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Effect of Seed Treatments and Storage Period on Seed Health Parameters of Pea (*Pisum sativum* L.) under Ambient Storage Conditions

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ABSTRACT

Background: Pea (*Pisum sativum* L.) is an important leguminous crop utilized as vegetable and pulse, being an important source of proteins. Pea seeds harbour various mycoflora both in field and during storage, which plays important role in reducing seed viability, germination and vigour inflicting considerable losses in yield and quality. There is scarcely any recommendation available to maintain seed quality during storage of pea seeds, hence present study was carried out.

Methods: Seeds of pea (cvs. Azad P. 1 and Arkel) were treated with Captan and Carbendazim 50%WP fungicides @ 2.0 g/kg seed and stored under ambient conditions. The samples were drawn at three months interval to assess the effect of storage on seed quality parameters. The vigour index I, per cent germination and speed of germination of these seed samples were determined using ISTA rules. The detection of associated mycoflora in pea (*Pisum sativum* L.) seed samples was carried out by standard blotter method as recommended by International Seed Testing Association.

Result: Total fourteen fungal species were found associated with different pea seed samples. Out of these, maximum incidence of *Alternaria alternata* (21.36%) followed by *Aspergillus flavus* (15.53%), *A. fumigatus* (14.56%), *A. niger* (11.60%) and *Rhizopus stolonifer* (9.71%), were recorded on untreated seed of pea cv. Azad P. 1, after 18 months of storage. The germination, speed of germination and vigour index of stored seed reduced with increase in storage period, whereas fungal incidence increased with the increasing storage period. Among the tested cultivars, germination in pea (cv. Arkel) remained above IMSCS even after 18 months of storage under ambient storage conditions. The maximum per cent germination (99.0) was found in pea cv. Azad P. 1 seeds treated with Captan at 3 months of storage, whereas minimum per cent germination (49.5) was found in untreated seed of pea cv. Azad P. 1 after 18 months of storage. The maximum speed of germination (23.88) was found in Captan treated seed of pea cv. Arkel at 0 month of storage and minimum speed of germination (3.52) was found in untreated seed of pea cv. Azad P. 1 at 18 months of storage. The maximum vigour index I (2339) was found in pea cv. Azad P. 1 (Captan treated seed) at 3 months of storage and minimum vigour index I (431) was found in untreated seed of pea cv. Azad P. 1 after 18 months of storage.

Key words: Mycoflora, Pea (*Pisum sativum* L.), Seed quality, Seedling vigour, Storage.

INTRODUCTION

Pea (*Pisum sativum* L.) belonging to family Leguminosae is an important legume crop widely cultivated throughout the world. Being a cool season crop, It is extensively grown in temperate zone but restricted to cooler altitudes in the tropics and winter season in subtropics. Pea is a dual-purpose crop, when its pods are harvested in green stage it is called as garden pea or green pea and when these pods are harvested after their maturity and are utilized as *dhal*, these are called as field pea (Chandel and Kumar, 2017). Like green beans, cultivars of pea are not considered as pulses when they are used as vegetables and are classified into green peas and field pea or 'dry pea' (Calles, 2016). Pea contains enriched levels of amino acids, lysine and tryptophan. Pea is rich source of protein (25%), sugars (8-12%), amino acids, carbohydrates, vitamin A and C, calcium and phosphorus besides having small quantity of iron. In addition, its cultivation is always beneficial to soil and our ecosystem due to its nitrogen fixing capabilities through which it contributes to the soil health and also helps to restrict the use of chemical fertilizers in subsequent crops (Stagnari *et al*., 2017).

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Seed-borne or seed associated infections have been established to pose high adverse effect on the germination, growth and productivity of different crop plants and pea is not an exception (Kumar and Gupta, 2020). Pea besides its high susceptibility to pre-emergence damping off and to post-

emergence root and foot rots, harbour various mycoflora both in fields and during storage, capable of causing considerable losses in yield and quality of pea (Jadon *et al*., 2020). Pea seeds loose its viability at faster rate during improper storage and exhibit poor field emergence and plant stand after sowing in next crop season. There is scarcely any recommendation available on storage of pea seeds. Keeping this in view, two popular garden pea cultivars *viz.,* Azad P. 1 and Arkel was selected to study the effect of seed treatments and storage period on seed health parameters of pea (*Pisum sativum* L.).

