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Full Length Research Paper

Fludioxonil effectively controls *Sclerotinia sclerotiorum* incited decay in stored carrots in Israel

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Carrot is an important crop in Israel, subjected to postharvest decay, incited in cold storage by the fungus *Sclerotinia sclerotiorum*, which may cause up to 7% loss. The fungicide iprodione, which had been used until recently to limit decay was phased-out in 2020. Hence, in the present study, the fungicide fludioxonil was examined as a replacement. In two cold storage trials with artificial inoculations, fludioxonil (0.1%; v/v) treated carrots had 43 and 37% and 69 and 58% less (*P<0.0001*) decay than carrots treated with chlorinated (200 ppm) water control or with the commercial reference tiabendazole, respectively. In two long-term commercial trials, without inoculations, carrots treated with fludioxonil (0.1%; v/v) had 98 and 96% less (*P<0.0001*) decay than carrots treated with chlorinated water as control. Following the result of this study, fludioxonil at a rate of 0.1% (v/v) was approved for use in stored carrots in Israel and was incorporated into commercial production as a replacement of iprodione starting January 2021.

Key words: Carrots, *Sclerotinia sclerotiorum*, iprodione, fludioxonil, cold storage, postharvest.

INTRODUCTION

Carrot is an important crop in Israel grown over a total area of 2200 ha. Carrots are harvested and marketed fresh between November and June. During the remainder of the year the roots are marketed from cold storage. To extend shelf life and limit decay development, freshly harvested carrots go through a series of sanitation procedures. Initially, the carrots are washed with water jets to remove the bulk of dirt from the field. Next, they enter a polishing drum, which scrapes off remnants of dirt. This step is followed by 10 to 15 min of hydrocooling sanitation in chlorinated (200 ppm NaClO) and chilled (4°C) water in Jacuzzi baths. The sanitized and chilled carrots are then transferred, via conveyor belts, into troughs filled with the fungicide iprodione at rate of 0.1% (volume per volume, v/v) and are finally packaged in big bags of approximately 1,500 kg and placed in cold storage at 1°C until marketed.

In Israel, carrots may be stored for up to 6 months and are susceptible to decay incited by *Sclerotinia sclerotiorum* (Papoutsis and Edelenbos, 2021). *Sclerotinia sclerotiorum* (Lib.) de Bary is an ascomycete fungus, prevalent worldwide and known for its wide host range and aggressive nature (Purdy, 1979). In Israel the fungus is primarily active from fall to spring, where it affects various crops (Ben-Yephet et al., 1993), including carrot. The fungus is commonly carried from the field into

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storage on the carrots' surface or in colonized crowns, where it develops over time into a watery soft rot, deteriorating the produce (Kora et al., 2003, 2005). Despite the vigorous sanitation practices, the fungus survives and decay develops, causing up to 7% loss annually.

Since the early 1990s and until recently, the fungicide iprodione was the sole fungicide in Israel used commercially to control postharvest decay of carrots incited by *S. sclerotiorum*. Iprodione is a Fungicide Resistance Action Committee (FRAC) code 2 group dicarboxamide, which affects signal transduction in fungi (FRAC code list® 2022; https://www.frac.info/). In Israel, the fungicide was approved for use to control white mold caused by *S. sclerotiorum*; gray mold caused by *Botrytis cinerea*; watery rot caused by *Rhizopus stolonifer*; early blight caused by *Alternaria alternata*, and other disease-causing pathogens of vegetable crops, horticultural crops, grapes, and citrus. Nonetheless, its use in Europe has been terminated and consequently, as of January 2021 the fungicide was phased-out in Israel.

The phase-out of iprodione placed the carrot industry with an immediate need for a replacement to sustain marketing. Therefore, it was suggested to examine the efficacy of the fungicide fludioxonil, which is used in Israel for the prevention of decay development in stored pomegranate (Porat et al., 2015). With this in mind, the present study tested the hypothesis that the fungicide fludioxonil can commercially replace the fungicide iprodione and limits decay incited by *S. sclerotiorum* in cold-stored carrots, as well as, extend storability and shelf life. The goal of the study was to provide evidence of efficacy for the purpose of approving the fungicide for commercial use by the Israeli Plant Protection and Inspection (PPIS) regulatory agency.

