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EFFECT OF PLANT EXTRACTS AND FUNGICIDES ON ASSOCIATED PATHOGENS OF CAPSICUM (*Capsicum annum*) SEED IN STORAGE

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Abstract

The effects of seed treatment on the prevalence of seed borne pathogens and quality of capsicum seed was studied with Autostin 50 WP (Carbendazim), Dithane M-45 (Mancozeb) @ 2.2 g/kg, Rovral50 WP (Iprodione) @ 2.0 g/kg, Provax-200 WP (Carboxin+ Thiram) @ 3.0 g/kg, Garlic extract @ 1:1, Garlic extract @ 1:2 , Neem leaf extract @ 1:1, Neem leaf extract @ 1:2, Zinger rhizome extract @ 1:1, Zinger rhizome extract @ 1:2 under storage condition. Prevalence of seed-borne pathogens, seed germination, speed of germination and vigour index of seedling were recorded at 0 day, 75 days and 150 days after storage.The fungi *Alternaria sp., Aspergillus sp., Fusarium sp., Carvularia sp., Colletotrichum sp., Penicillium sp. and Rhizopus sp.* were identified from the treated seeds during different storage periods, where the untreated control showed maximum prevalence of the pathogens throughout the storage period. Provax-200WP (Carboxin + Thiram) treated seeds were free from infection though Garlic extract @ 1:1 w/v, Zinger rhizome extract @ 1:2 w/v and Zinger rhizome extract @ 1:1 w/v gave better germination, speed of germination, seedling dry weight and vigour index.

Keywords: Prevalence, incidence, seed treatment, germination, vigour index.

Introduction

Bell pepper (*Capsicum annuum* L.) is an important spice-cum vegetable crop grown under various agro climatic conditions in Bangladesh and worldwide. Loss in vigor and viability as well as disease is a major problem in storage of capsicum seed. There are so many features like loss of seed vigor and viability, low germination and pathogens prevalence which may impair seed health status and quality during storage (Justice and Bass, 1979; Ahmed, 1982 and Asalmol *et al.*, 2001). However, its production is often hindered by various biotic and abiotic stresses, among which seed-borne pathogens play a significant role. Pathogens propagated by seeds cause significant losses in seed yield and quality, resulting in lower germination capacity and even damage to seedlings (Sarika *et al*, 2019). Among various hindering factors, diseases are predominant for the crop. Chili and pepper suffer from many diseases caused by fungi, bacteria, viruses, nematodes

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and also abiotic stresses. Fungal diseases play a vital role in reducing the germination of pepper. Among the fungal diseases, damping off, anthracnose or fruit rot, powdery mildew and leaf spots are the most prevalent ones. The fungi *Fusarium oxysporum*, *Sclerotium rolfsii*, *Aspergillus niger* and *Rhizoctonia solani* are soil inhabiting pathogens with a wide host range and very difficult to control. These pathogens are frequently transmitted through seeds (Fakir, 1998). *Aspergillus* spp. *Penicillium* spp. and *Fusarium* spp. can cause loss of quality of capsicum seeds in storage. Ahmed and Hossain (1985) stated that the fungus can affect seed germination and seedling growth. Seed-borne fungi adversely affect seeds in storage and causes discolor of seeds, enhance biochemical changes, and accumulate toxins, and results in loss of weight (Neergaard, 1979). Thus, management of pathogens infection in seeds during storage is one of the crucial factors to maintain proper germination, vigour and health status of seeds. Among recommended practices, seed treatment probably the cheapest and easiest method of plant disease control at seedling stage. Treatment of seeds with chemicals has been proved to be effective in reducing seed-borne infection (Dubey and Singh, 2005; Barua *et al.,* 2007). Application of fungicides effectively inhibits *F. oxysporum* and *A. niger* and contributes to higher seed germination and vigour index (Patra, 2017; Saranya *et al*., 2017). Plant extract is a promising alternative for pathogens control associated with seeds, reducing costs and impacts to the environment (Silva *et al.,* 2019). Various authors also reported that fungicides and plant extracts application significantly reduced seed-borne infection and increased the percentage germination and vigor index (Saranya *et al.,* 2017) of capsicum seed. Plant extracts act directly or indirectly on pathogens growth in the seed due to their bioactive compounds. Pre-storage seed treatment may reduce the prevalence of seed-borne pathogens and protect seeds from the invasion of mainly fungi in storage condition.

