International Journal of Tropical Agriculture
Volume 44, Numbers (1-2) • 2026 : pp. 15-21
ISSN: 0254-8755
https://DOI:10.47509/IJTA.2026.v44i1-2.04

Comparative Study on Seed Germination and Effect of Trichoderma harzianum Rifai on Fresh and Stored Seeds of Different Crops

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Abstract: Seed-borne fungal pathogens adversely affect seed viability, germination, and seedling establishment during storage and cultivation. The present investigation was conducted to evaluate the germination percentage of fresh and stored seeds of wheat, paddy, pigeon pea, and chickpea using different germination methods, namely blotter method, plastic pot method, multi-pot tray method, and rolled paper towel method. The observations were recorded after 8–10 days of incubation. Results revealed that fresh seeds exhibited higher germination percentages compared to stored seeds across all tested crops and methods. Among the methods evaluated, the plastic pot method proved to be the most suitable for assessing seed germination.

The effect of seed treatment with *Trichoderma harzianum* Rifai was also studied using plastic pot and multi-pot tray methods. Seeds treated with *T. harzianum* showed significantly improved germination percentages over untreated control seeds in both fresh and stored conditions. Treated seeds also demonstrated enhanced seedling growth and vigor. The study highlights the beneficial role of *T. harzianum* as a biological seed treatment agent for improving germination and seedling development.

Keyward: Seed-borne fungi, *Trichoderma harzianum*, seed germination, plastic pot method, blotter method, rolled paper towel method, seed treatment

INTRODUCTION

Fungal pathogens represent a major group of disease-causing organisms affecting agricultural crops worldwide. These pathogens can infect plants at various growth stages; however, their association with seeds is particularly important because seeds serve as the primary propagating material for many crop species. Seed-borne fungi may occur externally as spores or internally within seed tissues, thereby reducing seed quality, germination, and storage life.

Seed infection by fungal pathogens not only lowers seed viability but also acts as a potential source of inoculum for introducing diseases into new cultivation areas. Several researchers have reported that seed mycoflora significantly influences seed deterioration during storage and negatively affects germination and seedling vigor (Suryanarayana and Bhombe, 1961; Rai *et al*., 1997). Srivastava and Jaiswal (2000) observed that *Alternaria brassicae* reduced cauliflower seed germination, while Ammara *et al*. (2000) reported the involvement of seed-borne pathogens in poor germination of wheat seeds.

Biological control agents such as *Trichoderma harzianum* have gained importance due to their antagonistic activity against several plant pathogens and their ability to enhance plant growth. Das and Das (2005) reported effective suppression of white rot disease in

Article History: Received: 03 March 2026; Revised: 11 April 2026; Accepted: 18 April 2026; Published: 20 May 2026

French bean using *T. harzianum*. Harman *et al*. (2010) demonstrated that seed treatment with *T. harzianum* improved seedling vigor and reduced oxidative stress in germinating seeds. Similar observations regarding enhanced germination and seedling growth following seed treatment with *Trichoderma* spp. have also been reported by Nayaka *et al*. (2008) and Sultana *et al*. (2012).

Keeping these aspects in view, the present investigation was undertaken to:

1. Determine the germination percentage of fresh and stored seeds of wheat, paddy, pigeon pea, and chickpea using different germination methods.
2. Evaluate the effect of *Trichoderma harzianum* seed treatment on germination and seedling growth.

MATERIAL AND METHODS

Determination of seed germination (%) of fresh and stored seed

(A) Plastic pot method: Plastic pots containing sterilized soil mixture were used to evaluate seed germination under glasshouse conditions. The soil mixture consisted of four parts peat supplemented with the required quantity of fertilizer and sterilized at 95°C for 30 minutes in an autoclave. Fresh and stored seeds of wheat, paddy, pigeon pea, and chickpea were sown separately in the pots. Germination percentage was recorded after 8–10 days of sowing.

(B) Multi-pot tray method (Khare, 1996): Multi-pot trays were filled with sterilized peat-based soil mixture containing fertilizer. Fresh and stored seeds of the selected crops were sown in separate cavities and maintained under glasshouse conditions. Germination counts were recorded after 8–10 days.

(C) Blotter method (de Tempe, 1953): Three layers of moist blotting paper were placed in sterilized Petri dishes (9 cm diameter). Sterilized distilled water was used to moisten the blotters. Seeds were placed uniformly on the blotting paper and incubated at 25 ± 1°C. Three replications were maintained for each treatment. Germination percentage was calculated after one week.

(D) Rolled paper towel method (Agrawal, 1994): Good-quality paper towels measuring 46 × 29 cm were moistened with sterilized distilled water. Seeds were arranged on the towel and covered with another moist towel sheet. The towels were carefully rolled to ensure proper seed placement and wrapped with polyethylene sheets to prevent moisture loss. The rolled towels were kept upright and incubated at 25 ± 1°C. Germination observations were recorded after incubation.

