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Treatment with Thiabendazole can enhance germination, vigor and reduce incidence of fungi in zucchini seeds

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Abstract

Some chemical treatments might be required to eradicate or reduce the presence of fungi in seeds. However, these treatments can impair seed quality depending on the dose used. The purpose of the present study was to evaluate the control of fungi and physiological quality of 'Aline' hybrid zucchini seeds treated with the fungicide Thiabendazole. Fifteen treatments, were set up as a 3x5 factorial system (3 seed lots x 5 Thiabendazole doses: 0%, 0.12%, 0.2%, 0.3% and 0.4% active ingredient), were evaluated, in a completely randomized experiment design, with four replications. The commercial product used was Tecto® SC (485 g L^{-1} of Thiabendazole). Seed germination, germination first count (GFC) and incidence of fungi in seeds were assessed. The averages were compared with Tukey test at 5% probability. Some fungi species (*Alternaria* sp., *Aspergillus* spp., *Cladosporium* sp., *Colletotrichum* sp., *Curvularia* spp., *Penicillium* spp. and *Phoma* sp.) were detected in the pathology analysis and a general decrease of fungi incidence was observed with an increase in the Thiabendazole dose. Regardless of the dose, Thiabendazole fungicide did not affect total germination (99.6% on average) or vigor (99.3% on average in GFC). So, it can be recommended that treatment of zucchini seeds with Thiabendazole with dose 0.3% or 0.4% can control the observed fungi.

Keywords: *Cucurbita pepo*; fungicide; physiological quality; seed pathology; seed treatment.
Abbreviations: a.i._active ingredient, DAS_days after sowing, GFC_Germination First Count, SGT_Standard Germination Test

Introduction

Seed performance in field is determined mainly by it physiological and sanitary quality and association of pathogens and seeds can severely affect seed quality, reducing potential in terms of germination, vigor, seedling emergence, field sanity and production (David et al., 2014). It is not always possible to obtain seed lots 100% guaranteed free of pathogens. It is also not possible to ensure that the soil or substrate will be clear of fungi. Therefore, seed treatment is advisable in most cases, especially for vegetable hybrid seeds, where the cost of treatment is very low compared to the high price for these types of seeds.

Seed chemical treatment aims to eradicate pathogens related to them and/or to protect seeds against soil pathogens, especially in the event of germination (Mancini and Romanazzi, 2014). Seed treatment effectiveness depends on, among other factors, seed species and seed vigor, which may vary from lot to lot (Menten and Moraes, 2010). However, the treatment should not impair seed physiological quality (Cardoso et al., 2015a). In infected seed lots, the proper seed treatment can enhance seed germination and emergence as observed in many species (Kaiser and Hannan, 1987; Carvalho et al., 2004; Campos et al., 2009; Poletine et al., 2012), but in very high doses it can cause phytotoxic effects (Kaiser and Hannan, 1987). According to Menten and Moraes (2010), there are 19 active ingredients of fungicides registered for seed treatment in Brazil, although this registration is specific for only some species. One of these active substances is Thiabendazole, a member of the Benzimidazole chemical group, registered in Brazil for seed treatment in only a few crops. According to the Ministry of Agriculture, Livestock and Supply (MAPA, 2015), the commercial product Tecto® SC, active ingredient Thiabendazole, is a systemic fungicide registered for soybean and sunflower seed treatment; for avocado, banana, citrus, papaya, mango, and melon fruits in postharvest treatment and plant treatment of avocado, pineapple, coconut, pea, snap pea, papaya, mango, passion fruit, melon, and sweet pepper species. Thus, considering the Cucurbitaceae family, this fungicide has only been registered for treating melon for spraying plants or postharvest treatment in fruits, targeting control of fungi *Colletotrichum orbiculare*, and *Didymella bryoniae*. Therefore, this product has not been registered for vegetable seed treatment. Seed of several species have been treated with this fungicide. It has been found that this fungicide can control many fungi, such as *Ascochita lentis*, *Aspergillus* sp., *Botrytis* sp., *Curvularia* sp., *Fusarium* sp., *Leptosphaeria maculans*, *Penicillium* sp (Maude et al., 1984; Kaiser and Hannan, 1987; Pinto, 2000; Mendes et al., 2001; David et al., 2014; Cardoso et al., 2015b). Also, it has been reported that this fungicide does not impair seed physiological quality in species, such as alfalfa (Mendes et al., 2001), lentil (Chang et al., 2008), melon (Cardoso et al., 2015b), maize (Pinto, 2000; Fessel et al., 2003; Carvalho et al., 2004), soybean (Pereira et al., 2011) and wheat (Garcia

Júnior et al., 2008). In regard to zucchini, no studies on the use of this fungicide to treat seeds were found.

