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# Studies on Seed Longevity of Pigeonpea (*Cajanus cajan* L.) and Chickpea (*Cicer arietinum* L.) under Ambient Storage Condition

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## ABSTRACT

**Background:** Pulses are very crucial to meet the requirements of dietary proteins in the human diet especially for the predominantly vegetarian population in India. Pigeonpea [*Cajanus cajan* (L.) Millsp.] and chickpea (*Cicer arietinum* L.) are important pulses grown in India. The seeds of these crops are highly vulnerable to various microorganisms, both in the field and during storage, which play an important role in reducing the seed viability, germination and vigour causing considerable loss in yield and quality. There is no suitable recommendation available to maintain seed quality during storage of pulses, hence present study was undertaken.

**Methods:** Seeds of pigeonpea and chickpea were treated with Captan and Carbendazim 50% WP fungicides @ 2.0 g/Kg of seed and stored under ambient condition. The samples were drawn at three months interval to assess the effect of storage on seed quality parameters. The vigour index I, seed germination and speed of germination of these seed samples were determined using ISTA rules. The detection of seed mycoflora in pigeonpea and chickpea seed samples was carried out by the blotter test as recommended by the International Seed Testing Association.

**Result:** Total sixteen fungal species were recorded on stored seeds. Maximum incidence of *Aspergillus fumigatus* (19.83%), *A. flavus* (14.88%), *A. niger* (12.40%), *Alternaria alternata* (11.57%), *Fusarium oxysporum* (8.26%), *Rhizopus stolonifer* (7.44%) and *Penicillium notatum* (5.79%) were recorded on untreated seed of pigeonpea cv. P. 2001 after 18 months of storage. The per cent germination, speed of germination and vigour index of the stored seeds decreased with the increase in the storage period whereas the total number of fungi increased with the storage period, irrespective of treatments. Among the tested cultivars, germination in pigeonpea (cv. P. 991) remained above IMSCS (>75%) upto 18 months of storage under ambient storage condition. However, the germination of pigeonpea cv. P. 2001 seed could be maintained above IMSCS (>75%) up to 12 months only. In chickpea (local cultivar) seed, the germination remained above IMSCS (>85%) up to 9 months only under ambient storage, however seed treatment with either Captan or Carbendazim could prolong the longevity upto 12 months of storage.


**Key words:** Chickpea, Mycoflora, Pigeonpea, Pulses, Seed quality, Seedling vigour, Storage.

## INTRODUCTION

Pulses are one of the important group of crops in cropping systems of several developing countries in Asia, Africa and Latin America. Pulses constitute a rich source of dietary proteins in the human diet providing essential amino acids to predominantly vegetarian population in India. These are excellent source of proteins (20-25%) and carbohydrates (50-60%) and fairly good source of thiamin, niacin, calcium and iron (Jadon *et al*., 2020). In addition, pulses contain other essential nutrients such as calcium, iron and lysine that help the body fight vitamin and mineral deficiencies and diseases. India is the largest producer of pulses contributing approximately 25% of the world pulses production. In 2017-18, India produced 25.42 million tonnes total pulses from 29.81 million hectares area with 853 kg/ha average pulse production (Anonymous, 2019). However, our country is also biggest importer of the pulses, due to low productivity and losses caused by biotic and abiotic stresses. In most of the tropical countries one fifth of the harvested pulses are stored at large scale in warehouses in urban area and bulk of produce is retained by farmers in rural areas (Srivastava and Sagar, 2016).

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In addition to their nutritional importance, pulses being leguminous crops also contribute to soil fertility due to their nitrogen-fixing ability and thereby may help in reducing usage of chemical fertilizers in subsequent crops (Stagnari *et al*., 2017). Thus, pulse crops can potentially help to improve health and nutrition, reduce poverty and hunger and enhance ecosystem resilience (Jadon *et al*., 2020). Realizing the importance of pulses in the human diet, 2016 was celebrated as the International Year of Pulses (IYP) by FAO. To meet the growing demand and to raise per capita

availability of pulses, countries made efforts to increase production and explore trade opportunities to augment domestic supply.