MATERIALS AND METHODS

Seeds of two important cultivars of pea *viz*., Azad P. 1 and Arkel were treated with fungicides *viz*., Captan (2 g per Kg seed) and Carbendazim 50% WP (2 g per Kg seed). Prior to these fungicidal seed treatments, the all seeds including fungicide untreated seed were treated with Deltamethrin insecticide to avoid insects' infestation especially bruchids (*Callosobruchus* spp.) infestation 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 separately (one bag for each treatment for a variety) and seed quality parameters such as per cent germination, vigour index I, speed of germination and seed mycoflora were assessed at 3 months interval. Seeds were subjected to seed health testing using blotter technique. For per cent seed germination 100×4 seeds were tested between layers of paper towel substrata at 25°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 determining speed of germination, three layers of blotting paper presoaked in sterile distilled water were placed in each Petri dish. One hundred seeds were taken at random as per ISTA rules and 10 seeds per Petri dish were placed. Three replicates of each treatment were prepared. Each day numbers 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 seed germinated on day 1 to day x.

1 to X = Number of days.

Detection of seed fungi using standard blotter method

The seed mycoflora in pea seeds were detected by the standard blotter test as recommended by the International Seed Testing Association (ISTA, 1966). Triple layers of blotting paper presoaked in sterile distilled water were placed in each Petri dish. As per ISTA rules one hundred Seeds were selected randomly, of which 10 seeds per Petri dish were placed on triple layer of blotting paper (Fig 1). These Petri dishes were incubated at 27 ± 2°C under diffused light for ten days. After 10 days of incubation, the plated seeds were examined under a stereoscopic binocular microscope for the associated fungi. The fungi which emerged on the seed surface were sub cultured on PDA for identification following the keys outlined by Gilman (1957); Booth (1971); Ellis (1971); Subramanian (1971) and Alexopoulos and Mims (1979).

Statistical analysis

All the data was analyzed statistically. The data on vigour index I, per cent germination and speed of germination was analyzed using completely randomized design (CRD) with P= 0.01 level of significance.

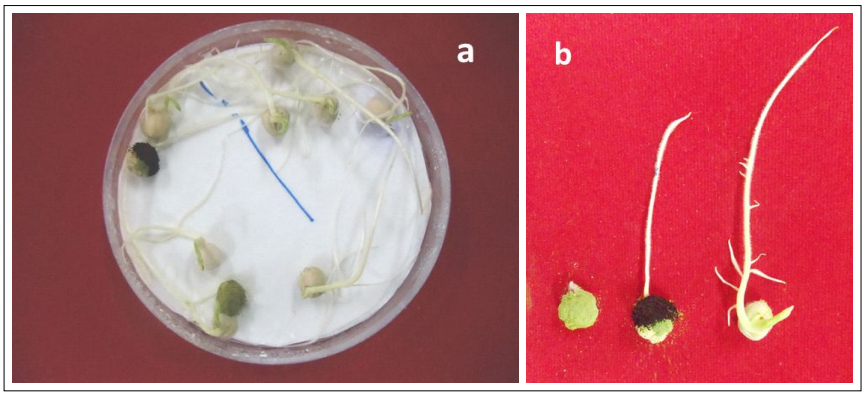


Fig 1: Detection of seed fungi on untreated pea seeds using standard blotter method (a), Incidence of mycoflora on untreated pea seeds under standard blotter method after 10 days of incubation (b).

RESULTS AND DISCUSSION

Seed Germination

Among the two cultivars of pea, higher seed germination (90.2%) was found in pea cv. Azad P. 1 as compared to pea cv. Arkel (82.4%) irrespective of storage period and seed treatments. Highest seed germination was recorded after 3 months of storage in both the cultivars, which got decreased with increase in the storage period, irrespective of seed treatments (Fig 2). The germination of untreated seed of pea cv. Azad P. 1 could be maintained above (>75%) Indian minimum seed certification standard (IMSCS) up to 12 months only under ambient storage but fungicide treatment with Captan or Carbendazim could extend the germination above IMSCS upto 15 months, however in pea cv. Arkel, seed germination was maintained above IMSCS upto 18 months of ambient storage, irrespective of seed treatments, though higher seed germination was recorded in Captan (84%) and Carbendazim (81%) treated seed as against 75.75% in untreated seed at 18 months of ambient storage. The difference in per cent germination between the seed treatments with Captan and Carbendazim was statistically non-significant.