Therefore, present research work was undertaken to evaluate the effect of seed treatment with chemical fungicides and botanical agents on prevalence of major seed borne fungi, seed germination rate, seedling health, and seedling vigour index of capsicum during storage.

Materials and Methods

The research work was executed in the Laboratory of Seed Technology Division as well as Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh during October 2020 to June 2021. The experiment was laid out in Completely Randomized Design (CRD) with 11 treatments including a control and each treatment was replicated 4 times. Seeds of chili var. BARI Mishtimorich-1 were treated with fungicides namely Autostin 50 WP

(Carbendazim) @ 2.2 g/kg (T_1), Dithane M-45 (Mancozeb) @ 2.2 g/kg (T_2), Rovral (Iprodione) @ 2.0 g/kg (T_3), Provax-200WP (Carboxin+ Thiram) @ 3.0 g/kg of seeds (T_4), and botanicals viz. Garlic extract @ 1:1 w/v (T_5), Garlic extract @ 1:2 w/v (T_6), Neem leaf extract @ 1:1 w/v (T_7), Neem leaf extract @ 1:2 w/v (T_8), Zinger rhizome extract @ 1:1(T_9), Zinger rhizome extract @ 1:2 w/v (T_{10}) and untreated control (T_{11}). The botanicals of 1:1 (w/v) ratio were made in, 100 ml of distilled water with 100 g plant parts and so on. Seeds were treated with fungicides and plant extracts with specified dose as well as distilled water for one hour, respectively. Each treatment consisted of twenty grams of capsicum seed. All the treated seeds were dried back to original moisture content at room temperature. The treated seeds were then kept in storage (15 ^{0}C temperature and 40-60% RH) and seed health status, germination rate and vigor index were tested according to standard methods (ISTA, 1996) just before storage (0 day), 75 days and 150 days after storage (DAS).

Seed health test: Seed health test of capsicum seeds was carried out by standard blotter method (ISTA, 1996). In this method, three layers of blotter papers (Whatman No. 1) were soaked in sterilized distilled water and placed on the bottom of the 9 cm diameter Pyrex glass Petri dish. Four hundred seeds from each treatment were taken randomly and then placed on the moist blotter paper at the rate of 25 seeds per plate. The experiment was laid out following Completely Randomized Design (CRD) with four replications. The Petri-dishes with seeds were then incubated at 25 ± 2 ^{o}C temperature in 12/12 hours alternating cycles of light and darkness for seven days. After incubation, the seeds were examined under stereo-microscope for the presence of seed-borne pathogens and identified by observing their growth characters. In case of confusion, temporary slide was prepared and examined under a compound microscope and identified the pathogens using appropriate keys of Mathur and Kongsdal (2003). Results were expressed in percentage of seeds infected by the pathogens.

Germination percentage: Seed germination test was conducted in the Seed Technology Division laboratory of BARI using three layered moistened blotter paper on Petri-dishes. Hundred seeds were placed on each petri dish and kept at 25^{0}C temperature for fourteen days for germination. Four replications were used for germination test. The first and final count was taken on 4th and 14th day of the test, respectively. Germination percentage was counted by using the following formula:

$$\text{Germination (\%)} = \frac{\text{Normal seedlings germinated}}{\text{Seeds kept for germination}} \times 100$$

Germination speed: Speed of germination was computed by recording daily observations on 100- seeds sown in sand medium until the final count day (14

days). The germination speed (GS)was calculated as total number of seedlings emerged on day basis, and the mean was calculated as suggested by Copeland (1976)

$$GS = \frac{\text{No. of germinated seeds at first count}}{\text{Days of first count}} + \cdots + \frac{\text{No. of germinated seeds at final count}}{\text{Days of final count}}$$

Seedling vigour index: For calculating seedling vigour index, 10 normal seedlings were randomly selected on 14th day of germination test and seedlings were dried in an oven at 71 ºC for 72 hr. Then cooled and weighed in an electronic weighing balance and expressed in mg. Vigour index was calculated on the basis of mean seedling dry weight by adopting the formula (Abdul-Baki and Anderson, 1973).

Vigour index = Germination (%) × Seedling dry weight (mg).