Pigeonpea [*Cajanus cajan* (L.) Millsp.] and chickpea (*Cicer arietinum* L.) are important pulses grown in India. The seeds of these crops are highly vulnerable to various microorganisms, both in the fields and during storage, which play an important role in reducing the seed viability, germination and vigour causing considerable loss in yield and quality. Among seed microflora, fungi constitute an important group of microorganisms responsible for causing seed deterioration (Kumar *et al*., 2020; Kumar and Gupta, 2020). Pigeonpea and chickpea seeds loose viability at faster rate due to improper storage and exhibit poor germination and plant growth after sowing in next crop season. Not much information is available to maintain seed quality during storage of pulses. Thus, two major pulses crop seeds *viz.,* chickpea and pigeonpea, were selected to study their health status and also to assess the effect of storage period and seed treatments on seed longevity under ambient storage condition.

## MATERIALS AND METHODS

Seeds of two major pulse crops *viz.,* pigeonpea (cvs. P-991 and P-2001) and chickpea (local variety) were treated with fungicides *viz.,* Captan (2.0 g/kg seed) and Carbendazim 50% WP (2.0 g/kg seed). Prior to these fungicidal seed treatments, all the seeds including fungicide untreated seed were treated with deltamethrin insecticide to avoid insects' infestation especially bruchids infestation (*Callosobruchus* spp.) in the seeds stored under ambient condition. 4 ml of Deltamethrin was prepared in 500 ml of water and 5 ml of this solution was used to mix in 1.0 Kg of seed. The untreated seed (without fungicide) was used as control to assess the effect of these fungicides on seed during storage. The research was conducted during 2013-2015 at ICAR-Indian Agricultural Research Institute, Regional Station, Karnal. These seeds were stored under ambient conditions in cloth bags and seed quality parameters such as per cent germination, vigour index I, speed of germination and seed mycoflora were assessed in the seeds at every 3 months interval. For per cent seed germination 100×4 seeds were tested between layers of paper towel substrata (Fig 1a and b) at 25±1°C temperature by keeping in seed germinator as per ISTA (1999). For seedling vigour test, the vigour index I was determined using following formula (Abdul Baki and Anderson, 1973).

Seedling vigour index I =
Standard germination (%) × Seedling length {shoot + root length (cm)}

For speed of germination test, blotting papers were soaked in sterile distilled water and three layers were placed in each petri dish. One hundred seeds were taken at random as per ISTA rules. Out of 100 seeds, 10 seeds per petri dish were placed. Three replicates of each treatment were prepared. Each day, number of germinated seeds were recorded and the speed of germination was calculated by using following formula:

$$\text{Speed of germination} = N_1/1 + N_2/2 + \ldots\ldots + N_x/x = N$$

Where;
$N_1$ to $N_x$ = No. of seeds germinated on day 1 to day x.
1 to X = Number of days.

### Detection of seed fungi using standard blotter test method

The detection of seed mycoflora in pigeonpea and chickpea seeds was carried out by the blotter test as recommended by the International Seed Testing Association (ISTA, 1966) with some modifications (Kumar *et al*. 2020). Blotting papers were soaked in sterile distilled water and three layers were placed in each petri dish. One hundred seeds were taken at random as per ISTA rules. Out of 100 seeds, 10 seeds per petri dish were placed. The petri dishes were then incubated at 28 ± 2°C under diffused light for seven days. After seven days of incubation, the plated seeds were examined under a stereoscopic binocular microscope for the observation of fungi. The fungi which emerged on the seed surface were sub cultured on PDA plates for proper identification following the keys (Gilman, 1957; Booth, 1971; Ellis, 1971; Subramanian, 1971; Alexopoulos and Mims, 1979).

## RESULTS AND DISCUSSION

Initially at the time of storage, per cent seed germination, seedling vigour and speed of germination was high in pigeonpea (cvs. P. 991 and P. 2001) and chickpea (cv. local variety) crops. But with increase in the storage period there was decrease in all the parameters in all the cultivars.

### Seed germination

The germination of pigeonpea cv. P. 2001 seed could be maintained above (>75%) Indian minimum seed certification standard (IMSCS) up to 12 months of ambient storage, however cultivar P. 991 of pigeonpea retained its per cent seed germination above IMSCS upto 18 months of storage (Fig 2). In case of chickpea seed, the germination of local cultivar could be maintained above (>85%) IMSCS up to 9 months only under ambient storage, however seed treatment with either Captan or Carbendazim could extend this period of standard germination (above IMSCS) up to 12 months of storage in chickpea local cultivar seed (Fig 3). However,