Given this background, the purpose of the present work was to evaluate physiological quality (germination and vigor) and the control of fungi in different lots of zucchini seeds treated with different doses of Thiabendazole.

Results and Discussion

Seeds germination and vigor

There was no interaction between factors (lots and fungicide doses), indicating mutual independence for germination and first count. The results for both variables are shown in Table 1.

Although all three zucchini seed lots display excellent quality, with minimum germination rate above 99%, lot 78021 showed lesser germination (99.1%) compared to the other two lots (76788 and 79999) (Table 1). The rates demonstrated for germination on three lots are much higher than the minimum standard rate of 75% for zucchini required for commercialization in Brazil by the Ministry of Agriculture, Livestock and Supply. However, according to Cardoso et al. (2014), due to higher cost of hybrid seeds and competition between companies for the market, the official standards are outdated in relation to market practices. It is rare to find cucurbit hybrid seeds in the market with less than 90% germination rate. Even with this reference frame, all the lots can be considered to have excellent physiological quality. The first seed count (4^{th} DAS) is considered a vigor test. The samples with faster germination and higher percentage of normal seedlings on this date are considered more vigorous (Baalbaki et al., 2009). Lot 78021 displayed less vigor in this feature than lot 76788, assessed by GFC (Table 1). However, even the lessest vigorous lot showed high germination at 4^{th} DAS, 98.7% on average. Considering Thiabendazole doses, there was no difference in germination (average of 99.6%) and vigor (first count, average of 98.7%), showing that, in the doses evaluated, the fungicide did not affect physiological quality of zucchini seeds in any lot (Table 1). According to Groot et al. (2006, 2008), Lobo (2008) and Menten and Moraes (2010), sensitivity to fungicide treatment can vary according to the initial quality of seed lots. In this study, despite differences in germination between lots, none of the seeds in any of the lots were affected by the fungicide treatment, regardless of the dose. Garcia Júnior et al. (2008) found no phytotoxic effect for this fungicide on wheat seeds, verifying germination and seedling emergence similar to untreated control seeds. This was, also, observed by Mendes et al. (2001) in alfalfa seeds, Gally et al. (2004) in soybean seeds, and Cardoso et al. (2015b) in melon seeds. However, Fessel et al. (2003) reported that high doses of this fungicide could impair maize seed quality. In contrast, Carvalho et al. (2004) found higher maize seed germination in different treated lots compared to the untreated control lot, because the fungicide reduced incidence of "damping off" caused by the fungus *Stenocarpella maydis* presents in seeds. The positive effects in germination and/or seedling emergence with seed treatment using this fungicide were also described for papaya seeds (Campos et al., 2009) and castor bean seeds (Poletine et al., 2012). Also, Kaiser and Hannan (1987) verified an increase in seedling emergence for lentil seeds treated with Thiabendazole, mainly due to control of fungus *Ascochita lentis*. Nevertheless, in very high doses, this fungicide caused phytotoxic effects in lentil seedlings. In this research, considering the assessed doses (0 to 0.4%), the fungicide did not affect germination and vigor for zucchini seeds. These contrasting results reported by different authors show the importance of conducting studies for each different species because the results may not always be the same.

Mancini and Romanazzi (2014) emphasized that vegetable seed treatment is not a substitute for using pathogen-free seeds. However, due to the difficulty of obtaining lots 100% pathogen-free and considering the presence of pathogens in the soil or substrate where the seeds are cultivated, treating seeds is the most effective method for achieving maximum seedling emergence.

Seed pathology analysis

Regarding treated seed sanitary, pathogenic species were not detected in the seeds, notably *Didymella bryoniae*, one of the main seedborne pathogens in zucchini. For saprophytic fungal species, non-significant interaction was verified for *Cladosporium* sp. (Table 2), with a higher incidence in lots 76788 and 79999, with no difference between them. Regardless of the lot, this fungus incidence decreased as the fungicide dose increased. Only the lower dose (0.12%) showed no difference against untreated control seeds. For *Colletotrichum* sp., there was fungus incidence only in lot 79999 (Table 2) and there was no difference between doses, despite no detection with higher doses, which is probably due to low incidence, even in control (dose 0). Among other saprophytic fungal species, *Penicillium* sp., *Aspergillus* spp., *Curvularia* sp. and *Phoma* sp., the interactions between lots x doses were significant (Table 3). In general, these fungi decreased in seeds with increasing fungicide doses. However, there was no significant difference between doses for some lots, and in some cases the fungus was not detected. But, in general, the fungicide showed a positive effect in controlling this kind of fungus species in zucchini seeds. There have been no reports on zucchini seed treatment with this fungicide and related control of its fungi. Although, there have been reports on controlling *Fusarium oxysporum* in alfalfa seeds (Mendes et al., 2001), *Aspergillus* sp. and *Penicillium* sp. in peanut seeds (Barbosa et al., 2013), *Ascochita lentis* in lentil seeds (Kaiser and Hannan, 1987), *Aspergillus niger, A. favus, Botrytis ricini, Curvularia* sp., *Penicillium* sp. and *Rhizopus* sp. in castor bean seeds (David et al., 2014), *Leptosphaeria maculans* in cabbage seeds (Maude et al., 1984), *Fusarium graminearum* in wheat seeds (Garcia Júnior et al., 2008), *Fusarium moniliforme* (Pinto, 2000), *Stenocarpella maydis* (Carvalho et al., 2004) in maize seeds, and *Penicillium* sp., *Aspergillus* spp. and *Curvularia* sp. in melon seeds (Cardoso et al., 2015b). These studies demonstrate that Thiabendazole is effective in controlling several fungi in seeds of different species. In the present study, doses 0.3% and 0.4% always showed or the best results, compared to control without treatment, or total control, i.e., 0% of incidence and can be recommended for zucchini seed treatment.

