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Short Communication

Effect of seed treatments on the seed health status of onion seeds during storage

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Onion seed has been regarded as having a short storage life amongst the vegetable crops; reported between 6 to 24 months under normal conditions by various workers; the seeds cannot be stored safely for longer periods as it loses its viability very fast, normally within a year and thus, poses a serious problem for the carry over stock [1-3]. The longevity of seeds of several species was studied under varied atmospheric conditions and onion seeds were found to be poorest keepers [4-5]. Several important factors affect the shelf life of the seeds namely; seed moisture content and storage environment, which increase the rate of seed deterioration and ultimately results in reduction and loss of viability. Seeds are referred to as 'microcosm of microbes', which have the potential to carry a wide variety of pests and pathogens including fungi, bacteria and nematodes. High storage temperatures, high seed moisture or relative humidity and pre-storage infection are congenial for the growth and development of these micro-organisms.

Many of these pathogens can affect the quality of the seeds, which is the major deciding factor in achieving the production targets and cause diseases in seedlings developing from such infected/contaminated seeds [6-7]. Amongst the various pests and pathogens found in/on the seeds, fungi predominate which can be divided into two groups viz., field fungi and storage fungi. Field fungi invade the seeds during development or after physiological maturity in the field. These include mainly species of *Alternaria, Cladosporium, Drechslera, Fusarium* and *Helimentho sporium*. In contrast to field fungi, the storage fungi invade the seeds under conditions that are encountered during storage. Storage fungi, including species of *Aspergillus* and *Penicillium* are mainly responsible for loss in germination and vigour, discolouration and production of mycotoxins, resulting in the deterioration of seed quality [8-10]. These fungi reduce the seed germination, develop heat in seed lots and cause discoloration of seeds. A greater number of colonies of mould fungi were observed at relative humidity above 70 percent and at 20^{0}C storage [11]. It was noted that fungi cannot develop on seeds if the relative humidity of the air is less than 65 percent [12]. The different species of fungi like *Aspergillus niger, A. flavus, A. ochraceus, Penicillium cyclopium, Curvularia lunata, Drechslera australiensis, Fusarium* spp., *Alternaria alternata, Stachybotrys atra, Chaetomium globosum* and *Rhizopus stolonifer* were isolated from onion seeds [13]. Also, the infestation with seed borne fungi during storage is one of the major factors for quick loss of viability. The reduction in percentage of germination and vigour is attributed to production of fungal metabolites in onion seed storage [14]. The effect of fungi on vegetable crop seeds in conditions of increased relative humidity and temperature was studied and it was concluded that the presence of fungi accelerated the process of seed damage [15].

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Seed treatments provide economical and relatively non-polluting delivery systems as compared to other field application systems because only a small amount of material is applied per hectare and in the immediate contact with the target site [16]. Therefore, it has been considered one of the least expensive, safe and most effective means of controlling many plant diseases. It has been established that fungicidal seed treatments helps not only in controlling the seed borne fungi, but also enhances the germinability and longevity of the seed in soybean [17-18] and onion [19-20]. The superiority of moisture impervious packaging for the seed storage is well established [21-22]. Though there are numerous reports regarding the effect of seed treatments on germination and vigour parameters, there is limited literature available related to seed health status vis-a-vis seed treatments during storage. In view of above, it was envisaged to undertake comprehensive investigations to analyze the seed health status of onion seeds treated with fungicides, botanicals and bio-agents during storage under different packaging materials and storage environments.