Seed Treatment with *Trichoderma harzianum* Rifai

Fresh and stored seeds of wheat, paddy, pigeon pea, and chickpea were treated with a spore suspension of *Trichoderma harzianum*. For preparation of the suspension, a 10 mm mycelial disc from a 7-day-old culture was inoculated into 100 ml potato dextrose broth and incubated at 25 ± 1°C for seven days.

After incubation, 30 ml sterile distilled water was added and the culture was shaken on a rotary shaker at 100 rpm for 30 minutes to obtain a uniform spore suspension. Spore concentration was adjusted to 10^6 spores/ml using a haemocytometer. Seeds were pre-soaked in water for four hours and coated with 7% acacia gum to facilitate adhesion of spores. The treated seeds were immersed in the spore suspension for 30 minutes and shade dried for one hour before sowing. Control seeds were treated with sterile distilled water.

Evaluation Methods

(A) Plastic Pot Method: Treated and untreated seeds were sown in sterilized soil-filled plastic pots under glasshouse conditions. Germination was recorded after 8–10 days.

(B) Multi-Pot Tray Method: Similarly, treated and untreated seeds were evaluated

in sterilized multi-pot trays maintained under glasshouse conditions. Germination counts were taken after 8–10 days.

RESULT AND DISCUSSION

Germination of Fresh and Stored Seeds by Different Methods

The germination percentages of fresh and stored seeds of wheat, paddy, pigeon pea, and chickpea were evaluated using four different methods. In all methods, fresh seeds exhibited better germination than stored seeds.

(A) Plastic pot method: Among all methods tested, the plastic pot method recorded comparatively higher germination percentages and proved effective for evaluating seed viability. Fresh seeds of wheat, paddy, pigeon pea, and chickpea showed superior germination compared to corresponding stored seeds.

(B) Multi-pot tray method: Fresh seeds exhibited higher germination percentages than stored seeds in all crops tested under the multi-pot tray method.

(C) Blotter method: The blotter method also demonstrated higher germination in fresh seeds than in stored seeds. Reduced germination in stored seeds may be attributed to the activity of seed-borne fungi and deterioration during storage.

(D) Rolled paper towel method: The rolled paper towel method showed comparatively good germination results, particularly in fresh seeds. However, stored seeds consistently showed lower germination percentages.

The reduction in germination of stored seeds may be due to deterioration caused by seed-associated fungi during storage. Similar observations were reported by Suryanarayana and Bhombe (1961), Rai *et al*. (1997), and Singh *et al*. (2011).

Effect of *Trichoderma harzianum* on Seed Germination

Seed treatment with *Trichoderma harzianum* significantly improved germination percentages in both fresh and stored seeds compared to untreated controls.

(A) Plastic pot method: Treated seeds of wheat, paddy, pigeon pea, and chickpea recorded higher germination percentages than untreated seeds. Improved seedling vigor and healthy growth were also observed in treated plants.

(B) Multi-pot tray method: Similarly, in the multi-pot tray method, *T. harzianum*-treated seeds showed enhanced germination compared to untreated seeds. The beneficial effects of *Trichoderma* treatment may be attributed to suppression of seed-borne pathogens and promotion of seedling growth.

These findings are consistent with previous studies by Harman and Taylor (1989), Das and Das (2005), Mohamedy and El-Baky (2008), Harman *et al*. (2010), and Sultana *et al*. (2012), who reported improved seed germination and seedling vigor following biological seed treatment.

CONCLUSION

The present study demonstrated that fresh seeds of wheat, paddy, pigeon pea, and chickpea showed higher germination percentages than stored seeds across all germination methods tested. Among the evaluated methods, the plastic pot method was found to be the most effective and reliable for assessing seed germination.

Seed treatment with *Trichoderma harzianum* significantly enhanced germination percentage and seedling vigor in both fresh and stored seeds compared to untreated controls. The study confirms the potential of *T. harzianum* as an effective biological seed treatment agent for improving seed quality, reducing the adverse effects of seed-borne fungi, and promoting healthy seedling establishment.

