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Research Paper

Improving Quality and Storage Life of Watermelon, Capsicum and Okra Seeds by Polymer and Fungicide Film Coating

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Abstract

Seed-film coating is a process of applying useful materials to form a continuous layer of thin coating over the seed. An experiment was conducted to study the efficacy of seed-film coating on improving seed quality, storability and the effectiveness of the control of seed-borne pathogens and seed contaminants. A thin film coat containing a combination of a film coating liquid (a readymade liquid including a polymer and colorants) + distilled water + Thiram 80% WP was applied on watermelon, capsicum and okra seeds from selected lots. Five different seed-film coating formulations were tested with a non-coated control for each seed lot. Seed quality parameters namely germination percentage, seed moisture percentage, seed health status and seedling vigour were evaluated just after film coating 4 and 11 months after storage using standard protocols. All seed coating formulations tested showed a significant positive effect on the control of seed-contaminating fungi, increasing germination percentage and enhancing seedling vigour compared to non-coated control while no significant difference (*P*>0.05) was observed among different seed coating formulations on each tested seed lot. Therefore, seed-film coating formulations including 10 g of film coating liquid + 50 mL of distilled water + 0.5 g of Thiram for 1 kg of watermelon seeds, 25 g of film coating liquid + 90 mL of distilled water + 0.5 g of Thiram for 1 kg of Capsicum seeds and 4 g of film coating liquid + 14 mL of distilled water + 0.5 g of Thiram for 1 kg of Okra seeds are recommended as effective seed-film coatings for improving seed quality and enhancing storage life.

Keywords: Seed-borne pathogens, Seed-film coating, Seed germination, Seed storage

Introduction

Production of genetically pure seeds and preservation of the quality of seeds until sowing are the two most important aspects in maintaining the continuous supply of high-quality seeds to producers. Maintenance of high seed quality during seed storage is of great significance (Sushma, 2013). Seed deterioration is an undesirable and unfavorable characteristic for seed industry and viable agriculture. Several biological factors namely pest infestation and infection by seed-borne pathogens contribute heavily to seed deterioration thus making it difficult to maintain seeds viability during storage. Furthermore, seed quality and viability also depend upon the initial quality of the seed and method of storage.

Seed coating is one of the most economical approaches for improving the performance of seeds (John *et al*., 2005). It is a process of applying useful materials to form a continuous layer of thin coating over the seed without altering the shape or size of seeds by employing water as the solvent (Dixit *et al*., 2018). Such formulations consist of a mixture of polymer, plasticizer, and colorants that are commercially available as ready-to-use liquids (John *et al*., 2005; Verma and Verma, 2014). Seed coating has long been a practice specially used in seed of vegetables, forest plants and ornamentals. This advanced technology allows the application of a combination of nutrients, fungicides, insecticides, herbicides and beneficial microorganisms on to the seed. Seed treatment with various combinations of polymers and pesticides requires only a small financial investment but assured an excellent return to the investment (Corlett *et al*., 2014).

The positive and significant responses of seed coating alone or in combination with pesticides, bio-agents etc. on germination, seedling growth, field performance, enhanced yield, control of seed-borne infections and increasing seed storability have been reported by several workers (John *et al*., 2005; Mehrabi and Chaichi, 2012; Sushma, 2013; Corlett *et al*., 2014; Verma and Verma, 2014; Dixit *et al*., 2018). Furthermore, reducing the exposure of agricultural workers to pesticides, eliminating or minimizing product dust off, minimizing leaching of pesticides from the treated seeds, enabling accurate and even dose of chemicals and reducing chemical wastage were also stated as positive effects of seed coating. Polymer-coating makes the sowing operation easier due to smooth seed handling. The addition of colorants helps in visual monitoring of accurate placement enhance the appearance, marketability and consumer preference (Verma and Verma, 2014).

Considering the benefits of seed coating using polymer-based material, this study was conducted with the objectives of studying the efficacy of seed-film coating on improving seed quality, storability and the effectiveness of the control of seed-borne pathogens and seed contaminants in seeds produced in Sri Lanka and to establish an appropriate seed-film coating technology for crop seeds.

Materials and Methods

Seed samples for the study were collected from Seed and Planting Materials Development Centre (SPMDC), Peradeniya, Sri Lanka. The study was conducted at Central Seed Testing Laboratory (CSTL), Peradeniya of Seed Certification Service (SCS), and SPMDC, Peradeniya from 2017 to 2019.

