1, showed that the seed moisture content after imbibition differs among the seed lots tested. However, the percentages of water taken up did not differ depending upon the seed lot (different seed treatments). Water uptake during imbibition was maximum (50.89%) in lemon leaf treated seed, which contained the lowest moisture content before imbibition. The minimum water uptake (44.08%) was recorded in the untreated seed. Treatments and initial moisture content (after storage) of seeds may cause differences in the process of imbibition, and the amount of water required for the initiation of metabolism and germination. The imbibition of water into the dry seed is an essential part of germination; which has also been documented as a potentially hazardous period (Woodstock, 1988). The imbibition of water converts the seed from a quiescent body with a very low or non-detectable respiratory rate into a metabolizing organism, active in respiration and in biosynthesis, and capable of growth (Mayer & Poljakoff-Mayber, 1974). The amounts of water taken up by the seeds during imbibition prior to germination

in the present study appeared within the expected range depending on their initial moisture contents (Table 1).

1000 seed weight before and after imbibition

1000 seed weight before and after imbibition were not varied significantly among the treatments. The lowest (2.996 g) 1000 seed weight before imbibition was recorded in mixture of leaves and the highest (3.047 g) was recorded in bleaching powder treatment. The lowest (4.328g) 1000 seed weight after imbibition were recorded in untreated seed and the highest (4.568g) was recorded in neem leaf powder treatment (Table 1).

Table 1. Initial moisture content and extra water are taken up during imbibition of onion seeds of different treatments stored at ambient conditions

Pre-storage seed invigoration treatment	% moisture before imbibition	% moisture after imbibition	Water uptake (%)	1000 seed wt. (g) before imbibition	1000 seed wt. (g) after imbibition
Bleaching Powder (T1)	7.97	38.01	48.526	3.0477	4.525
Mancozeb (T2)	9.17	39.613	49.511	3.021	4.515
Red Chilli Powder (T3)	9.13	39.99	46.785	3.046	4.4713
Neem Leaf Powder (T4)	7.57	39.707	49.205	3.062	4.5687
Lemon Leaf Powder (T5)	7.24	42.74	50.892	3.0207	4.558
Mixture of leaves (T6)	7.43	38.233	47.843	2.9967	4.4303
Control (Untreated) (T7)	7.92	37.703	44.087	3.005	4.328
LSD 5%	* 0.57	* 1.96	NS	NS	NS
CV (%)	4.05	2.85	11.61	1.41	3.42

* Significant at 5% level; NS= Not significant. *Values are the means of four replicates, each of 1000 seeds (Initial moisture of seed during storage was 7.0%)

Seed viability

Samples of the seeds from the different treatments available were subjected to the tetrazolium test. The seed samples tested showed good viability. The highest viability was recorded when the seeds were treated chemically (97% and 96% viability in bleaching powder and Mancozeb respectively) (Table 2) The tetrazolium test is a biochemical test, which differentiates the living and dead tissues of seed by the presence or absence of a red stain known as formazan. A germination test can give an apparently erroneous result because some seeds may be in a state of dormancy, but the tetrazolium test includes all the seeds, which are alive either active or dormant (Porter *et al.*, 1947).



Fig. 1. Different stained condition of onion seed after viability test

Germination test (Normal seedling, abnormal seedling, and dormancy)

Laboratory germination tests are performed in optimum conditions, but field conditions are seldom optimal. Some indications of the probable performance of a seed lot in the field may be obtained by subjecting the seeds to some degree of stress before or during the laboratory germination period. A comparison of germination percentages in Tables II shows that seed from all treatments had slightly changed their relative positions (initial germination percentage before storage was 99%) with the best performance in the germination test (86%) shown in the lemon leaf powder treated seed. Seeds treated with neem leaf powder, a mixture of leaves, and bleaching powder were in the middle, and seeds stored with mancozeb and without any treatment gave the poorest performance. Seedling abnormalities vary slightly with the treatments. The highest abnormalities were recorded in the seedlings resulting from treatment T6 (mixture of leaves) and the lowest in the T4 treatment (Mancozeb). Abnormalities were similar to those from all other treatments. Considering the level of dormancy chemically treated seeds showed the highest dormant condition (17% and 16% in bleaching powder and Mancozeb, respectively). This might be due to some chemical interactions between the treatments along with the seed. Seed treated with lemon leaf powder and neem leaf powder resulted in the lowest (5% and 6%, respectively) dormant condition of the seed (Table 2).