Table 1: Germination test of fresh and stored seeds by different methods

S.No.	Crop Seed	Blotter Method		Multi-Pot		Plastic Pot		Rolled Paper Towel Method	
		Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
1.	Wheat	93.00 (9.69)	82.00 (9.11)	92.00 (9.64)	81.00 (9.05)	90.00 (9.53)	82.00 (9.10)	95.00 (9.79)	89.00 (9.48)
2.	Paddy	90.00 (9.53)	80.00 (8.99)	89.00 (9.48)	80.00 (8.99)	88.00 (9.43)	79.00 (8.94)	93.00 (9.69)	87.00 (9.38)
3.	Pegeon pea	88.00 (9.43)	75.00 (8.71)	82.00 (9.10)	72.00 (8.54)	85.00 (9.27)	72.00 (8.54)	85.00 (9.27)	80.00 (8.99)
4.	Gram	88.00 (9.43	77.00 8.83	82.00 (9.10)	70.00 (8.42)	81.00 (9.05)	72.00 (8.54)	90.00 (9.53)	85.00 (9.27)
	CD(5%)	0.19	0.26	0.29	0.16	0.28	0.19	0.29	0.30

Table 2: Effect of *Trichoderma harzianum* seed treatment on germination of fresh seeds by plastic pot method and multi-pot method

S.No.	Crop Seed	Plastic Pot Method				Multi-Pot Method			
		Fresh	Control	Stored	Control	Fresh	Control	Stored	Control
1.	Wheat	95.00 (9.79)	90.00 (9.53)	87.00 (9.38)	82.00 (9.10)	96.00 (9.84)	92.00 (9.64)	86.00 (9.32)	81.00 (9.05)
2.	Paddy	92.00 (9.64)	88.00 (9.43)	85.00 (9.27)	79.00 (8.94)	94.00 (9.74)	89.00 (9.48)	86.00 (9.32)	80.00 (8.99)
3.	Pegeon pea	90.00 (9.53)	85.00 (9.27)	83.00 (9.16)	72.00 (8.54)	89.00 (9.48)	82.00 (9.10)	75.00 (8.71)	72.00 (8.54)
4.	Gram	89.00 (9.48)	81.00 (9.05)	79.00 (8.94)	72.00 (8.54)	88.00 (9.43)	82.00 (9.11)	74.00 (8.65)	70.00 (8.42)
	CD(5%)	NS	0.23	0.17	0.29	0.25	0.21	0.20	0.36

