academicJournals

Vol. 10(35), pp. 3472-3476, 27 August, 2015
DOI: 10.5897/AJAR2015.10135
Article Number: 55146E755159
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Germination, vigor, and fungi incidence in melon seeds treated with Thiabendazole

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Received 8 July, 2015; Accepted 21 August, 2015

Chemical treatment is known to effectively eradicate or at least reduce the presence of fungi in seeds. However, this treatment should not impair seed quality. The purpose of the present study was to evaluate the physiological and sanitary quality of Cozumel hybrid melon seeds treated with the fungicide Thiabendazole. The experiments were conducted under a completely randomized design, consisting of 15 treatments in a 3×5 factorial system (three seed lots × five Thiabendazole doses: 0, 0.12, 0.2, 0.3 and 0.4% a.i.). The commercial product used was Tecto® SC (485 g L^{-1} of Thiabendazole). Four replications of 50 seeds were used for germination and vigor tests (evaluated by germination test first count: GFC). Eight replications of 50 seeds were performed for seed pathology analysis, amounting to 400 seeds per treatment. The average results were compared by Tukey test at 5% probability. The results showed differences in germination between lots, with lower total germination in lot 84538 (90.8%). There was no difference in Thiabendazole dosage regarding both total germination (94.8% on average) and GFC (93.2% on average), showing that the fungicide did not affect the physiological quality of seeds. Pathogenic species were not detected in the sanitary analysis, only saprophytic fungal species (*Alternaria* sp., *Aspergillus* spp., *Curvularia* spp and *Penicillium* spp.) and a general reduction in fungi incidence was observed with the increase of Thiabendazole doses.

Key words: *Cucumis melo*, seed treatment, fungicide.

INTRODUCTION

The association between fungi and seeds can severely affect seed quality, reducing germination, vigor, seedling emergence and productive potential. It is not always possible to obtain seed lots 100% guaranteed free of pathogens. It is also not possible to ensure that the sown soil or substrate will be clear of fungi. Therefore, seed treatment is advisable in most cases, especially for vegetable hybrid seeds, because treatment cost is very low compared to the high price this type of seeds carries (Cardoso et al., 2015).

Seed treatment has been efficient in preventing plant disease outbreaks caused by pathogenic agents in seeds, particularly fungal agents. Chemical seed treatment aims to eradicate these pathogens and/or to protect against soil pathogens, especially during germination. Furthermore, seed treatment can help reduce the volume of fungicides needed to control the diseases. Seed treatment can eliminate the need of foliar

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use of fungicide products in field crops (Mancini and Romanazzi, 2014).

It must be emphasized that beyond the control of pathogens and/or protection of the seeds, the treatment should not impair seed physiological quality (Cardoso et al., 2015). Seed treatment effectiveness depends on, among other factors, seed species and vigor, which may vary from lot to lot (Menten and Moraes, 2010).

According to Menten and Moraes (2010), there were 19 fungicides active ingredients registered for treating seeds 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 few cultures.

According to the Ministry of Agriculture, Livestock and Supply (MAPA, 2014), 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 postharvest treatment and aerial part of avocado, pineapple, coconut, pea, snap pea, papaya, mango, passion fruit, melon and sweet pepper crop treatment.

Thus, considering the Cucurbitaceae family, this fungicide has only been registered for treating melon culture, to the control of fungi *Colletotrichum orbiculare* and *Didymella bryoniae*, and recommended for spraying plants or postharvest treatment in fruits. Therefore, this product has not been registered for vegetable seed treatment. It has been reported that this fungicide does not impair seed physiological quality of several species, such as alfalfa (Mendes et al., 2001), lentil (Chang et al., 2008), maize (Fessel et al., 2003; Carvalho et al., 2004), soybean (Pereira et al., 2011) and wheat crops (Garcia Júnior et al., 2008). In regard to melon, no studies on the use of this fungicide to treat seeds were found.

Thus, the purpose of the present work was to evaluate physiological (germination and vigor) and sanitary (incidence of fungi) quality in seeds of different lots of melon seeds treated with five doses of Thiabendazole.