Three crops propagated through seeds namely, capsicum (*Capsicum annuum*), okra [*Abelmoschus esculentus*] and watermelon [*Citrullus lanatus*] were selected for the study. Based on the prior analysis for seed quality parameters conducted at the CSTL and Seed Health Testing Unit (SHTU) capsicum var. CA8 (Lot No. V/1/17/ALU/St/005), okra var. Haritha (Lot No. V/1/16/MIL/St/004) and watermelon var. Thilini (Lot No. R/1/17/MIL/St/) were used.

Seed-film coating was done at the Vegetable Seed Centre (VSC) at Peradeniya. A film coating liquid (ready-made liquid containing a polymer and colorants) + fungicide (0.5 g Thiram 80 WP) in 50, 90, 14 mL distilled water was used for watermelon, capsicum, okra seeds respectively. These rates were selected as the most effective based on the results of the initial experiments. Readymade film coating liquid with different colours (inert matter) was used for seed coating. Five different seed-film coating formulations (T_1, T_2, T_3, T_4 and T_5) were evaluated with a non-coated control (T_0) for each seed lot (Table 1). The dosage of polymer applied to seeds is limited by their adherence on to seeds and ranges from 0.6 – 1.4% of seed weight. Polymer materials are marginally more viscous than most chemicals that are used as pesticides in seed treatments (Corlett *et al*., 2014). Therefore, a seed coating machine was used at VSC to apply the coating with high precision.

The moisture content of seeds would increase after film coating and thus, the film-coated seed lots were separately air-dried. A seed dryer was used at 35 – 40 °C for 4 – 10 hours depending on the size of seeds to reduce the moisture content below the maximum allowable levels of 9, 11, and 12% for capsicum, okra, and watermelon respectively according to the Seed Certification Standards (DOA, 2009).

Table 1. Amounts of seed film coating components used per kg seed

Treatment	Polymer (g)			Fungicide (g)			Distilled water (ml)		
	Water melon	Capsicum	Okra	Water melon	Capsicum	Okra	Water melon	Capsicum	Okra
T_o	0	0	0	0	0	0	0	0	0
T_1	10	25	4	0.5	0.5	0.5	50	90	14
T_2	13	28	5	0.5	0.5	0.5	50	90	14
T_3	15	30	6	0.5	0.5	0.5	50	90	14
T_4	17	32	7	0.5	0.5	0.5	50	90	14
T_5	20	35	8	0.5	0.5	0.5	50	90	14

The dried seed lots were separately stored in cold stores at 15 °C temperature at the VSC at Peradeniya. Seeds from each treatment from each seed lot were sampled at 4 and 11 months after film coating and handed over to relevant laboratories to study the quality parameters namely germination percentage, moisture percentage and seedling vigour and seed health status.

The test protocols recommended by the International Rules for Seed Testing (ISTA) was used to evaluate seed germination (Chapter 5, ISTA, 2014) and seed moisture content (Chapter 9, ISTA, 2014). Seed health was evaluated using the standard blotter method (Chapter 7, ISTA 2017) to detect the percentage of infection of different fungal pathogens in each treatment. Identification of seed inhabiting fungi was done using morphological characters. Characteristics of Mycelia conidiophores and spore etc. were observed through a stereo-binocular microscope at a maximum magnification of x100 and compound microscope at magnifications from x 40 to x 400 using the standard guides and manuals (Mathur and Kongsdal, 2003).

Enzyme- Linked Immunosorbent Assays (ELISA) were performed to detect the presence of seed-borne viral pathogens in capsicum and watermelon seed lots However, okra seeds were not used for the study as okra is not affected by seed-borne viruses. For each assay, 100 seedlings were used from each treatment in two replicates. Visual colour development and absorbance values obtained through an ELISA reader (Multiskan FC Microplate Photometer, Thermo Scientific) were used for comparisons.

A washing test was used to determine the presence of bacterial pathogens in watermelon seed lots by using 100 randomly selected seeds from each treatment. These seeds were suspended in sterile distilled water + Tween 20 (0.02% v/v) in a conical flask. Flasks were shaken in an orbital shaker at 100-125 rpm for 2½ hours at room temperature. Serial dilutions were prepared from each seed extract. A 100 µl from each dilution was cultured in semi-selective media for the detection of Bacterial Fruit Blotch (BFB) pathogen. Plates were incubated for 4 days under 40 °C temperature. Incubated culture plates were examined under stereo-microscope to detect the presence of BFB pathogen, *Acidovorax avenae* sub sp. *citrulli.* Other suspected bacterial colonies in different treatments were cultured on Nutrient Agar media for isolation and determination of the pathogenicity.