MATERIALS AND METHODS

Fungicide formulations

Fludioxonil (Scholar® SC; www.syngenta.com) was examined at 0.025, 0.05 and 0.1% v/v and was compared in efficacy to iprodione (Rovral® WP; www.ag.amf.com) at the commercial standard rate of 0.1% v/v. The fungicide thiabendazole (TBZ; Tecto® 500 SC; www.syngenta.com) and chlorinated water (200 ppm NaClO) were used as a commercial reference and as untreated control, respectively.

Fungal inoculum for artificially inoculated trials

Sclerotinia sclerotiorum was recovered from cold-stored decaying carrot onto 1% Potato Dextrose Agar containing 0.1 g/L of chloramphenicol. The S*. sclerotiorum* isolate was coded 'SSC-4' and was maintained at 18°C in the dark until used. Freshly harvested carrots, not treated with fungicides, were collected from the packing house following hydrocooling and commercial sanitation (NaClO at 200 ppm). The roots were transferred to the laboratory enclosed in plastic bags to prevent contamination. In the laboratory, the crowns and root ends were excised and the carrots were re-sanitized (NaClO 200 ppm) for 5 min and then washed twice in sterilized di-ionized water. The carrots were dissected into 5 cm long segments and a 1 cm deep well was drilled into the center of each carrot segment using a cork borer (Ø=6 mm). Then, the well was filled with 3 agar plugs (Ø=6 mm) removed from the growing edges of a 7-day-old *S. sclerotiorum* culture. The inoculated carrots were incubated at 24°C in the dark and were ready for use as inoculum units 10 days later.

Artificially inoculated fungicide efficacy trials

Two replicated trials with artificial inoculations were performed. Artificially inoculated trial 1 was executed at 'Gezer Sheluhut' commercial packing house, Kibbutz Sheluhot, Israel. Trial 1 included six treatments: (i) Fludioxonil at 0.025% v/v; (ii) Fludioxonil at 0.05% v/v; (iii) Fludioxonil at 0.1% v/v; (iv) Iprodione at 0.1% v/v; (v) TBZ at 0.1% v/v and (vi) Chlorinated water (NaClO, 200 ppm). Artificially inoculated trial 2 was performed at 'Yaham' commercial storage facility located at Kibbutz Sa'ad, Israel and examined three treatments: (i) Fludioxonil at 0.1% v/v; (ii) TBZ at 0.1% v/v and (iii) Chlorinated water (NaClO, 200 ppm). Fungicide solutions were prepared in 60 L plastic cans in local tap water. Freshly harvested carrots were subjected to commercial sanitation procedures and were removed immediately following the hydrocooling stage. The carrots were dipped in each fungicide solution for 30 s. Then, eight fungicide-treated carrots were packaged into 5 kg macro-perforated polyethylene retail bags. Each bag was inoculated with a single carrot segment infected by *S. sclerotiorum* (inoculum unit). Trials 1 and 2 included 10 and 5 replicated bags per fungicide treatment, respectively. The bags were organized in a completely randomized design (CRD) in plastic crates and placed in the commercial cold storage at 1°C. Decay development was monitored over time and was scored per each replicated bag visually using the following 0 to 5 ordinal scale: 0 = no decay; 0.1 = 0.1 to 1% decay without exudates; 0.5 = 1^+ to 5% decay without exudates; 1 = 5^+ to 10% decay without exudates; 2 = 10^+ to 25% decay without exudates; 3 = 25^+ to 50% decay with exudates; 4 = 50^+ to 75% decay with exudates; 5 = 75^+ to 100% decay with exudates (Madden et al., 2007). The decay scores were used to calculate the area under the decay progress curve (AUDPC) using the formula: $AUDPC = \{[\sum ((Y_i + Y_{i+1}) \div 2) \times (t_{i+1} - t_i)]$; where Y_i and Y_{i+1} are respectively the decay scores at t_i and t_{i+1} (Campbell and Madden, 1990; Madden et al., 2007).