MATERIALS AND METHODS

Seeds of three lots of Cozumel hybrid melon were analyzed concerning physiological and sanitary qualities after treatment with the fungicide Tecto® SC. This product belongs to the chemical group of Benzimidazole and the active ingredient (a.i.) is Thiabendazole (485 g/L of Thiabendazole). The experiments were conducted independently (one for sanitary and another for physiological quality), under a completely randomized design, with 15 treatments, according to a 3x5 factorial system (three seed lots: 67594, 84538 and 84539 × five fungicide doses: 0.0; 0.12; 0.2; 0.3 and 0.4% a.i.).

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. After treatment and drying, the seeds 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 pieces of paper towel dampened with 2.5 times their weight of distilled water and covered with another piece 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 to 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 8th day after sowing (DAS), with values expressed in percentages.

Seed vigor

Germination First Count (GFC) tests: normal seedlings were counted on the 4th 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 treatments. 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. The results were converted in arc sin $\sqrt{(x/100)}$ for statistical analysis.

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

RESULTS AND DISCUSSION

There was no interaction between factors (lots and fungicide doses), indicating mutual independence for germination and vigor. The results for both variables are shown in Table 1. Although, all three melon seed lots offer excellent quality with minimum germination rate above 90%, lot 84538 showed lesser germination (90.8%) compared to the other two lots (67594 and 84539) (Table 1). The rates demonstrated for germination on three lots are much higher than the minimum standard rate of 70% for melon required for marketing in Brazil by the Ministry of Agriculture, Livestock and Supply (MAPA). 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 Cucurbitaceas seeds in the market with less than 85% germination rate. Even with this reference frame, all the lots can be considered to have good physiological quality.

The first seed count is considered a vigor test. The samples with faster germination and higher percentage of normal seedlings on this date are considered more vigorous (Marcos Filho, 2005; Baalbaki et al., 2009). There was no difference between lots in this feature

Table 1. Germination and vigor test of ‘Cozumel’ melon seed lots treated with different doses of Thiabendazole.

Treatments	Germination (%)	Vigor (%)
Seed lots		
67594	98.1^{a1}	94.2^{a}
84538	90.8^{b}	90.6^{a}
84539	95.5^{a}	94.9^{a}
Doses (%Thiabendazole)		
0.00	94.7^{A2}	92.8^{A}
0.12	96.5^{A}	94.0^{A}
0.20	97.3^{A}	96.5^{A}
0.30	93.0^{A}	91.3^{A}
0.40	92.5^{A}	91.5^{A}
F_{lots}	7.43**	0.45^{ns}
F_{doses}	1.45^{ns}	1.47^{ns}
C.V. (%)	6.40	6.51

[1]Seed lot averages followed by the same lowercase letter do not differ by Tukey test at 5% probability. [2]Thiabendazole dose averages followed by the same uppercase letter do not differ by Tukey test at 5% probability. [ns] = non-significant by F test at 5% probability and ** = significant by F test at 1% probability.

(Table 1), with an average of 93.2% rate, indicating high seed vigor.

Considering Thiabendazole dosage (Table 1), there was no difference in germination (average of 94.8%) and vigor (first count, average of 93.2%), showing that the fungicide did not affect physiological quality of melon seeds in any lot. 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 among 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 of this fungicide on wheat seeds, verifying germination and seedling emergence similar to untreated control seeds, as observed by Mendes et al. (2001) with alfalfa seeds and Gally et al. (2004) with soybean seeds. Yet, Fessel et al. (2003) reported that a very high concentration of 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 fungicide reduced the incidence of "damping off" caused by the fungus *Stenocarpella maydis* in seeds. 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*. But, in high doses, this fungicide caused phytotoxic effects on lentil seedlings. The positive effects on germination and/or seedling emergence with seed treatment using this fungicide were also described in papaya seeds (Campos et al., 2009) and castor bean seeds (Poletine et al., 2012). These contrasting results for each crop show the importance of conducting studies for each different species because the results may not always be the same.