Statistical Analysis: The collected data on different parameters were analyzed statistically by analysis of variance (ANOVA) using Statistix 10 software program. The significance of the difference among the treatment means was calculated by LSD (Least Significance Difference) test.

Results and **Discussion**

Effect of seed treatment on the prevalence of seed- borne fungi :

All the capsicum seeds sample were infected with different levels of infection except T_4 (Table 1). There were seven different fungi viz., *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., *Carvularia* sp., *Colletotrichum* sp., *Penicillium* sp. *and Rhizopus* sp. were identified from the seeds placed in blotter method in laboratory conditions. Asalmol *et al.,* (2001) reported that *Aspergillus flavus, Rhizopus stolonifer, Fusarium moniliforme, Colletotrichum capsici and Aspergillus niger* were the predominant seed borne fungi in the chili seeds. The results also supported the findings of Solanke *et al.* (2001) who reported presence of *Fusarium moniliforme, Colletotrichum capsici*, *Aspergillus niger* (van Tieghem), *Aspergillus flavus* (Linkex Fries), *Alternaria alternata and Curvularia lunata* from the seed samples of different chili cultivars.

The prevalence of fungal pathogens in seeds increased with the increase in storage time. The prevalence of pathogens was minimal just after treatment (0 DAS) with different fungicides and botanical agents. The untreated control showed highest incidence of all the observed pathogens in an upward trend with storage period. The lowest disease incidence was found in T_5 and T_{10} treatments with only one pathogen, *Colletotrichum* sp. The treatment T_5 and T_{10} showed identical results throughout the storage period. There was no pathogenic infection in T_4 treatment even at 150 days after storagethat was treated with Provax-200 (Carboxin + Thiram) @ 3.0g/kg.

Table 1. Effect of different treatments on percent incidence of different pathogens in capsicum seeds at different storage period

Treatments	Isolated pathogens	Percent incidence of fungi		
		0 DAS	75 DAS	150 DAS
T_1	*Alternaria* sp.	-	2.15	5.33
	Carvularia sp.	-	3	4
	Fusarium sp.	-	3.55	5. 57
T_2	*Colletotrichum* sp.	1.25	3.45	7.10
	Fusarium sp.	-	4	6.50
T_3	*Carvularia* sp.	-	2	5
	Alternaria sp.	-	3	7
	Colletotrichum sp.	-	4	9
T_4	-	-	-	-
T_5	*Colletotrichum* sp.	-	1.25	2.75
T_6	*Penicillium* sp.	-	2.0	4.0
	Rhizopus sp.	-	2.0	5.0
	Aspergillus sp.	1.55	3.10	9.15
T_7	*Colletotrichum* sp.	-	2.0	5.1
	Aspergillus sp.	1.33	2	4.25
T_8	*Alternaria* sp.	-	2	5
	Colletotrichum sp.	1.00	2.50	6.50
T_9	*Alternaria* sp.	-	2.15	5.0
	Rhizopus sp.	-	1.50	2.0
	Fusarium sp.	1.25	1.50	2.75
T_{10}	*Colletotrichum* sp.	-	1.50	2.25
Control	*Alternaria* sp.	1.50	4.25	7.25
	Penicillium sp.	1.25	3.0	5.0
	Fusarium sp.	1.75	3.75	7.50
	Colletotrichum sp.	1.50	4.0	9.15
	Aspergillus sp.	1.50	3.2	8.0

DAS- Days after storage; T_1- Autostin 50 WP @ 2.2 g/kg, T_2 -Dithane M-45 @ 2.2 g/kg, T_3 - Rovral @ 2.0 g/kg, T_4-Provax 200 WP @ 3.0 g/kg of seeds, T_5 -Garlic extract @ 1:1 w/v, T_6 -Garlic extract @ 1:2 w/v, T_7 -Neem leaf extract @ 1:1 w/v, T_8 -Neem leaf extract @ 1:2 w/v, T_9 -Zinger rhizome extract @ 1:1, T_{10} -Zinger rhizome extract @ 1:2 w/v and T_{11} -untreated control.