Materials and Methods

The experiments were conducted in the Vegetable Seeds Laboratory in the Department of Horticulture and Seed Disease Laboratory of the Department of Plant Protection of the Agriculture Sciences College (FCA) of the Universidade Estadual Paulista (UNESP), in Botucatu, São Paulo State, Brazil.

Plant materials

Seeds of three lots of Aline hybrid zucchini (Sakata®) were analyzed concerning physiological and sanitary qualities after

Table 1. Germination and first count in standard germination test in three lots of 'Aline' hybrid zucchini seeds treated with different doses of Thiabendazole.

Treatments	Germination (%)	First Count (%)
Lots		
76788	99.9 a[1]	99.7 a
78021	99.1 b	98.7 b
79999	99.7 a	99.5 ab
Doses (%)		
0.00	99.7 A[2]	99.5 A
0.12	99.7 A	99.5 A
0.20	99.2 A	99.2 A
0.30	99.8 A	99.5 A
0.40	99.5 A	98.8 A

[1]Lot averages followed by the same lowercase letter do not differ by Tukey's test at 5% probability. [2]Thiabendazole dose averages followed by the same uppercase letter do not differ by Tukey's test at 5% probability.

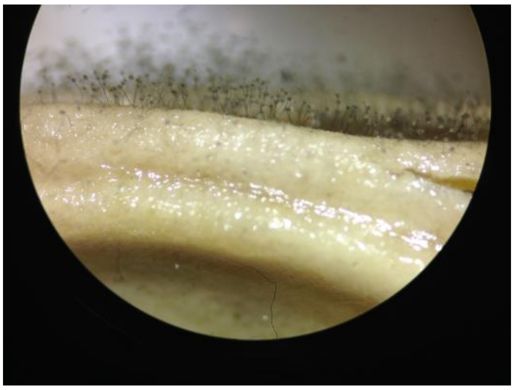

Fig 1. *Cladosporium* sp. in zucchini seeds without fungicide treatment.

Table 2. Incidence of *Cladosporium* sp. and *Colletotrichum* sp. in three lots of 'Aline' zucchini seeds treated with Thiabendazole doses.

Treatments	*Cladosporium* sp.	*Colletotrichum* sp.
Lots		
76788	11.72 a[1]	0.00 b
78021	2.85 b	0.00 b
79999	13.34 a	1.39 a
Doses (%)		
0.00	15.81 A[2]	1.16 A
0.12	10.19 AB	0.82 A
0.20	5.87 B	0.34 A
0.30	7.50 B	0.00 A
0.40	7.14 B	0.00 A

[1] Lot averages followed by the same lowercase letter do not differ by Tukey's test at 5% probability.
[2] Thiabendazole dose averages followed by the same uppercase letter do not differ by Tukey's test at 5% probability.

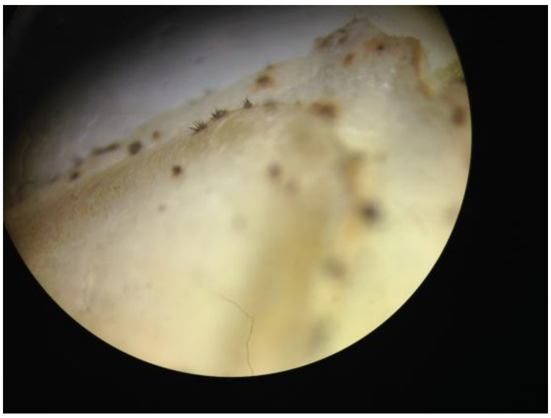

Fig 2. *Colletotrichum* sp. in zucchini seeds without fungicide treatment.