**Fig 1 (a) and (b):** Germination of pigeonpea seed in Paper Towel.

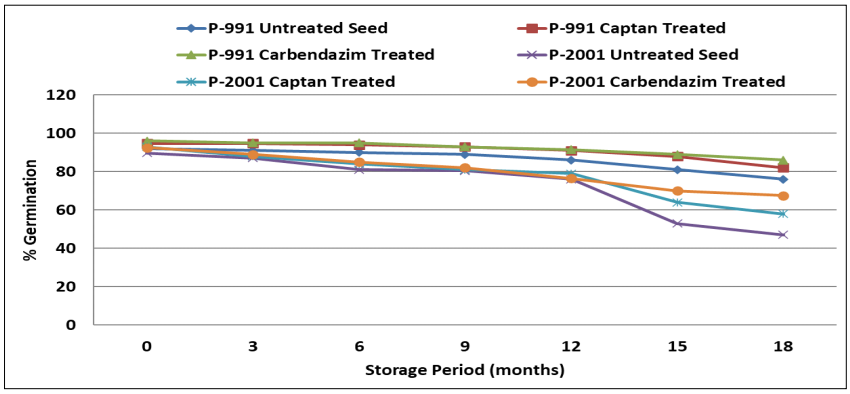


**Fig 2:** Effect of seed treatment and storage period on pigeonpea seed germination under ambient storage conditions.

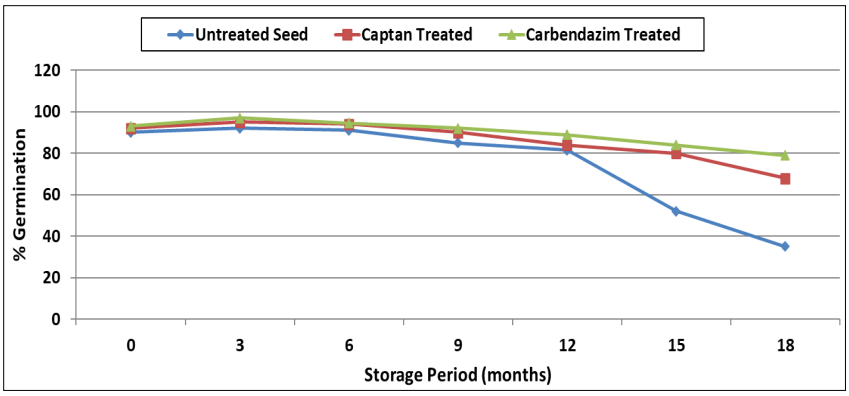


**Fig 3:** Effect of seed treatment and storage period on chickpea seed germination under ambient storage conditions.

higher seed germination was observed in seeds treated with Captan or Carbendazim as against untreated seed in all the cultivars, irrespective of crops and storage duration.

Comparatively higher seed germination was noticed in pigeonpea cv. P. 991 than in cv. P. 2001 and chickpea (local cultivar). The per cent germination of the seeds of different cultivars of pigeonpea and chickpea decreased drastically with increase in the storage period which may potentially be due to the fungi growing on the stored seeds, along with loss in the content of carbohydrate, protein, amino acids *etc*., that induces increased moisture content, free fatty acid content and enhances other biochemical changes (Srivastava *et al*., 2013, 2014; Pedireddi *et al*., 2018). With increase in storage period, the seed germination decreased and the load of fungal flora increased in all the cultivars. The increased presence of seed mycoflora contributes to reduced germination and thereby affects seed quality and longevity under storage as has been reported earlier by Gupta (2010), Mehta *et al*. (2015) and Gupta and Kumar (2020).

### Seedling vigour

Seedling vigour also followed a trend similar to seed germination. It was higher initially upto 6 months of storage and thereafter declined with the fall in seed germination in all the cultivars. Comparatively higher seedling vigour was noticed in chickpea (local cultivar) followed by pigeonpea cv. P. 991 and pigeonpea cv. P. 2001 (Fig 4; Table 1). In pigeonpea, maximum vigour index I (1898) was found in pigeonpea cv. P. 991 (Captan treated seed) at 3rd month of storage and minimum vigour index I (744) was found in untreated seeds of pigeonpea cv. P. 2001 after 18 months of storage (Table 1). In chickpea, maximum vigour index I (2610) was found in Carbendazim treated seed of local cultivar at 3rd month of storage and minimum vigour index I (753) was found in untreated seed of chickpea (local cultivar) after 18 months of storage (Table 1). However, as expected higher seedling vigour was observed in seeds treated with Captan or Carbendazim as against untreated seed in all the cultivars, irrespective of crops and storage duration. The seedling vigour is challenged with the increase in storage of pulse seeds and it decreases due to direct reduction in the per cent seed germination and also due to increased incidence of seed mycoflora during storage thus adversely affect the seedling growth parameters (Gupta, 2010; Kumar *et al*. 2013, 2014).