The per cent germination of seeds of the two cultivars of pea decreased considerably with the increase in storage period which may be potentially contributed to increased occurrence of fungal flora on stored seeds coupled with the loss in the content of carbohydrate, protein, amino acids *etc*., that induce increased moisture content, free fatty acid content and enhances other biochemical changes in the seed (Srivastava *et al*., 2013, 2014; Pedireddi *et al*., 2018). With the increase in period of seed storage, the seed germination decreased and the load of fungal flora increased. The increased storage period contributes to reduced germination in pea (Chandel and Kumar, 2017; Gupta and Kumar, 2020).

Seedling vigour

Seedling vigour of both cultivars of pea 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 both the cultivars. Among the two cultivars of pea, higher seedling vigour (1789.48) was found in pea cv. Azad P. 1 as compared to Pea cv. Arkel (1705.67) irrespective of treatments (Table 1; Fig 3). However, as expected higher seedling vigour was observed in seeds

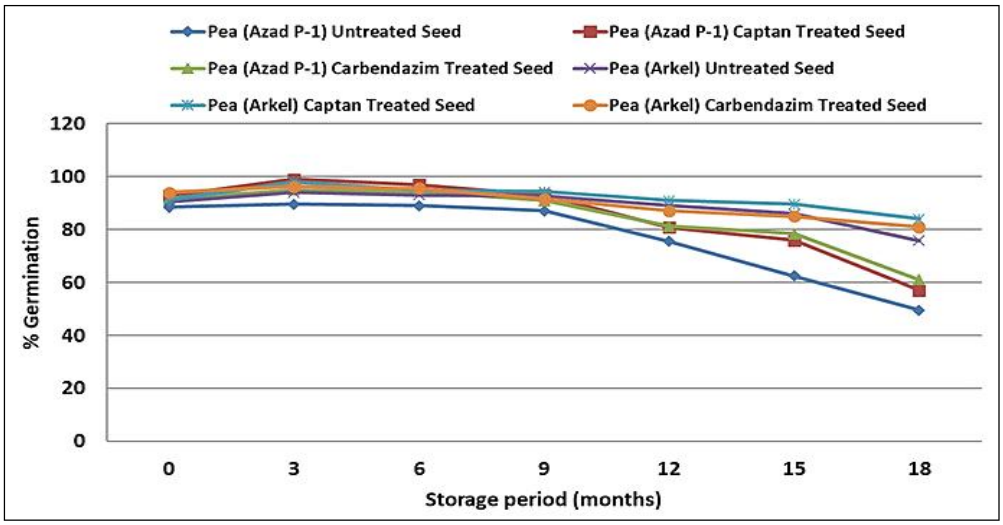


Fig 2: Effect of seed treatments and storage period on pea seed germination under ambient storage conditions.

Table 1: Effect of seed treatments and storage period on seedling vigour index I of pea cultivars under ambient conditions.

Crop/	Seed	Storage duration (months)						
Variety	treatment	0	3	6	9	12	15	18
Pea (Azad P. 1)	Untreated	1540^{a}	1554^{a}	1685^{a}	1507^{a}	1469^{a}	821^{a}	431^{a}
	Captan	2329^{b}	2339^{b}	2307^{b}	2178^{b}	1733^{b}	1380^{b}	905^{b}
	Carbendazim	2259^{c}	2290^{b}	2231^{c}	2194^{b}	1956^{c}	1442^{c}	1269^{c}
Pea (Arkel)	Untreated	1898^{d}	1960^{c}	1942^{d}	1803^{c}	1414^{a}	1198^{d}	1092^{d}
	Captan	2058^{e}	2082^{d}	2075^{e}	1923^{d}	1847^{d}	1702^{e}	1533^{e}
	Carbendazim	2005^{e}	2024^{d}	2077^{e}	1994^{e}	1820^{d}	1677^{e}	1455^{f}
CD (at 1.0%)		62.60	59.08	57.75	44.95	75.83	51.51	61.47
Sem±		17.75	16.75	16.37	12.74	21.49	14.60	17.43