A randomly selected 100 seeds from each treatment of each seed lot was used for the determination of seedling vigour. Test tube slants were prepared by pouring 10 ml of 2% water agar, sterilized in an autoclave under 15 psi at 121 °C for 20 min. Twenty-five seeds were placed in test tubes at a rate of one seed per test tube with four replicates for each treatment and incubated at 28 °C for 10 days. After 10 days, the seedlings were taken out from each test tube and the root length was measured from the collar region to the tip of the primary root. The shoot length was measured from the collar region to the tip of the leaves. Seedling Vigor Index (SVI) was calculated using the Equation 1 (Baki and Adnerson, 1973).

SVI = Seed germination % × Seedling length [Shoot length (cm) + Root length (cm)] Eq. 1

Samples were drawn from stored seed lots at 4 and 11 months after film coating, and subjected to laboratory testing. Germination percentage, moisture percentage, SVI and seed associated fungi were determined in each treatment of watermelon, capsicum and okra seed lots at different storage periods (*i.e.*, initial - after seed coating/ before storing, four months after storage, and eleven months after storage) as described above. Quality parameters of watermelon seeds were evaluated until 4 months of storage as locally produced seeds are normally stored for subsequent growing season.

All laboratory experiments were conducted in Complete Randomized Designs (CRD) with a maximum of four replicates. The data obtained on various parameters were statistically analyzed using analysis of variance (ANOVA) and mean separation was done using Duncan's Multiple Range test (P=0.05) of the SAS statistical analysis system version 9.1.

Results and Discussion

Effect of seed-film coating on germination percentage

Germination percentages of film-coated watermelon seed lots were not significantly different (P>0.05) from the non-coated control during the early stages of seed storage (Table 2). However, with increasing storage time, the germination percentage of the watermelon seed lot was reduced in non-coated control (88%) compared those of the film- coated seeds. Among seeds lots treated with different seed coating formulations, the germination percentages were not significantly different (P>0.05).

The germination percentage of capsicum seeds in non-coated control was significantly lower (P<0.05) than the film-coated seed lots at the time of storage after seed coating, 4 months after seed coating and 11 months after seed coating.

Table 2. Germination percentages of watermelon, capsicum and okra in different treatments at different storage times

Treatment	Germination %							
	Watermelon		Capsicum			Okra		
	Initial (before storing)	4 months after treatment	Initial (before storing)	4 months after treatment	11 months after treatment	Initial (before storing)	4 months after treatment	11 months after treatment
T_0	96.0 ^{a}	88.0 ^{b}	84.0 ^{b}	79.0 ^{c}	77.0 ^{b}	94.0 ^{c}	96.0 ^{ab}	81.0 ^{c}
T_1	96.0 ^{a}	99.0 ^{a}	92.5 ^{a}	96.5 ^{a}	92.5 ^{a}	99.0 ^{a}	98.0 ^{a}	94.0 ^{ab}
T_2	99.0 ^{a}	97.0 ^{a}	89.5 ^{a}	95.5 ^{ab}	93.5 ^{a}	95.0 ^{bc}	94.0 ^{b}	97.0 ^{a}
T_3	97.0 ^{a}	96.0 ^{a}	92.0 ^{a}	94.5 ^{ab}	95.5 ^{a}	98.0 ^{ab}	97.0 ^{ab}	95.0 ^{ab}
T_4	97.0 ^{a}	97.0 ^{a}	87.5 ^{ab}	93.0 ^{b}	93.5 ^{a}	97.0 ^{abc}	97.0 ^{ab}	92.0 ^{b}
T_5	97.0 ^{a}	95.0 ^{a}	92.0 ^{a}	94.0 ^{ab}	93.0 ^{a}	98.0 ^{ab}	97.0 ^{ab}	95.0 ^{ab}

Within a column, means followed by the same letters are not significantly different by the DMRT at p = 0.05

The lowest seed germination percentage was shown in the non-coated control (77%). Significantly higher germination percentages (>90.0%) were recorded in all film-coated seed lots.

In the case of okra seeds, the germination % among all seed coating treatments and the non-coated control was not significantly different ($P>0.05$) at the initial storage and 4 months after storage. However, a drastic drop in the germination percentage was observed in non-coated control at 11 months after storage ($P<0.05$) compared to that of the film-coated seed lots. The lowest germination percentage was recorded in the non-coated control (81%).