Table 3. Incidence of fungi species in three lots of 'Aline' zucchini seeds treated with Thiabendazole doses.

		Lots	
Dose (%)	76788	78021	79999
		Penicillium spp.	
0.00	8.13 b A[1]	17.71 a A	22.94 a A
0.12	1,02 b B	1.02 b B	11.05 a B
0.20	2.03 a B	0.00 a B	1.02 a C
0.30	1.02 a B	2.03 a B	3.05 a C
0.40	2.03 a B	0.00 a B	0.00 a C
		Lots	
Dose (%)	76788	78021	79999
		Aspergillus spp.	
0.00	3.90 b A	0.00 c A	10.31 a A
0.12	0.00 a B	0.00 a A	0.00 a B
0.20	0.00 a B	0.00 a A	0.00 a B
0.30	0.00 a B	0.00 a A	0.00 a B
0.40	0.00 a B	0.00 a A	0.00 a B
		Lots	
Dose (%)	76788	78021	79999
		Curvularia spp.	
0.00	0.00 b A	0.00 b A	8.30 a A
0.12	0.00 b A	0.00 b A	2.03 a B
0.20	0.00 b A	0.00 b A	1.02 a B
0.30	0.00 b A	0.00 b A	0,00 b B
0.40	0.00 b A	0.00 b A	0,00 b B
		Lots	
Dose (%)	76788	78021	79999
		Phoma spp.	
0.00	0.00 b A	32.30 a A	0.00 b A
0.12	0.00 b A	28.95 a A	0.00 b A
0.20	0.00 b A	20.30 a B	0.00 b A
0.30	0.00 b A	10.84 a C	0.00 b A
0.40	0.00 b A	6.52 a C	0.00 b A

[1]For each fungus, averages followed by the same letter, uppercase letter for doses (columns) and lowercase letters for lots (lines) do not differ by Tukey's test at 5% probability.

treatment with the fungicide Tecto® SC. This product belongs to the chemical group of Benzimidazoles and the active ingredient (a.i.) is Thiabendazole (485 g/L of Thiabendazole).

Treatments and experimental design

The experiments were conducted independently (one for sanitary and another for physiological quality), in a completely randomized design, with 15 treatments, according to a 3x5 factorial system (three seed lots: 76788, 78021, and 79999 x five fungicide doses: 0.0%; 0.12%; 0.2%; 0.3% and 0.4% a.i.).

Fungicide treatments

The application of fungicide was done in rotating pans with a central disk inside the pan located in the center, also rotating, but in the opposite direction to distribute the product. The treatment duration was 20 minutes. After treatment, seeds were dried at 30oC during 2 hours, and were evaluated for physiological quality and incidence of fungi.

Seed germination

Standard Germination Test (SGT) was done according to Seed Analysis Standards (ISTA, 2004; Brasil, 2009). The seeds were distributed over two sheets of paper towel dampened with 2.5 times their weight of distilled water and covered with another sheet of moistened paper towel. The sheets were rolled and placed in a germination chamber in a vertical position at 25±1 °C, relative humidity between 80-95%, without light. There were four replications with 50 seeds, amounting to 200 seeds per treatment. The counting of normal seedlings was performed on the 8^{th} day after sowing (DAS), with values expressed in percentages.

Seed vigor

Germination First Count (GFC) tests: normal seedlings were counted on the 4^{th} DAS during the SGT, as described previously, according to Brasil (2009), with values expressed in percentages.

Seed pathology analysis

The "blotter test" method was used to evaluate the incidence of fungi in seeds. It involved distributing ten seeds placed equidistantly over three sheets of filter paper, previously moistened, positioned on Petri dishes. Eight replications of 50 seeds were performed, totaling 400 seeds per treatment. The experimental plot consisted of five dishes with ten seeds. The dishes were kept at 20 ± 2 °C for a twelve-hour photoperiod under white fluorescent light for seven days. After incubation, the seeds were individually evaluated under a magnifier and the results were expressed in percentage of seeds with fungus. No pathogenic species were detected, only saprophytic ones, such as *Cladosporium* sp. (Figure 1), *Colletotrichum* sp. (Figure 2), *Penicillium* spp., *Aspergillus* spp., *Curvularia* spp., and *Phoma* spp. The results were converted in arc sin $\sqrt{(x/100)}$ for statistical analysis.

Statistical analysis

The data obtained for all variables evaluated were subjected to analysis of variance and the averages were compared by Tukey's test at 5% probability.

Conclusion

The fungicide Thiabendazole promotes reduction of saprophytic fungus in zucchini seeds without impairing their germination and vigor in the assessed doses. It can be recommended zucchini seeds treatments with Thiabendazole with dose 0.3% or 0.4% that had better efficiency to control the observed fungi.

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