*The figures indicated with same letter in the same columns were not significantly different.

treated with Captan or Carbendazim as against untreated seed in both the cultivars, irrespective of storage duration. The seedling vigour is challenged with the increase in storage of pea seeds, it gets decreased due to direct reduction in per cent germination and also due to increased incidence of seed mycoflora during storage which adversely affect the seedling growth parameters (Kumar *et al*., 2014; Chandel and Kumar, 2017; Wang *et al*., 2018).

Speed of germination

Among two cultivars of pea, the higher speed of germination was found in pea cv. Arkel (15.98) as against pea cv. Azad P. 1 (14.19) irrespective of treatments. The speed of germination also decreased in all the treatments with increase in the storage duration, however, seeds treated with Captan or Carbendazim maintained higher speed of germination than untreated seed at all storage periods (Fig. 4), but in maximum of the months' intervals of seed storage the differences between the speed of germination in both Captan and Carbendazim treated seeds were statistically non-significant. These results are in conformity with Pandita and Nagarajan (2002), who reported similar trend in garden pea (*Pisum sativum*). Garoma *et al*. (2017) also concluded that longer storage period had an adverse effect on germination speed in maize parental lines.

Seed mycoflora

In all, fourteen fungal species were found associated with pea seed samples of both varieties. These included *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger*, *Chaetomium globosum*, *Cladosporium herbarum*, *Curvularia lunata*, *Colletotrichum* sp., *Drechslera* sp., *Fusarium oxysporum, Fusarium moniliforme*, *Penicillium* sp., *Macrophomina phaseolina* and *Rhizopus stolonifer*. Out of these, five fungi *i.e*., *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger* and *Rhizopus stolonifer*, were found in high frequencies. Initially the fungal incidence was low in both treated and untreated seeds and it increased with the increase in storage period. Maximum incidence of *Alternaria alternata* (21.36%) followed by *Aspergillus flavus* (15.53%), *A. fumigatus* (14.56%), *A. niger* (11.60%) and *Rhizopus stolonifer* (9.71%), were recorded on untreated seed of pea cv. Azad P. 1 after 18 months of storage. The results obtained during the present study on occurrence of fungal species were in agreement with Ozgonen and Gulcu (2011); Singh *et al*. (2015); Dinler and Gunay (2018) and Pszczółkowska

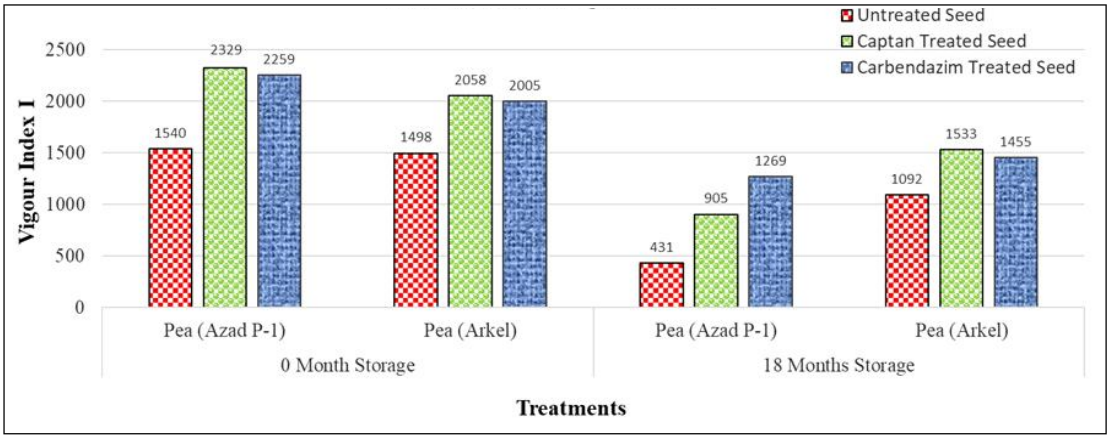


Fig 3: Effect of seed treatments and storage period on seedling vigour of pea seed under ambient storage conditions.