### Speed of germination

Among two cultivars of pigeonpea, the maximum speed of germination (11.09) was found in Captan treated seed of pigeonpea cv. P. 991 as against 8.39 in untreated seed at 0 month of storage which reduced to 7.37 and 6.13, respectively. The speed of germination was 7.45 in untreated seed of pigeon pea cv. P. 2001 at 0 period of storage which reduced to 3.95 at 18 months of storage (Fig 5). Whereas,

**Table 1:** Effect of seed treatment and storage period on seedling vigour index I of pigeonpea and chickpea seed under ambient conditions.

| Crop | Variety | Seed treatment | Storage period (months) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 3 | 6 | 9 | 12 | 15 | 18 |
| Pigeon pea | P-991 | Untreated | 1747 | 1775 | 1716 | 1689 | 1625 | 1504 | 1478 |
| | | Captan | 1887 | 1898 | 1883 | 1851 | 1733 | 1619 | 1567 |
| | | Carbendazim | 1758 | 1784 | 1790 | 1773 | 1707 | 1635 | 1603 |
| | P-2001 | Untreated | 1352 | 1444 | 1391 | 1264 | 1187 | 926 | 744 |
| | | Captan | 1578 | 1596 | 1614 | 1570 | 1517 | 1258 | 865 |
| | | Carbendazim | 1614 | 1649 | 1643 | 1582 | 1544 | 1333 | 955 |
| Chickpea | Local Variety | Untreated | 2309 | 2317 | 2277 | 1802 | 1639 | 1082 | 753 |
| | | Captan | 2438 | 2577 | 2561 | 2364 | 1786 | 1626 | 1347 |
| | | Carbendazim | 2489 | 2610 | 2581 | 2452 | 2356 | 1795 | 1510 |
| | LSD 0.01 | | 73.134 | 54.752 | 90.507 | 92.752 | N/A | 52.007 | 98.130 |
| | Sem± | | 20.731 | 15.521 | 25.656 | 23.006 | 26.793 | 14.742 | 27.817 |

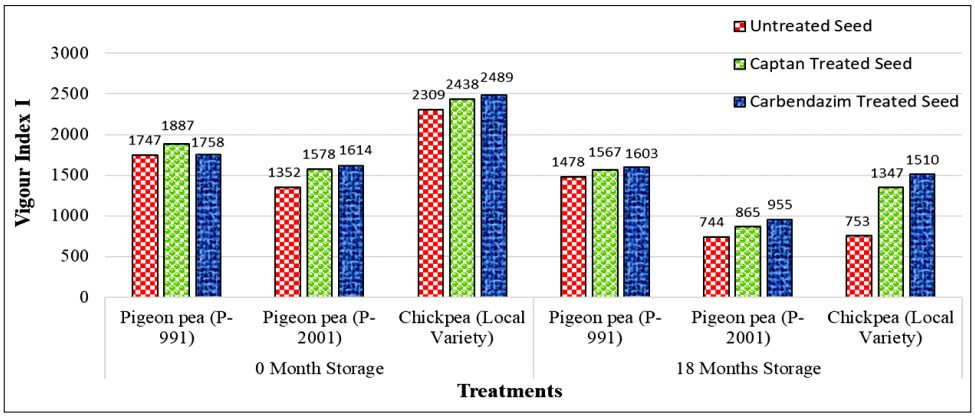


**Fig 4:** Effect of seed treatment and storage period on seedling vigour index I of pigeonpea and chickpea seeds under ambient storage conditions.