Commercial fungicide evaluation trials

Two replicated commercial trials were carried out without artificial inoculations, as those were prohibited by the packing houses and did not reflect commercial practices. Commercial trial 1 was carried out at 'Gezer Ba'Gilboa' packing facility located at Kibbutz Maale' Gilboa', Israel, and included three treatments: (i) Fludioxonil at 0.1% v/v; (ii) Iprodione at 0.1% v/v; and (iii) Chlorinated water (NaClO 200 ppm). Commercial trial 2 was carried out at 'Yaham' commercial storage facility located at Kibbutz Sa'ad, Israel, and included two treatments: (i) Fludioxonil at 0.1% v/v and (ii) Chlorinated water (NaClO 200 ppm). Freshly harvested carrots were subjected to commercial sanitation and hydrocooling procedures and were transferred via conveyor belts into the fungicide dipping baths for 15 to 30 s of exposure to each tested fungicide. The fungicide baths and conveyor belts were washed thoroughly with tap water between treatments to prevent fungicide cross-contaminations. In commercial trial 1, the carrots were packaged in big bags weighing 1100 to 1300 kg, and in commercial trial 2, the carrots were packaged in 25 kg mesh bags. The fungicide-treated carrots were stored at 1°C. Trials 1 and 2 were carried out, respectively, with 3 and 4 replications per fungicide