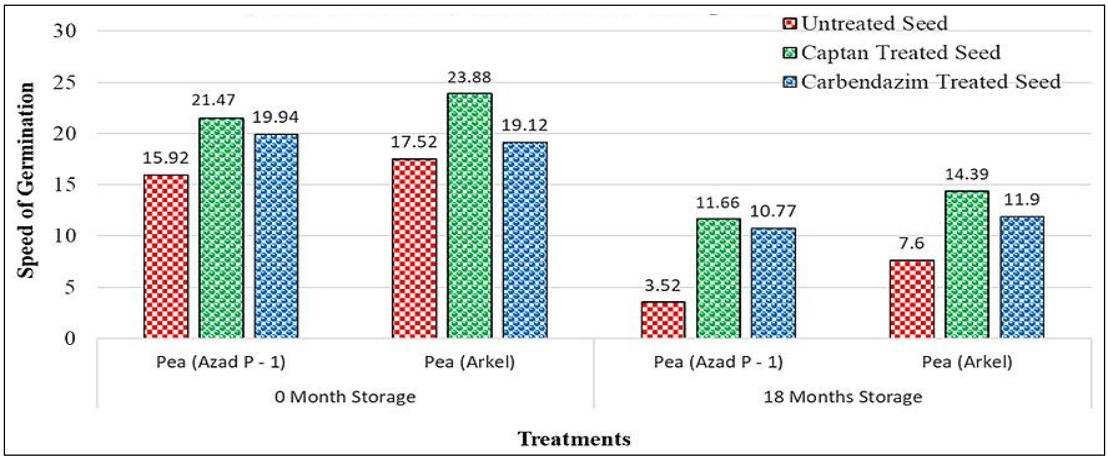


Fig 4: Effect of seed treatments and storage period on speed of germination in pea seed under ambient storage conditions.

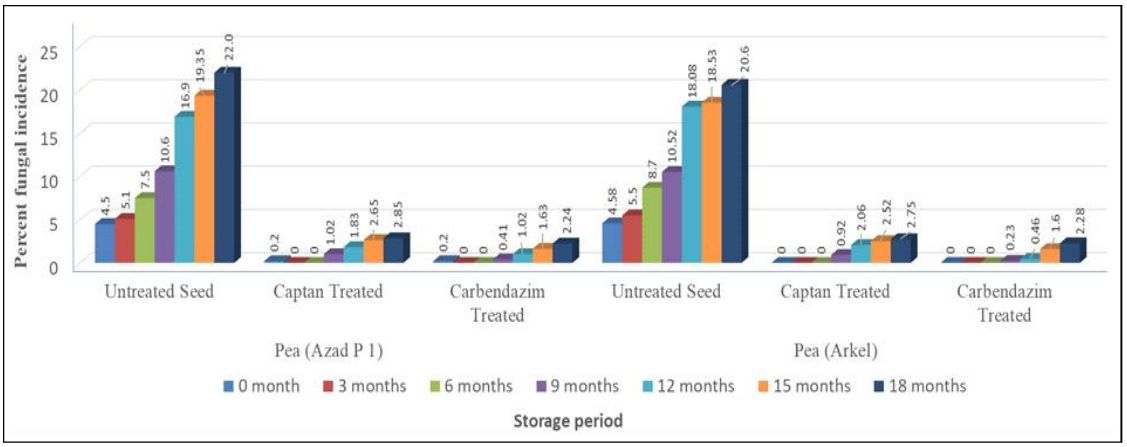


Fig 5: Effect of seed treatments and storage period on incidence of seed fungi on pea seeds under ambient storage conditions.