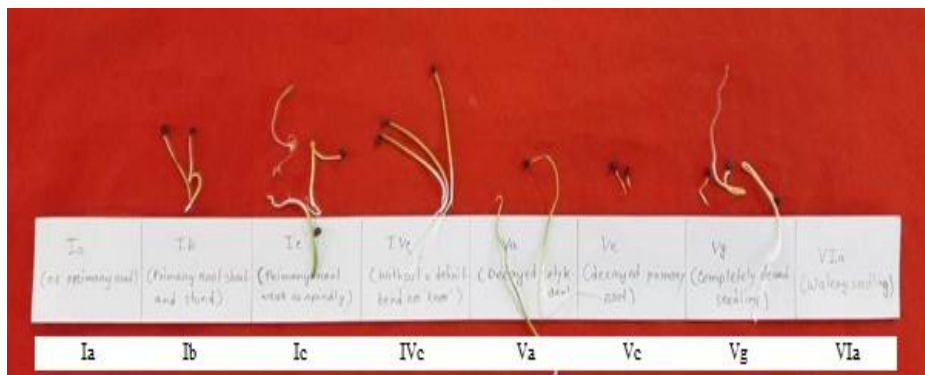


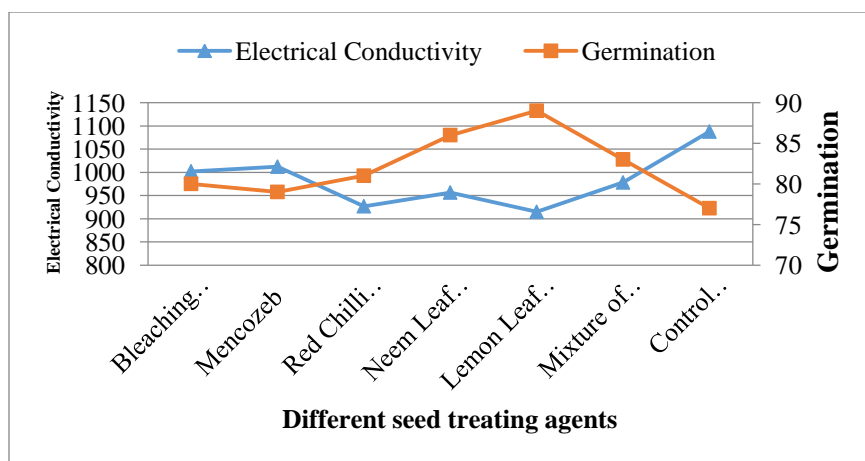
Fig. 2. Different types of abnormal seedlings of onion that were found at the period of last counting days of germination test

Table 2. Germination potential after twelve days of germination

Pre-storage seed invigoration treatment	Viability (tetrazolium test)	Number of Normal Seedling	Number of abnormal Seedling	Dormancy
Bleaching Powder	97	74	6	17
Mancozeb	96	77	3	16
Red Chilli Powder	90	75	5	10
Neem Leaf Powder	92	80	6	6
Lemon Leaf Powder	94	83	6	5
Mixture of leaves	90	75	8	7
Control (Untreated)	90	72	5	13
LSD 5%	6	5	3	2
CV (%)	3.66	3.41	27.57	9.86

*Fig. are the means of four replicates of 100 seeds for germination test, and two replicates of 50 seeds each for tetrazolium test.

The close relationship between the EC of different treatment's germination percentages was illustrated in Fig. 3. The highest ($1087\mu\text{s}/\text{cm}$) solute leakage was recorded in the untreated seed which indicates the lowest germination percentage which was statistically identical with the chemically treated seeds. And the lowest ($914\mu\text{s}/\text{cm}$) solute leakage was recorded in Lemon leaf powder treatment (T5) which was statistically identical with all the treated seeds of crude plant materials. (Fig. 3). The relation between solute leakage and normal germination percentages was highly negative in all treatments. This shows that conductivity detects that the germination differences among the treatments.

**Fig. 3.** Relationship between electrical conductivity and germination percentage

We measured the leakage of all treated seed samples measuring the overall mean of all seed leakage. Obviously, higher solute leakage would occur in samples with a high proportion of dead seeds (low germination percentages). Demir *et al.* (2008) and Mavi *et al.* (2016) were also reported that the average conductivity reading per gram seed weight in the bulk conductivity method is successful. A high correlation between EC measurements, germination, and dead seeds indicates that conductivity readings have the potential to provide a rapid assessment of standard laboratory germination. Information about the standard germination percentages of any lot can be obtained within 24 hours, compared to the 12-day germination test in this work for cress. It is easy to correlate directly germination with solute leakage and germination with the tetrazolium test. But it is highly difficult to correlate these three parameters. In this experiment, a high EC value was found in chemically treated seeds which indicates a low germination percentage, more precisely which indicates more dead seeds. But in the tetrazolium salt test, these two treated seeds showed high value, which indicates having minimum dead seeds. So it can be concluded like that, EC test will provide an assessment of germination but cannot provide the assessment of dead seeds.

Conclusion

Considering the experimental results, it could be concluded that pre-storage seed treatment with lemon leaf powder is an appropriate corrective measure to be chalked out to maintain the germination potential of onion seeds during storage.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this paper.

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