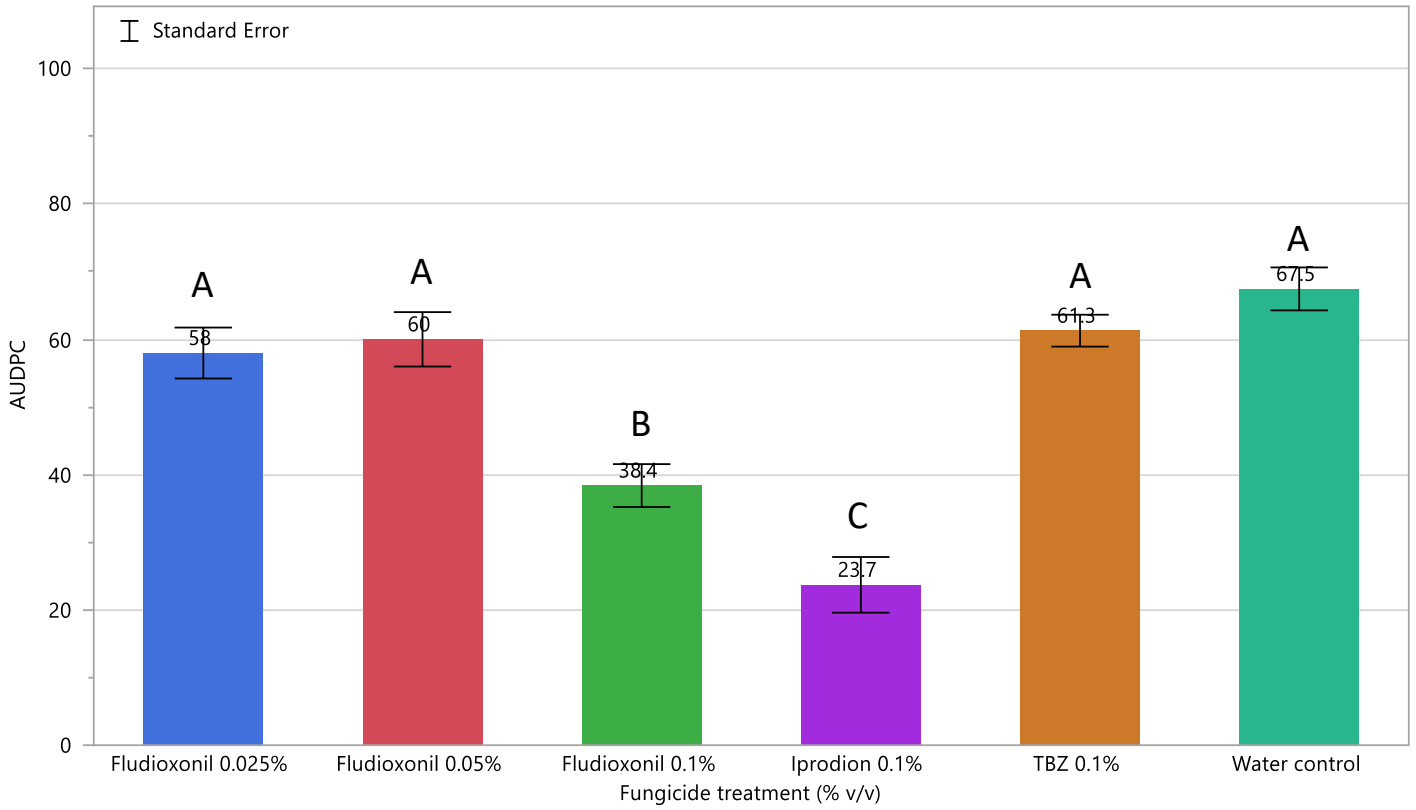

Figure 1. Mean area under the decay progress curve (AUDPC) that developed in cold storage (1°C) following inoculation with *Sclerotinia sclerotiorum* of carrots treated with fludioxonil at three different rates in comparison with iprodione at the standard commercial rate. The trial was terminated after 58 days in storage. Chlorinated water (NaClO 200 ppm) and thiabendazole (TBZ; 0.1% v/v) were used as control and commercial reference, respectively. The trial was carried out with 10 replications per treatment. Analysis of Variance was followed by Fisher's protected LSD test. Different upper-case letters signify statistical differences among treatments at 5% significance level.
Source: from author's work

treatment and were organized in a completely randomized design (CRD). Decay was quantified by weight as the incidence of rotted carrots using the formula: Incidence (%) = (Weight rotted / Total weight)*100.

Statistical analysis

Statistical analyses were performed at α = 0.05 in JMP 16 (SAS Institute Inc., Cary, NC, USA). In the artificially inoculated trials, the area under the decay progress curve (AUDPC) values were average-rank transformed, as they arose from an ordinal scale, and were subjected to analysis of variance (ANOVA) utilizing the Kruskal-Wallis procedure. In the commercial trials, decay incidence values were root-squared and subjected to arcsin transformation followed by parametric ANOVA or two-tailed Student's t-test. ANOVA was followed by Fisher's protected least significant difference (LSD) test for treatment's mean separation. Values presented in the result section were back-transformed (Zar, 1999).

RESULTS

The goal of the present study was to provide evidence of fludioxonil's efficacy to control decay incited by *S. sclerotiorum* in stored carrots for the purpose of approving the fungicide for commercial use by the Israeli regulatory agency. Four replicated trials, two with artificial inoculations and two commercial, absent inoculations, were carried out. Artificially inoculated trial 1 extended for 58 days in cold storage. Of the three fludioxonil rates tested, only 0.1% (v/v) limited decay incited by *S. sclerotiorum.* Carrots treated with fludioxonil at 0.1% had 43 and 37% less (*P<0.0001*) decay than those exposed to chlorinated water or tiabendazole, but not less (*P>0.05*) than iprodione (Figure 1). Artificially inoculated trial 2 extended was for 70 days and presented a similar outcome (Figure 2). Carrots treated with fludioxonil at 0.1% (v/v) had 69 and 58% less (*P<0.0001*) decay than carrots treated with chlorinated water control or with the commercial reference tiabendazole, respectively. In both trials, thiabendazole proved ineffective in preventing decay caused the by *S. sclerotiorum*. The commercial trials were carried out in two different packing houses, managed by different commercial growers, utilizing carrots that were grown at two different locations - in the north or in the south of the country. The fungicide-treated