Germination percentage of watermelon, capsicum and okra has declined with the increasing storage period in most of the treatments in the seed lots of all three crops. Similar findings were observed by Sushma (2013) where a progressive decline of the germination percentage was reported with an increase in the storage period in both coated and non-coated seeds of chickpea, attributing to the phenomenon of natural ageing, depletion of food reserves, and decline in metabolic activity of seeds due to respiration.

The significantly low germination percentage in the non-coated control of watermelon, capsicum and okra seed lots compared to the seed lots coated with different film coating formulations reflects a clear positive relationship of seed-film coating with the percentage germination of watermelon, capsicum and okra seeds. These observations are in line with the findings of various workers; where a positive and significant response of seed coating on germination percentage has been reported in chickpea (Sushma, 2013), maize (John *et al.*, 2005), soybean (Verma and Verma, 2014), and wheat (Dixit *et al.*, 2018).

Effect of seed-film coating on moisture percentage of seed lots

Seed moisture content in the non-coated control was comparatively higher than that of all seed coated treatments of all seed lots tested throughout the storage period (Table 3).

Table 3. Effect of seed-film coating on the moisture content of watermelon, capsicum, okra seed lot

Treatment	Seed Moistures %							
	Watermelon		Capsicum			Okra		
	Initial (before storing)	4 months after treatment	Initial (before storing)	4 months after treatment	11 months after treatment	Initial (before storing)	4 months after treatment	11 months after treatment
T_0	9.0 ^{a}	9.52 ^{a}	9.0 ^{a}	9.22 ^{a}	9.16 ^{a}	11.0 ^{a}	10.77 ^{a}	10.98 ^{a}
T_1	4.75 ^{e}	5.4 ^{bc}	6.10 ^{c}	6.41 ^{b}	6.59 ^{b}	9.15 ^{c}	8.92 ^{b}	9.35 ^{c}
T_2	5.3 ^{b}	5.28 ^{c}	5.99 ^{d}	6.19 ^{d}	6.41 ^{b}	9.07 ^{d}	8.90 ^{b}	9.32 ^{c}
T_3	4.75 ^{e}	5.30 ^{c}	5.99 ^{d}	6.32 ^{bc}	6.42 ^{b}	9.11 ^{cd}	8.93 ^{b}	9.55 ^{b}
T_4	5.06 ^{c}	5.02 ^{e}	6.06 ^{cd}	6.41 ^{b}	6.39 ^{b}	9.23 ^{b}	8.95 ^{b}	9.62 ^{b}
T_5	4.88 ^{d}	5.53 ^{b}	6.24 ^{b}	6.28 ^{cd}	6.40 ^{b}	9.05 ^{d}	8.90 ^{b}	9.48 ^{bc}

Within a column, means followed by the same letters are not significantly different by the DMRT at p = 0.05

The maximum moisture percentages were reported in non-treated control were 9.16% (watermelon), 9.2% (capsicum) and 11.24% (okra) (data not shown). The seed moisture percentage among film-coated treatments (T_1 to T_5) in each seed lot were similar (P>0.05; Table 3). Moisture content is a major factor that affects the deterioration of seed quality, especially during storage. During the process of seed-film coating, the coated seeds were dried well before storing and the moisture absorption into seeds during cold storage may be restricted due to coated polymer layer.

Effect of seed-film coating on seed-borne pathogens and seed contaminants

Aspergillus spp., *Rhizopus* spp., *Penicillium* spp., *Fusarium* spp., *Curvularia* spp., *Alternaria* spp., and other saprophytic fungi were detected by the blotter test in watermelon, capsicum, and okra seed lots. Total fungal infection as percentage values in each treatment of watermelon, capsicum and okra seed lots are illustrated in Figures 1, 2, and 3, respectively.