The seeds of onion cultivar Agrifound Dark Red were used as the experimental material and the seeds were obtained from three locations *viz.* Indore, Rajkot and Lasargaon. The initial seed quality parameters were investigated and depending upon the germination percentage and incidence of mycoflora, the seed lot from Rajkot was selected for further studies. The seed quality parameters recorded were; moisture content (9.53 percent), germination (89.5 percent), seedling length (13.58 mm), seedling dry weight (20.90 mg), whereas Vigour index I and II values were 1219 and 1876 respectively. The seeds were dried to safe moisture level of 6 percent and subjected to seed coating (with/without polymer coating) of Vitavax, Thiram, neem oil, *Trichoderma harzianum* and *Kalisena* and seed priming (GA_3 @ 100 ppm). Proper controls were maintained in each case. The polymer coating was done using the polymer, polykote procured from M/s Chemtura, The coated as well as uncoated seeds were packed in cloth bags (P-58 Pathanchhap Phagwara mill cloth) and vacuum sealed aluminum foil packets (12 micron outer layer of polyester; 12 micron middle layer of Aluminum foil and 250 gauge inner layer of polyethylene) and kept under two storage conditions viz., Low Temperature (15 ± 1^0C) - Low Humidity (30 ± 5%) condition (LTLH) as well as under ambient conditions for a period of one year. The samples were drawn and evaluated for seed health at an interval of 3 monthly intervals. The incidence of mycoflora associated with seeds was determined by following deep freeze method, a modification of Standard Blotter Method [23]. For each seed sample, four hundred seeds were used. Twenty five seeds per plate were placed on three well moistened blotters kept in plastic Petriplate of 10 cm diameter. These plates were incubated at 20 ± 1^0C for 24 h. and subsequently transferred to -20^0C for 24 h, followed by incubation at 20 ± 1^0C under alternating cycle of 12 h of darkness and 12 h light (Near Ultra Voilet) for five days. After incubation, the seeds were examined under stereo binocular microscope and compound microscope for the presence of seed borne fungi, which were identified with the help of relevant keys. The total number of seeds infected by specific seed borne fungus was recorded to determine percent incidence of seed borne fungi.

The effect of seed treatments on the percent incidence of fungi was studied in onion seeds during storage for one year and a total of 11 different fungi belonging to ten genera were recorded on the untreated seeds. However, the predominant ones included *Alternaria alternata* (Fr.) Keissler, *Fusarium moniliforme* Scheldon, *F. semitectum* Berk. & Rav., *Cladosporium cladosporioides* Penz.,

Curvularia lunata (Wakker) Boedjin, *Aspergillus flavus* Link ex Fries and *Penicillium spp.* Hence, the results are presented for these fungi only. The other fungi detected were *Rhizopus stolonifer, Stemphylium vesicarium, Botrytis alli, Epicoccum* spp. and *Memmloniela* spp., which occurred in very low frequency. Hence, the results have been presented for the predominating fungi only. Prior to seed drying and treatment, initial seed infestation with fungi was observed to be 23.25 percent; the maximum incidence was recorded for *Alternaria alternata* (5.5%), followed by *Fusarium moniliforme* (5.0%) *Cladosporium cladosporioides* (3.75%) and *A. niger* (3.25%). The results in table 1 reveal that the seed treatments were effective in reducing or eliminating the seed borne fungi. The slight reduction in seed borne fungi in untreated seeds may be attributed to the reduction in moisture content. Amongst the treatments, Thiram and Vitavax were superior as compared to other treatments. It has been observed that most of the fungi associated with onion seeds could be controlled by seed treatment with Vitavax (Carboxin) [19]. Fungicidal seed treatment has been established as an inexpensive method for disease control that can protect not only seedlings against a variety of fungal pathogens and but also improve field emergence [6] The bio-agents, Kalisena and *Trichoderma* also gave excellent control of *Fusarium* spp. and Kalisena was especially effective in controlling *A. flavus*. Similar results were obtained in earlier studies [24] and Kalisena was found to control the population of *A. flavus*. Use of antagonists like *Trichoderma harzianum, Aspergillus niger, Fusarium oxysporum and Bipolaris sorokiniana* have been reported to control seed-borne fungi of rice [25-26].

Moreover, the efficacy of bio-agents was further improved as a result of polymer coating in the present investigations. However, neem oil (T_4) and seed priming (T_7) treatments were not found effective. In fact, priming treatment was observed to favor the growth of fungi during initial storage and subsequently declined, thereafter. The deterioration in the health status of onion seeds after priming has been observed by previous workers [27-28]. The incidence of *Alternaria alternata* was maximum (2.41 percent), which increased gradually to 6.13 percent in cloth bags and 1.89 percent in aluminum foil packaging under ambient storage. Some of the fungi like *Alternaria alternata* and *A. niger* were present on the seeds before storage and were observed till the end of storage period. However, some of the fungi like *Fusarium* spp., *Curvularia lunata* and *Cladosporium cladosporioides* could not be detected after 9 months of storage. *Asperillus flavus* and *Penicillium spp* were not present initially and appeared during the subsequent storage and persisted even after 12 months of storage. The effect of storage containers as well as storage environment had profound impact on the incidence of seed borne fungi.