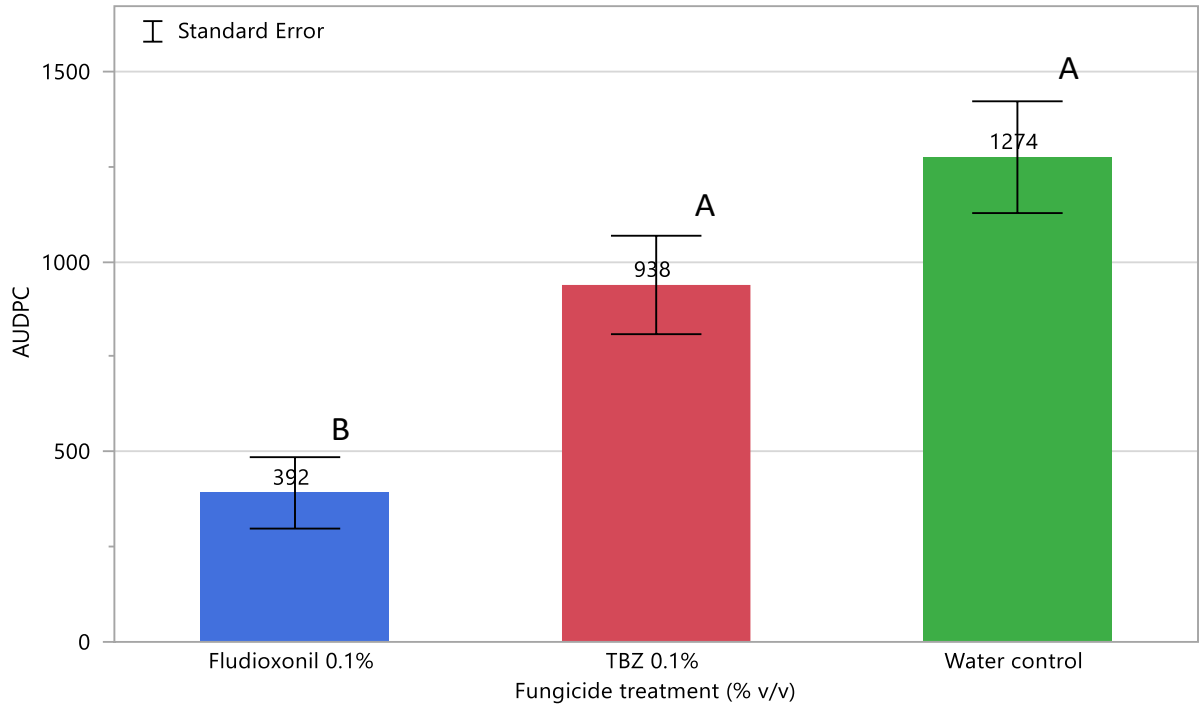

Figure 2. Mean area under the decay progress curve (AUDPC) that developed under cold storage conditions (1°C) in carrots, artificially inoculated with *Sclerotinia sclerotiorum* and treated with fludioxonil (0.1% v/v). Chlorinated water (NaClO 200 ppm) and thiabendazole (TBZ; 0.1% v/v) were used as control and commercial reference, respectively. The trial was terminated after 70 days in storage. The trial was carried out with 5 replications per treatment. Analysis of variance was followed by Fisher's protected LSD test. Different upper-case letters signify statistical differences among treatments at 5% significance level.
Source: from author's work

carrots were stored for 187 and 167 days, which were 5.5 and 6 months, respectively. In both trials, carrots treated with fludioxonil at 0.1% (v/v) had 98 and 96% less (*P<0.0001*) decay than those exposed only to chlorinated water control (Figures 3 and 4). Furthermore, under the non-inoculated commercial conditions, fludioxonil was as effective as (*P>0.05*) iprodione (Figure 3).

DISCUSSION

The fungicide iprodione was used in Israel for nearly 30 years for the prevention of decay development in stored carrots, caused by *S. sclerotiorum*. As a result of its elimination in Europe, it had been phased-out in Israel as of January 2021, leaving the carrot industry without an effective replacement, prohibiting long term storage of carrots for both the local market and export. The present study succeeded in identifying the fungicide fludioxonil at a rate of 0.1% (v/v) as an effective replacement. As a result of the study presented here, fludioxonil was regulatorily approved by the Plant Protection and Inspection Services (PPIS) in Israel, and entered commercial use.

The fungicide fludioxonil is a synthetic analog of the bacterial antifungal compound pyrrolnitrin, which is produced by bacterial species from the genus *Pseudomonas* (Arima et al., 1964; Gordee and Matthews, 1969). Fludioxonil is characterized to FRAC code 12 (Fungicide Resistance Action Committee; https://www.frac.info), disrupting osmotic signal transduction via improper activation of the Hog1-type mitogen-activated MAP/Histidin Kinase (Yoshimi et al., 2005) and association of the *Ssos4* gene (Li et al., 2021). Hence, causing osmotic stress in fungi and interfering with mycelial growth. Duan et al. (2014) demonstrated that in exposure to fludioxonil, *S. sclerotiorum* had an increase in mycelial membrane permeability, increased activity of the stress related enzymes phenylalanine ammonia lyase and peroxidase, and accumulation of glycerol. As well, a decrease in oxalate, which is associated with pathogenicity and in extracellular polymeric substances levels associated with the decomposition of organic matter (Op De Beeck et al., 2021).