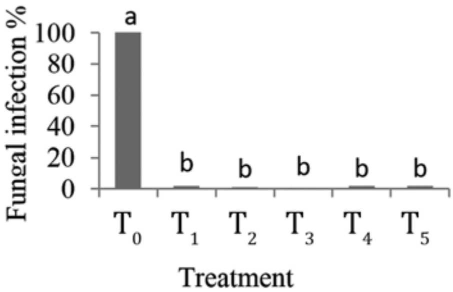

Figure 1. Average fungal infection percentages of water melon seed lots

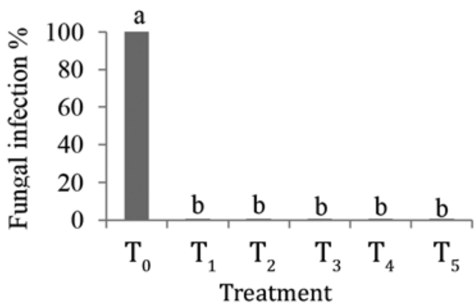

Figure 2. Average fungal infection percentages of capsicum seed lots

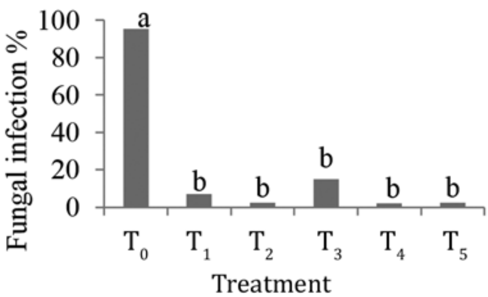

Figure 3. Average fungal infection percentages of okra seed lots

A similar pattern was observed in the presence of seed contaminating fungal pathogens and the infection % among six different treatments in watermelon, capsicum and okra seed lots. The percentage infection of seed contaminating fungal pathogens in non-coated control in each seed lot was significantly higher (P<0.05) than the film-coated seed lots. However, the presence of seed contaminants was low in film-coated seed lots.

Among seed contaminating fungal pathogens detected in this study, a high infection percentage of *Aspergillus* spp. was observed in tested seed lots, especially in non-coated control. A positive linear correlation between the presence of *Aspergillus* spp. and the reduction of seed germination percentage has been detected by previous studies conducted by Jayarathna *et al*. (2021).

Percentage infection of *Aspergillus* spp. was significantly higher (P<0.05) in the non-coated control (T_0) in each seed lot of watermelon (41.5%), capsicum (52.3%) and okra (59.9%) compared to that of respective film-coated seed lots. However, the *Aspergillus* infection among seed coated treatments in each seed lot was not significantly different (P>0.05; Figure 4).

The percentage infection of *Rhyzopus* spp. and other saprophytes also showed a similar behavior to *Aspergillus* spp., while the infection rates of *Penicillium* spp., *Fusarium* spp., *Curvularia* spp., *Alternaria* spp., in all treatments were relatively low (data not shown).

Irrespective of the treatments, *Zucchini yellow mosaic virus* (ZYMV) was detected by ELISA test, as a seed-borne viral pathogen in all coated and non-coated seed lots of watermelon at the initial stages of storage. The results suggest that seed coating formulations used may not have an impact on the control of ZYMV infection in watermelon. Seed-borne bacterial pathogens were not detected in any of the seed lots tested in the study.

The results revealed a significant positive effect of seed-film coating on the control of fungal contaminants, especially *Aspergillus* spp. The effects of different fungicide seed treatments on suppressing seed-borne fungal infections with the enhancement of germination of treated seeds was reported by Sushma (2013) for chickpea, which may be due to the pathogen- and pest-suppressive nature of chemicals.

Effect of seed-film coating on Seedling Vigor Index

Irrespective of the treatments, the seed vigor index (SVI) declined gradually in the majority of the treatments with increasing storage period of watermelon seed lot (Table 4). A significant difference of the initial SVI was not observed (P>0.05) among the treatments.

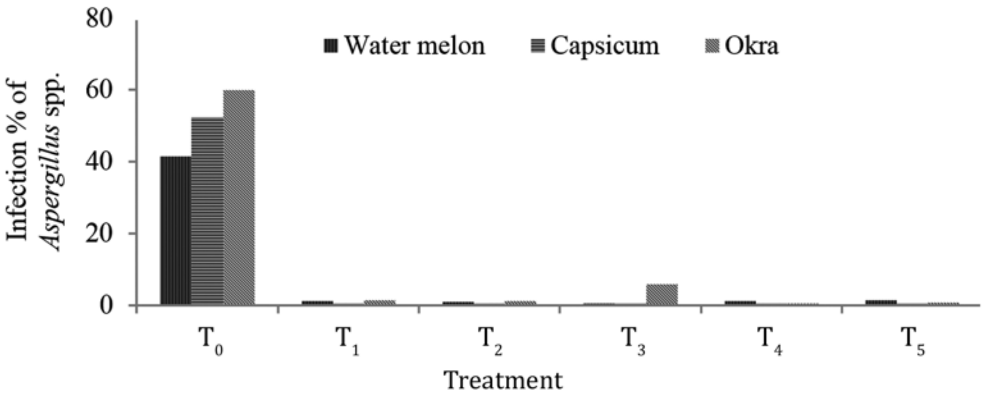

Figure 4. Percentage Infection of *Aspergillus* spp. in different treatments

Table 4. Effect of seed-film coating on Seeding vigour index (SVI) of watermelon, capsicum and okra seed lot