Effect of seed treatment on germination:

At the time of storage, the average initial germination of capsicum seeds was 84%. The treated seeds after 75 days of storage showed significant difference in

germination that ranged from 55.00 to 81.25% (Table 2). Maximum 81.25% seed germination was recorded in seed treated with Zinger rhizome extract @ 1:1 w/v. Seed treated with Neem leaf extract @1:2w/v showed 76.25% germination that was similar to seed treatment with Rovral. The lowest germination (55%) was recorded from the untreated control. In case of 150 DAS, considerably different results in germination rate were observed among the treatments. The higher germination(80.50%) was recorded in the seeds treated with Zinger rhizome extract @ 1:1 w/v followed by Neem leaf extract (74.50%) treated seeds @1:2 w/v while minimum germination was found in control treatment (50.50%).

Table 2. Effect of seed treatment on germination (%) of capsicum seeds at different storage period

Treatments	0 DAS	75 DAS	150 DAS
T_1	84.00	70.25	69.50
T_2		64.50	64.75
T_3		74.00	69.50
T_4		65.00	63.50
T_5		66.00	64.00
T_6		64.25	62.00
T_7		73.50	70.50
T_8		76.25	74.50
T_9		81.25	80.50
T_{10}		63.50	61.50
Control		55.00	50.50
LSD (0.05%)		2.7204	2.3592
CV (%)		2.47	4.54

DAS- Days after storage; T_1- Autostin 50 WP @ 2.2 g/kg, T_2 -Dithane M-45 @ 2.2 g/kg, T_3 - Rovral @ 2.0 g/kg, T_4-Provax 200 WP @ 3.0 g/kg of seeds, T_5 -Garlic extract @ 1:1 w/v, T_6 -Garlic extract @ 1:2 w/v, T_7 -Neem leaf extract @ 1:1 w/v,T_8 -Neem leaf extract @ 1:2 w/v, T_9 -Zinger rhizome extract @ 1:1, T_{10} -Zinger rhizome extract @ 1:2 w/v and T_{11} -untreated control.

Effect of seed treatment on germination speed:

Significant variation in germination speed of capsicum seed was observed in different storage period due to seed treatment with different fungicides and plant extracts **(**Figure 1). Initial germination speed of capsicum seed was 8.657 that reduced to 4.124 in untreated control after 75 DAS. Maximum speed of germination (7.625)was found in the seed treated with Zinger rhizome extract @ 1:1 w/v that was similar to seed treatment with Rovral (7.014) and Provex 200WP (7.180). Germination speed was gradually declined with the increase of storage period. After 150 DAS germination speed of capsicum was reduced to 3.478 in

untreated control. Significantly higher speed of germination (6.619)was found in the seed treated with Zinger rhizome extract @ 1:1 w/v that was also similar to seed treatment with Rovral (6.600) and Provex 200WP (6.365).

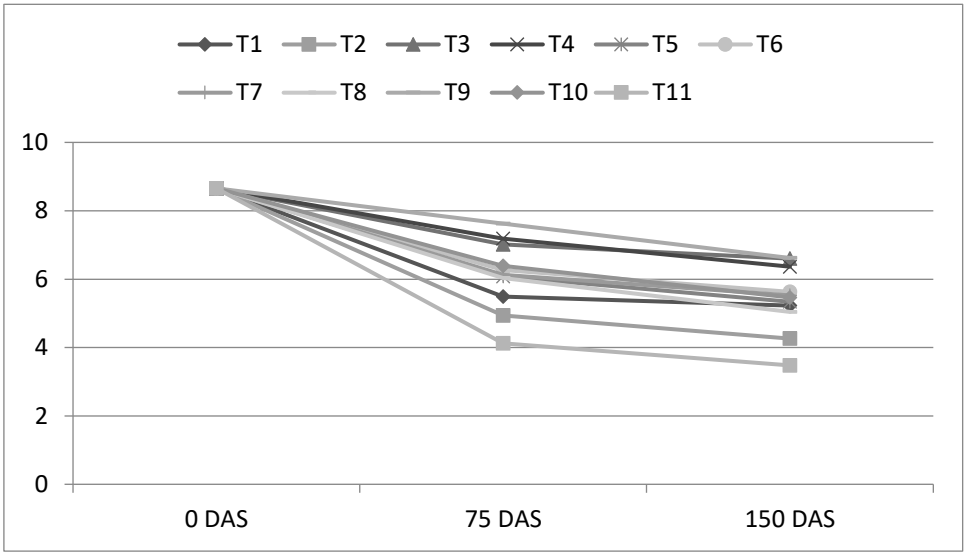

Fig. 1. Germination speed (GS) of capsicum seed due to different storage period.