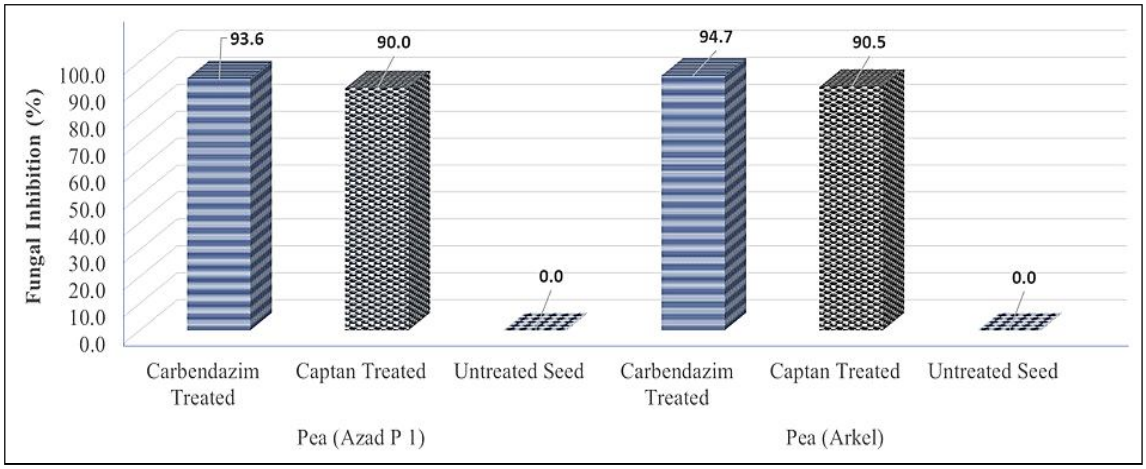


Fig 6: Efficacy of seed treatments against fungal load in pea (*Pisum sativum* L.) seeds.

et al. (2019) who reported similar fungal species on pea seeds. The maximum incidence of mycoflora associated with seed was found in untreated seed of pea cv. Azad P. 1 (22.0%) followed by untreated seed of cv. Arkel (20.6%) after 18 months of ambient storage. The fungal incidence in seeds treated with the fungicides was negligible during storage and remained restricted maximum upto 2.85% even after 18 months of ambient storage (Fig 5). Different cultivars of agricultural crops including pea respond differentially to the fungal seed infection and thereby may have different seed longevity under storage (Wilman *et al*., 2014; Pedireddi *et al*., 2018). 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 losses in seed morphology and seed viability (Srivastava *et al*., 2014; Singh *et al*., 2015; Kumar *et al*., 2020).

The overall efficacy of Carbendazim and Captan fungicides against seed fungi on both the cultivars was very good. Maximum fungal inhibition (94.70%) was achieved with the seed treatment using Carbendazim 50% WP on pea seed (Arkel variety) followed by Carbendazim 50% WP seed treatment of Azad P. 1 and Captan seed treatment of Arkel cultivar of pea resulting in 93.60% and 90.50% reduction in fungal occurrence on seed, respectively (Fig 6). These results were in conformity with Chaudhari *et al*. (2017), who managed the seed mycoflora of pigeonpea using seven fungicides and reported that 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 of three crops *viz*., mungbean, mustard and rice. Lamichhane *et al*. (2020) concluded that the seed treatments not only provide the protection against seed-borne fungi but also helps to manage the soil-borne inoculum of certain phytopathogens in case of peas and chickpeas.

CONCLUSION

Pea (*Pisum sativum* L.) is an important dual-purpose crop being utilized as vegetable and pulse. The longevity of seed varied with respect to cultivar of pea. With increase in the

storage period, there was decrease in seed germination, seedling vigour and speed of germination in seeds of both cultivars of pea, irrespective of seed treatment. The present study is helpful to understand the dynamics of seed associated fungal microorganisms under ambient storage conditions. Seed treatment with both fungicides *viz.,* Captan and Carbendazim improved seed germination, speed of seed germination and seedling vigour in pea seeds and reduced seed mycoflora thereby maintained seed quality during storage; however the differences among the Captan and Carbendazim treated seeds for these seed health parameters were mostly statistically non-significant. Therefore, it can be concluded that seed treatment with any of these two fungicides, offers the better seed health and seed longevity in pea under ambient storage conditions.

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