Treatment	Seedling vigour index							
	Watermelon		Capsicum			Okra		
	Initial (before storing)	4 months after treatment	Initial (before storing)	4 months after treatment	11 months after treatment	Initial (before storing)	4 months after treatment	11 months after treatment
T_0	1817 ^{a}	972 ^{e*}	253 ^{c*}	47 ^{d*}	318 ^{c*}	2186 ^{a}	2436 ^{a*}	1591 ^{c*}
T_1	1852 ^{a}	1845 ^{b}	588 ^{ab}	373 ^{a}	535 ^{ab}	2130 ^{a}	2463 ^{a}	1772 ^{bc}
T_2	1879 ^{a}	1601 ^{cd}	726 ^{a}	158 ^{cd}	576 ^{a}	2255 ^{a}	1959 ^{b}	2122 ^{a}
T_3	2002 ^{a}	2098 ^{a}	632 ^{ab}	316 ^{ab}	530 ^{ab}	2226 ^{a}	2493 ^{a}	1967 ^{ab}
T_4	1790 ^{a}	1687 ^{bc}	558 ^{b}	238 ^{bc}	358 ^{bc}	2038 ^{a}	2189 ^{ab}	1938 ^{ab}
T_5	1766 ^{a}	1451 ^{d}	520 ^{b}	416 ^{a}	545 ^{a}	2164 ^{a}	2530 ^{a}	1729 ^{bc}

Within a column, means followed by the same letters are not significantly different by the DMRT at p = 0.05

However, with increasing storage period, a significantly lower SVI ($P<0.05$) was recorded in the non-coated control than in the coated seed lots. A significant difference could not be observed among seed lots coated with different film-coating formulations (Table 4).

The non-coated seeds of capsicum showed a lower SVI ($P<0.05$) compared to that of the coated seed lots (Table 4). Similar findings were reported by Sherin *et al*. (2005), where the coating treatment showed a significant improvement in seed germination and vigour of maize over the control. Results of the present study are also in conformity with the findings of Sushma (2013) for chickpeas.

A clear difference of SVI was not observed among the coated and non-coated seed lots of okra ($P>0.05$) throughout the storage period (Table 4). Okra seeds also did not show a significant difference ($P>0.05$) in seed germination among treatments up to 4 months of storage. Therefore, the effect of seed coating on SVI of okra for long-term storage should be studied further.

According to the results of the seed quality parameters tested on watermelon, capsicum, and okra seed lots, all seed coating formulas tested showed a significant positive effect ($P<0.05$) on the control of seed contaminating fungi, increasing germination percentage, and enhancing seedling vigour compared to the non-coated control.

Sushma *et al*., (2013) also reported that, the polymer coat and seed treatment chemicals have increased shoot length, root length, seedling dry weight and lowered electrical conductivity of seeds under laboratory conditions and improved field performance (*i.e*., increasing of the number of branches, number of pods per plant, number of seeds per plant, seed weight per plant, and seed yield per hectare) in chickpea.

Since, no significant effect ($P>0.05$) was observed among the different seed coating formulations tested on seed quality parameters, the lowest application rate of seed coating liquid formulations (T_1) for each crop type tested can be recommended for seed-film coating of watermelon, capsicum, and okra seeds.

Conclusions

Film-coated seeds of watermelon, capsicum, and okra with five different formulations including a fungicide, different rates of film coating liquids and distilled water recorded higher germination percentages, higher seedling vigour and control of seed-borne contaminants and, reducing seed moisture content over control. Hence, seed-film coating formulations with 10 g of film coating liquid + 50 mL of distilled water + 0.5 g of Thiram for 1 kg of watermelon seeds, 25 g of film coating liquid + 90 mL of distilled water + 0.5 g of Thiram for 1 kg of capsicum seeds and 4 g of film coating liquid + 14 mL of distilled water + 0.5 g of Thiram for 1 kg of okra seeds can be recommended as an effective seed-film coatings suitable for improving quality of seeds and increase their storage life.

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