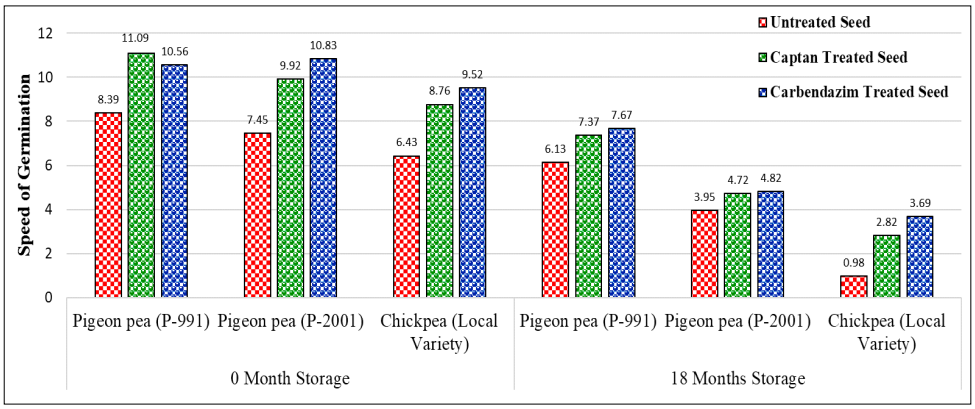


**Fig 5:** Effect of seed treatment and storage period on speed of germination pigeonpea and chickpea seed under ambient storage conditions.

in case of chickpea (local cultivar), the speed of germination was 6.43 at the time of initial storage which reduced to 0.98 after 18 months of storage (Fig 5). Seed treatment with both Captan and Carbendazim improved speed of germination in all the cultivars as against untreated seed. However, maximum speed of germination was found in Carbendazim treated seed of all the cultivars in both the crops at all storage durations. The increased presence of the associated fungi on seed certainly affects the germinability and vigour index of stored seeds and thereby the speed of germination. Garoma *et al.* (2017) concluded that longer storage period had an adverse effect on germination speed.

### Seed mycoflora

In all 16 fungi were found associated with seeds of different cultivars of both crops *viz.*, pigeonpea and chickpea.

The fungi recorded on these seeds were *Alternaria alternata, Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A ochraceous, Cercospora* species, *Chaetomium globosum, Colletotrichum* species, *Curvularia lunata, Fusarium oxysporum, Fusarium moniliforme, Mucor* species, *Penicillium notatum, Penicillium citrinum* and *Rhizopus stolonifer*. Initially, the fungal incidence was low in treated seed and well as untreated seeds and it increased with the increase in storage period. Maximum incidence of *Aspergillus fumigatus* (19.83%), *A. flavus* (14.88%), *A. niger* (12.40%), *Alternaria alternata* (11.57%), *Fusarium oxysporum* (8.26%), *Rhizopus stolonifer* (7.44%) and *Penicillium notatum* (5.79%), were recorded on untreated seed of pigeonpea cv. P. 2001, after 18 months of storage. In case of chickpea, maximum incidence of *Aspergillus fumigatus* (21.65%), *A. flavus* (13.40%), *A. niger* (12.37%), *Alternaria alternata* (10.31%), *Fusarium oxysporum* (8.25%), *Penicillium citrinum* (7.22%) and *Rhizopus stolonifer* (6.18%), were recorded on untreated seeds of chickpea (local cultivar), after 18 months of storage. These results obtained on occurrence of fungal species were in agreement with Patil *et al*. (2012), Margaret *et al*. (2013), Sontakke and Hedawoo (2014) and Chaudhari *et al*. (2017) who reported similar fungal species on pigeonpea and chickpea. The maximum incidence of mycoflora associated with seed was found in untreated seed of pigeonpea cv. P 2001 (20.93%) followed by untreated seed of cv. P 991 (19.42%) and untreated seed of chickpea local cultivar (17.92%) after 18 months of ambient storage. The fungal incidence in seeds treated with the fungicides was negligible during storage and remained below 3.0% even after 18 months of ambient storage (Fig 6). The fungal species associated with seed play crucial role in determination of seed longevity particularly under ambient storage conditions, as these are the primary causative agents of seed biodeterioration which actually cause both quantitative and qualitative loss in seed morphology and seed viability (Gupta, 2010; Srivastava *et al*., 2014; Singh, 2014; Kumar *et al*., 2020).

The overall efficacy of Carbendazim and Captan fungicides against seed fungi on both the crops was very good. Maximum fungal inhibition (94.50%) was achieved with the seed treatment using Carbendazim 50%WP on