DAS- Days after storage; T_1- Autostin 50 WP @ 2.2 g/kg, T_2 -Dithane M-45 @ 2.2 g/kg, T_3 - Rovral @ 2.0 g/kg, T_4-Provax 200 WP @ 3.0 g/kg of seeds, T_5 -Garlic extract @ 1:1 w/v, T_6 -Garlic extract @ 1:2 w/v, T_7 -Neem leaf extract @ 1:1 w/v,T_8 -Neem leaf extract @ 1:2 w/v, T_9 -Zinger rhizome extract @ 1:1, T_{10} -Zinger rhizome extract @ 1:2 w/v and T_{11} -untreated control.

Effect of seed treatment on dry weight of seedling:

The initial mean dry weight of 10 seedlings was 36 mg that decreased with the increase of storage period in all treatments (Table 3). The dry weight of capsicum seedlings ranged from 29.25 mg to 36.25 mg after 75 days of storage. Maximum dry weight of seedling was 36.25mg in seed treatment with zinger rhizome extract @1:1 w/v followed by 35.50 mg in Neem leaf extract @ 1:2 w/v that was statistically similar to Zinger rhizome extract @ 1:2 w/v (35.50 mg) and Neem leaf extract @ 1:1 w/v (35.00mg). At 150days of storage the seedling dry weight ranged from 27.00mg to 35.50mg. Statistically similar result was found in case of seedling dry weight from Neem leaf extract @ 1:2 w/v (34.25mg), Zinger rhizome extract @ 1:1 (35.50mg) and Zinger rhizome extract @ 1:2 w/v (33.50mg) where minimum seedling dry weight was recorded from control treatment.

Table 3. Effect of seed treatment on dry weight of capsicum seedlings at different storage period

Treatments	0 DAS	75 DAS	150 DAS
T_1	36.00	32.50	30.75
T_2		33.75	31.00
T_3		33.00	30.75
T_4		33.25	32.25
T_5		31.50	29.75
T_6		31.25	31.00
T_7		35.00	32.75
T_8		35.50	34.25
T_9		36.25	35.50
T_{10}		35.50	33.50
Control		29.25	27.00
LSD (0.05%)		1.8015	2.1323
CV (%)		3.76	4.68

DAS- Days after storage; T_1- Autostin 50 WP @ 2.2 g/kg, T_2 -Dithane M-45 @ 2.2 g/kg, T_3 - Rovral @ 2.0 g/kg, T_4-Provax 200 WP @ 3.0 g/kg of seeds, T_5 -Garlic extract @ 1:1 w/v, T_6 -Garlic extract @ 1:2 w/v, T_7 -Neem leaf extract @ 1:1 w/v,T_8 -Neem leaf extract @ 1:2 w/v, T_9 -Zinger rhizome extract @ 1:1, T_{10} -Zinger rhizome extract @ 1:2 w/v and T_{11} -untreated control.

Effect of seed treatment on seedling vigour index

Initial vigour index value of capsicum seedlings was 3025 before storing of seeds (Figure 2). A decreased trend of seedling vigour index value was found with the storage period increased. After 75 days of storage, the vigor index of capsicum seedlings was 2945 in case of Zinger rhizome extract @ 1:1w/v treated seeds and the lowest vigour index value 1610 was found in untreated control. In case of 150 days of storage, the vigour index value was decreased to 2858 in Zinger rhizome extract @ 1:1 w/v treated seeds that was significantly higher than the other treatments. Lower value of seed vigour index value (1362) was observed in control.

Provax-200, Rovral, Dithane M-45, Autostin 50 WP and the plant extracts were found effective in controlling seed-borne pathogens of capsicum seeds in storage condition and also improved seedling germination and seedling vigour index to a considerable extend. Provax-200WP (Carboxin+ Thiram) @ 3.0g/kg, Garlic extract @ 1:1 w/v, Zinger rhizome extract @ 1:1 w/v, and Zinger rhizome extract @ 1:2 w/v showed promising result in case of pathogens reduction, germination rate, speed of germination, seedling dry weight and vigour index throughout the storage period of capsicum seed. Efficacy of Autostin 50 WP, Rovral 50 WP and Provax-200 was reported against *F. oxysporum* and *Aspergillus* sp.