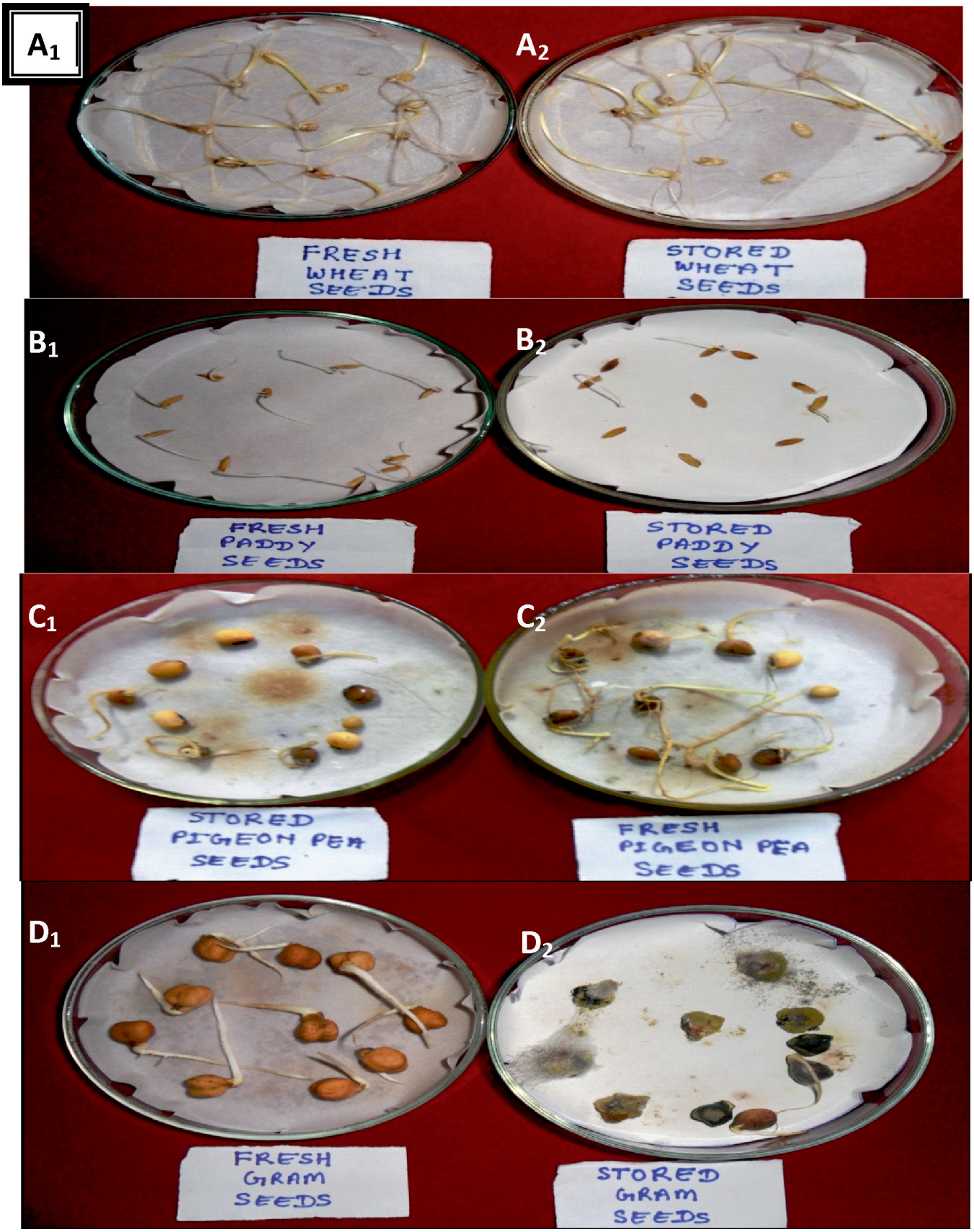

Plate 1: Germination test of fresh and stored seed by blotter method

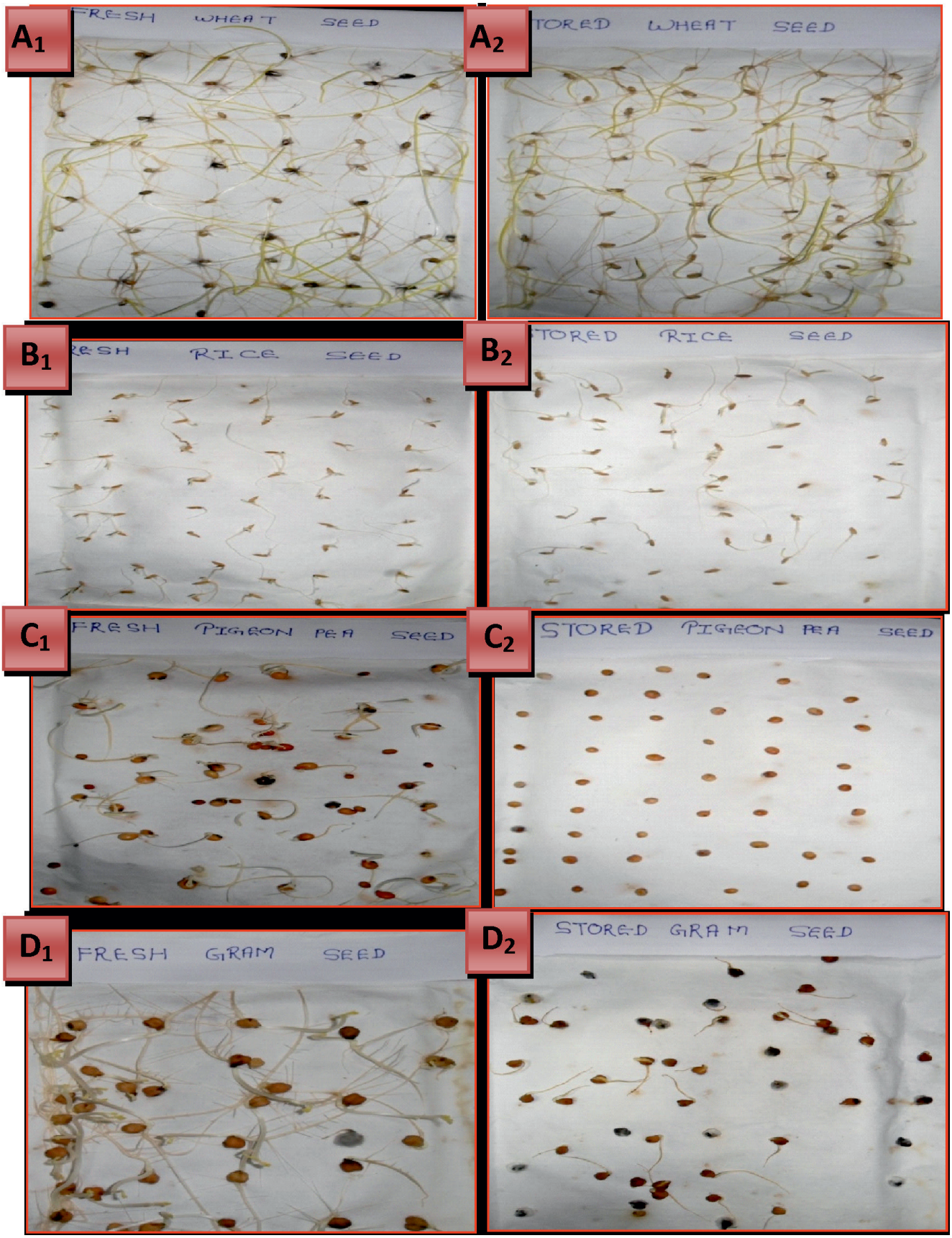

Plate 2: Germination test of fresh and stored seed by rolled paper towel method

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