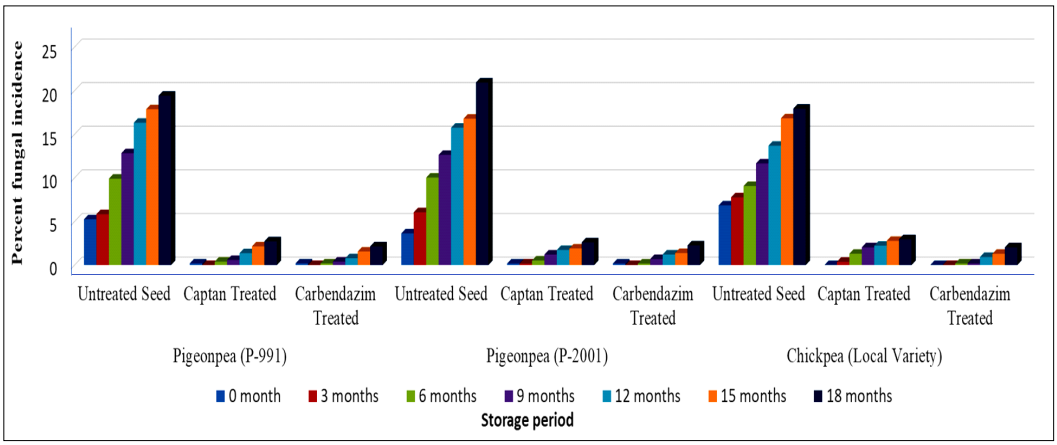


**Fig 6:** Effect of seed treatment and storage period on incidence of seed fungi of pigeonpea and chickpea seed under ambient storage conditions.

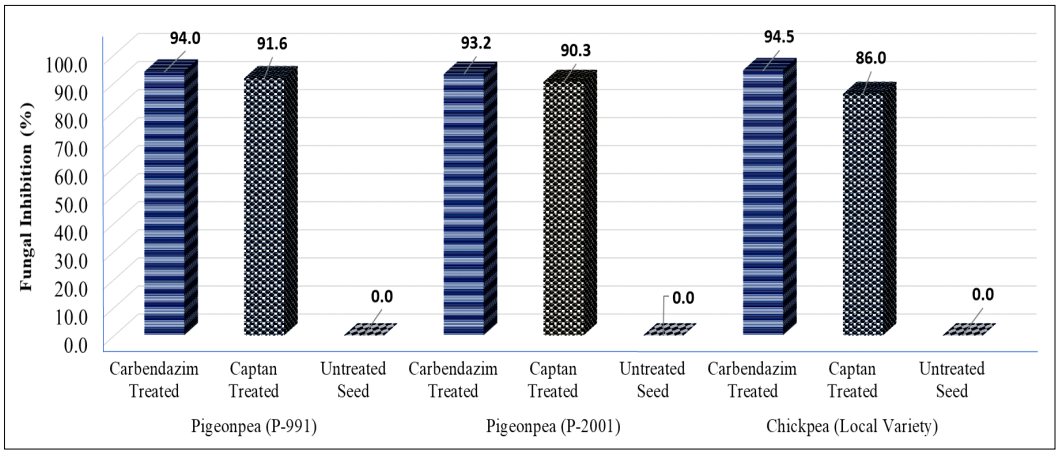


**Fig 7:** Efficacy of seed treatments against fungal load in pigeonpea and chickpea seeds.

chickpea seed (local cultivar) followed by Captan seed treatment of chickpea (local cultivar) resulting in 86.0% reduction in fungal occurrence on seed. Among pigeonpea cultivar Carbendazim seed treatment resulted in 94.0% and 93.20% fungal inhibition in pigeonpea cv. P. 991 and P. 2001, respectively. Whereas, Captan seed treatment resulted in 91.60% and 90.30% fungal inhibition in pigeonpea cv. P. 991 and P. 2001, respectively (Fig 7). These results are in similarity with Chaudhari *et al*. (2017), who managed the seed mycoflora of pigeonpea using seven fungicides and concluded Carbendazim 50% and Carbendazim 12% in combination with mancozeb 63% were the most efficacious treatments in managing seed associated fungi and also for enhancing the seed quality parameters. Pan *et al*. (2010) also reported improvement in germination and seed viability of fungicides treated seeds.

## CONCLUSION

The longevity of seeds varied with respect to crops and varieties. With increase in the storage period, there was decrease in per cent seed germination, seedling vigour and speed of germination in seeds of both the pulse crops, chickpea and pigeonpea, irrespective of cultivars or seed treatment. The study investigates maintenance of seed quality parameters with the application of Captan (a protective fungicide) and Carbendazim 50%WP (a protective and curative fungicide) during storage under ambient conditions which are the most probable conditions of seed storage available to the majority of farmers in our country. Seed treatment with both fungicides *viz.,* Captan and Carbendazim improved per cent seed germination, speed of seed germination and seedling vigour in chickpea and pigeonpea seeds and reduced seed mycoflora thereby maintained the seed quality during storage. This work helps to understand the dynamics of seed associated fungal microorganisms under ambient storage conditions.

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