Currently, fludioxonil is used in Israel exclusively and extensively for minimizing decay in stored carrots. This overwhelming application of fludioxonil poses significant management risks for insensitivity development and resistance build-up in the population of *S. sclerotiorum*.

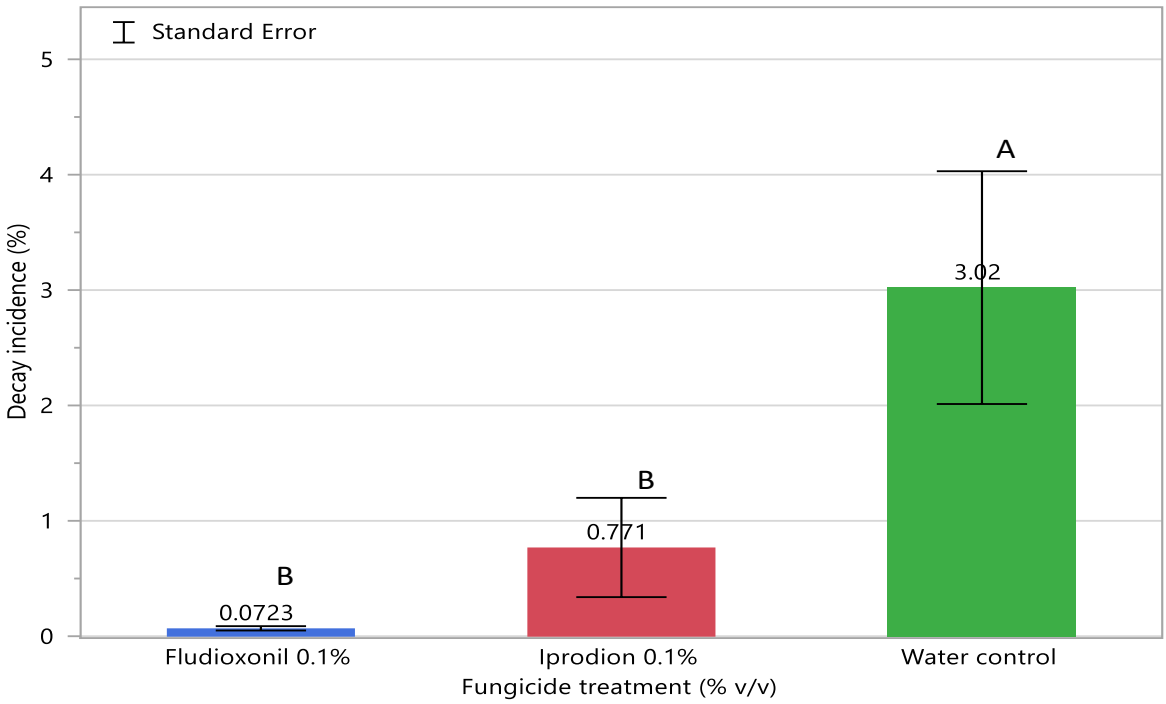

Figure 3. Incidence (%) of decayed carrots following 187 days of commercial storage (1°C) in big bags containing carrots that were treated with fludioxonil (0.1% v/v), iprodione (0.1% v/v) or chlorinated water (NaOCl 200 ppm). The test was carried out with 3 replications per treatment. Analysis of variance was followed by Fisher's protected LSD test. Different upper-case letters signify statistical differences among treatments at 5% significance level.
Source: from author's work