The treated seeds, regardless of dose and lot, showed no difference compared to untreated control seeds, both for germination test and vigor (Table 1). The discrepancy of reports on other species probably occurred for two reasons: (a) because the observed presence of fungi in seeds was small in all treatments, including in the control (Tables 2 and 3), (b) the treatment with this fungicide did not affect germination and vigor. Therefore, seed treatment with Thiabendazole, in the doses evaluated, did not affect seed germination and vigor.

Mancini and Romanazzi (2014) emphasize that vegetable seed treatment is no substitute for using pathogen free seeds. However, due to the impossibility of obtaining lots 100% free of pathogens and considering the presence of fungi in the soil or substrate where the seeds are sown, treating seeds is the most effective method to achieve maximum seedling emergence.

Regarding treated seed sanity, a pathogenic species was not detected in the seeds, notably *D. bryoniae,* one of the main seedborne pathogens in melons. However, there were saprophytic fungal species. For *Alternaria* sp., interaction between factors (seed lots and Thiabendazole doses) was non-significant. It was observed lesser incidence on lot 84539 than in lots 84538 and 67594 (Table 2). Regardless of the lot, this fungus incidence decreased as the fungicide dose increased. The lower dose (0.12%) showed no difference against untreated control seeds (Table 2).

Among other saprophytic fungal species, *Penicillium* sp., *Aspergillus* spp. and *Curvularia* sp., the interaction

Table 2. Incidence of *Alternaria* spp. in seeds of three lots of Cozumel melon treated with Thiabendazole doses.

Treatment	***Alternaria* spp.**
Seed lots	
67594	2.90[a1]
84538	3.22[a]
84539	0.61[b]
Doses (%Thiabendazole)	
0.00	5.03[A2]
0.12	3.05[AB]
0.20	1.78[B]
0.30	0.68[B]
0.40	0.68[B]

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

Table 3. Incidence of saprophytic fungal species in seeds of three lots of 'Cozumel' melon treated with Thiabendazole.

Dose (%Thiabendazole)	**Seed lots**		
	67.594	**84.538**	**84539**
		***Penicillium* spp**	
0.00	33.69[aA1]	18.66[b A]	37.50[aA]
0.12	19.03[aB]	18.25[aA]	25.86[aB]
0.20	13.93[bBC]	19.38[abA]	25.34[aB]
0.30	5.84[aC]	12.24[aA]	10.63[aC]
0.40	5.10[bC]	9.27[abA]	15.96a[BC]
Dose (%Thiabendazole)	**Seed lots**		
	67.594	**84.538**	**84539**
		Aspergillus spp.	
0.00	6.20[cA]	29.57[aA]	19.32[bA]
0.12	6.57[bA]	24.39[aA]	3.47[bB]
0.20	3.80[aA]	8.32[aB]	4.07[aB]
0.30	3.80[aA]	9.27[aB]	2.03[aB]
0.40	1.02[aA]	7.57[aB]	1.02[aB]
Dose (%Thiabendazole)	**Seed lots**		
	67.594	**84.538**	**84539**
		Curvularia spp.	
0.00	0.00[bA]	2.03bA	6.97[aA]
0.12	0.00[bA]	0.00bA	3.74[aAB]
0.20	0.00[aA]	0.00aA	2.03[aB]
0.30	0.00[aA]	0.00aA	1.02[aB]
0.40	0.00[aA]	0.00aA	0.00[aB]

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

between factors (seed lots and Thiabendazole doses) was significant (Table 3). These fungi incidence decreased in seeds with increasing fungicide doses. Thus, the fungicide showed a positive effect on the control of these fungi species in melon seeds.

There have been no reports on melon seed treatment with this fungicide and related control of its pathogens. But, 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) and *Stenocarpella maydis* (Carvalho et al., 2004) in maize seeds. These studies demonstrate that Thiabendazole is effective in controlling fungi in seeds of different species, as observed in the present study with melon seeds.

Conclusion

In general, the fungicide Thiabendazole promotes a reduction of saprophytic fungus in melon seeds without impairing their germination and vigor.

Conflict of Interest

The authors have not declared any conflict of interest.

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