In general,-less number of fungi was recorded in case of seeds stored in aluminum foil packets as compared to the cloth bags. Also, the seeds kept under ambient conditions were associated with higher incidence as compared to those under LTLH conditions This can be explained on the basis of studies that storage fungi develop at higher relative humidity and hence the higher moisture content provided favourable conditions for the growth of storage fungi in moisture pervious containers under ambient conditions [29]. The storage fungi affect the seeds adversely through the production of toxic metabolites [8].

Further, these seeds were found completely free from seed borne pathogens throughout the storage period. It was revealed that seeds stored in polyethylene bags could maintain germinability above the certification standards, irrespective of the

Table 1. Effect of seed treatments, storage environments and packaging materials on the seed health status of onion.

Seed Treatment	Period of storage (months)															
	Ambient conditions								LTLH							
	CB					AF			CB				AF			
	0	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12
T_1	18.25	35.75	45.25	16.0	21.75	15.25	14.5	5.0	3.5	21.25	18.25	8.0	5.25	11.0	7.25	2.5	1.5
T_2	4.25	3.5	2.25	1.25	0.5	2.0	0	0	0	2.75	0.5	0	0	1.0	0	0	0
T_3	2.0	3.25	2.75	2.75	1.5	1.75	0	0	0	2	0	0	0	0.5	0	0	0
T_4	16.0	36.5	48.25	21.75	38.75	14.75	18.5	8.25	8.0	18.25	23.0	18.5	21.0	10.5	8.25	6.0	4.75
T_5	7.75	9.75	10.25	7.0	8.75	4.25	5.25	2.0	1.75	6.25	5.25	5.25	3.25	2.0	0.5	0	0
T_6	3.0	7.25	5.25	5.0	4.25	2.5	1.0	0	0	4.0	2.5	0	0	0.25	0	0	0
T_7	12.5	24.75	30.25	13.25	20.75	10.0	8.75	1.75	2.5	9.25	9.0	6.0	3.0	7.5	4.25	2.0	1.25
T_8	6.25	13.25	16.75	9.5	11.5	4.5	4.25	2.25	1.5	4.75	5.0	6.5	5.25	4.25	1.25	0.5	0
T_9	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T_{10}	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T_{11}	14.5	20.0	25.75	9.5	16.25	11.25	20.0	5.5	4.25	16	11.5	9.25	11.75	5.5	6.0	5.25	6.0
T_{12}	1.5	4.75	6.5	4.5	2.25	0.5	0	0	0	3.75	2.5	0.75	1.0	0	0	0	0
T_{13}	1.75	1.75	2.5	4.25	3.25	0	0	0	0	1.5	0.75	0	0	0	0	0	0
T_{14}	6.25	14.25	10.0	6.25	13.5	6.0	4.25	4.75	5.0	6.0	5.25	4.5	1.25	4.75	2.5	0.75	1.0

T_1-Untreated Control T_2-Vitavax @ 2.5 g/Kg seed T_3-Thiram@ 3.0 ml//Kg seed T_4-Neem oil (10.0ml/Kg seed)
T_5-Apergillus niger (Kalisena) @ 4 g/Kg seed T_6-Trichoderma harzianum @ 4 g/Kg seed T_7-Primed seeds
T_8-Polymer alone T_9-Vitavax @ 2.5 g/Kg seed + Polymer T_{10}-Thiram @ 3.0 ml//Kg seed + Polymer

T_{11}-Neem oil (10.0ml/Kg seed) + Polymer T_{12}-Apergillus niger (Kalisena)@ 4 g/Kg seed+ Polymer

T_{13}-Trichoderma harzianum @ 4 g/Kg seed+ Polymer T_{14}- Primed seeds+ Polymer

chemical treatments [21]. The packing of seeds in polyjars and storing at room temperature or packing in poly bags and storing in deep freezer, after treating with Thiram was found effective for fungal free storage of onion seeds for two years [22].

It is evident from the present investigations that polymer coating with fungicides and bio-agents were effective for the maintenance of seed quality during storage of onion seeds. Hence, onion seeds should be dried to a moisture content below 6 percent and treated with Vitavax/ TMTD @ 0.25 percent or *Trichoderma harzianum* @ 0.4 percent, followed by storage in moisture impervious containers like a Alumninum foil packets at 15^{0}C temperatures and 30% RH for maintain seed health status during storage.

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