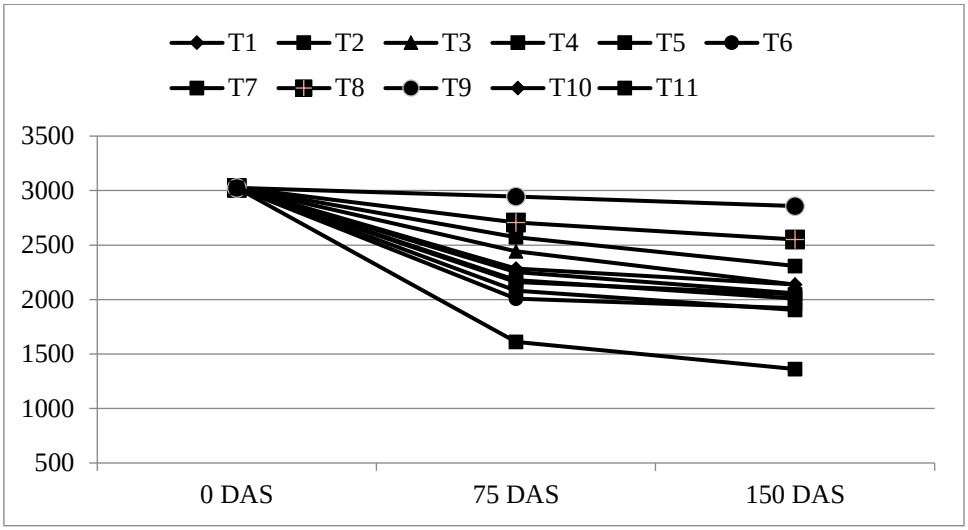

Fig. 2. Seedling vigor index at different storage period.

DAS- Days after storage; T_1- Autostin 50 WP @ 2.2 g/kg, T_2 -Dithane M-45 @ 2.2 g/kg, T_3 - Rovral @ 2.0 g/kg, T_4-Provax 200 WP @ 3.0 g/kg of seeds, T_5 -Garlic extract @ 1:1 w/v, T_6 -Garlic extract @ 1:2 w/v, T_7 -Neem leaf extract @ 1:1 w/v,T_8 -Neem leaf extract @ 1:2 w/v, T_9 -Zinger rhizome extract @ 1:1, T_{10} -Zinger rhizome extract @ 1:2 w/v and T_{11} -untreated control.

Fungicides like Carbendazim, Thiophanate-methyl, Benomyl, Prochloraz and Tebuconazole showed inhibiting effects against *F. oxysporum* and their application increased the emergence of seedlings (Song *et al*., 2004; Ozer and Koycu, 2004; Rajput *et al.,* 2006; Chandel and Deepika, 2010). Abbas *et al.* (2020) reported that seed treatment with Mancozeb 80% WP effectively control seed borne fungi and increase the seed germination in capsicum. Plant extract was a promising alternative for pathogens control associated with seeds, reducing costs and impacts to the environment (Silva *et al.,* 2019). Plant extracts act directly or indirectly on pathogens growth in the seed due to their bioactive compounds. In the present study; all the botanicals especially Zinger rhizome extract @ 1:1 w/v and Zinger rhizome extract @ 1:2 w/v significantly reduced seed borne fungal pathogens of capsicum in storage and enhanced the growth of capsicum seedling. The similar results in reducing percent seed borne fungal pathogens and enhancing growth characters of vegetable seedling were also found by others (Islam *et al*., (2006); Hossain *et al.,* (2005); Howlader (2003); Rahman *et al.,* (2012); Khan *et al.,* (1995).

Conclusions

Seed treatment with different plant extracts and fungicides greatly contributed to seed health status and quality of capsicum seed during storage period. It was revealed that Provax-200 (Carboxin + Thiram) @ 3.0g/kg was the most effective

fungicide which reduced seed-borne pathogen infection in capsicum seed during storage. Significantly higher germination rate, speed of germination, seedling dry weight and vigour index was found in capsicum seed treated with zinger rhizome extract @ 1:1 w/v up to 150 days of storage. Therefore, pre-storage seed treatments with Provax-200 (Carboxin + Thiram) @ 3.0g/kg was suggested as effective fungicide to control seed borne fungi where zinger rhizome extract@ 1:1 w/v was also good for germination rate, vigour index and quality of capsicum seeds in storage.

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