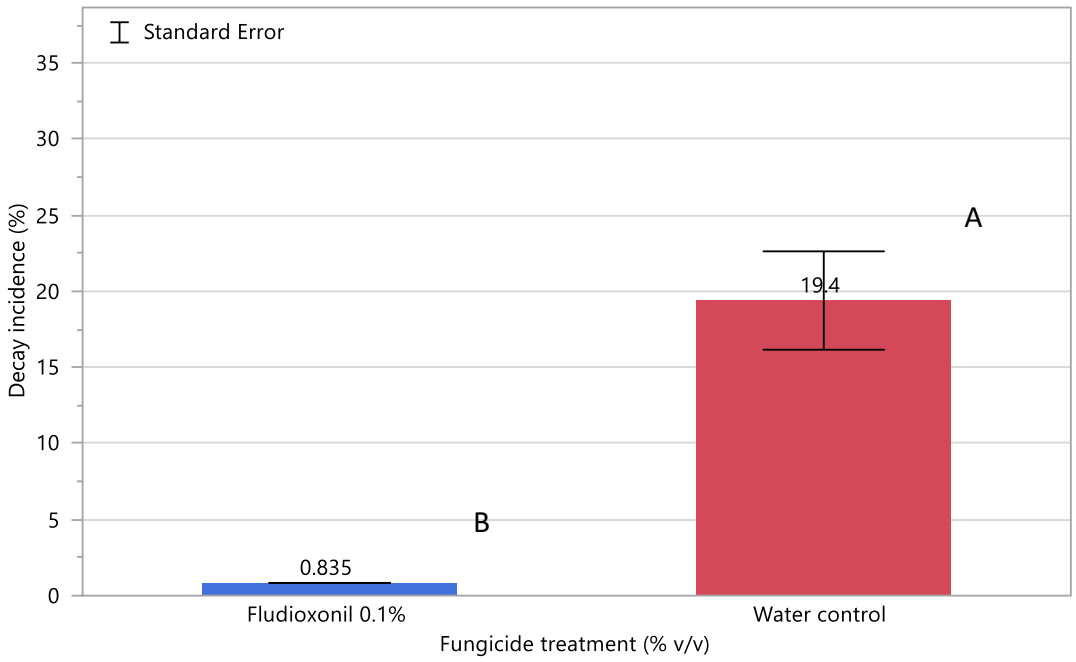

Figure 4. Incidence (%) of decayed carrots following 167 days of commercial storage (1°C) in 25 kg bags containing carrots that were treated with fludioxonil (0.1% v/v) or chlorinated water (NaOCl 200 ppm). The test was carried out with 4 replications per treatment. Different upper-case letters signify statistical differences among means following a two-tailed Student's t-test at 5% significance level.
Source: from author's work

To date, the information in the literature does not indicate resistance to fludioxonil in wild-type populations of *S. sclerotiorum*. Nonetheless, *in vitro* tests with *S. sclerotiorum* mutants suggested a moderate risk for insensitivity development (Kuang et al., 2011). Low sensitivity to fludioxonil has been reported in *Penicillium digitatum* and *P. italicum* with maximum inhibitory concentration (MIC) of ≥100 µg/ml and EC_{50} values of 0.1 to 1 and 1 to >100 µg/ml, respectively (Schirra et al., 2005). Resistance to fludioxonil also was reported in *Botrytis cinerea* from a strawberry field in Virginia, USA (Fernández-Ortuño et al., 2013). The resistant isolate grew *in vitro* on a discriminatory rate of 0.1 µg/ml and had an EC_{50} value of 0.26 µg/ml. On the contrary, the sensitive isolates were inhibited by the discriminatory dose with an EC_{50} of 0.01 µg/ml. Furthermore, the resistant isolate cause disease when strawberry fruits were artificially inoculated and treated with the recommended field application rate of 2.5 ml/liter fludioxonil. Keeping in mind that *B. cinerea* and *S. sclerotiorun* are closely related genetically (Amselem et al., 2011) and characterized to the fungal family *Sclerotiniaceae*, it is not unlikely for development of fludioxonil resistance genotypes in the latter. This information stresses the need to avoid the exclusive use of fludioxonil in Israel for preventing decay in cold-stored carrots and identify additional means for decay management. The focus may be additional chemical fungicides that could be used in alternation with fludioxonil or in mixtures. But, primarily bio-fungicides and other eco-friendly GRAS materials, such as plant extracts, essential oils, biostimulants and effective food additives (Elsherbiny and Taher, 2018; Papoutsis and Edelenbos, 2021) that will provide a sustainable decay control.

Conclusion

The present study reported the successful identification of the fungicide fludioxonil at a rate of 0.1% v/v as a replacement of iprodione for decay management in stored carrots in Israel. As a result of this research, fludioxonil was regulatorily approved and incorporated into commercial use. The reports of the potential for resistance development to fludioxonil in phytopathogenic fungi is alarming, requiring to continue exploring new materials to providing the carrot industry with additional means for decay control.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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