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Effect of Fungicides on *in vitro* Infestation Level of Radish, Carrot and Pepper Seeds

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Abstract: Through incubation of radish (*Raphanus sativus*), carrot (*Daucus carota*) and pepper (*Capsicum annuum*) seed samples on potato dextrose agar at 25°C, six species of fungi were determined as seedborne in these crops. *Alternaria dauci*, *A. Tenuissima*, *A. tenuis* and *Epicoccum* sp. were found to be transmitted by radish seed. For carrot seed, *A. Tenuissima*, *A. dauci*, *A. tenuis*, *Epicoccum* sp. and *Fusarium oxysporum* were identified. However, *Alternaria tenuissima* and *Botrytis cinerea* were isolated from pepper seeds. Seed treated with fungicides such as Maneb, Mancozeb, Benomyl and Thiophanate methyl could reduce the infestation levels of tested crops.

Key words: Radish, carrot, pepper, *in vitro*, fungicides

INTRODUCTION

Market vegetable seeds is a strategic sector, it serves more than 145 000 ha that require about 180 tons of seeds for the species produced by sexual way. These quantities are in the most major part imported from other countries (> 60%), whereas the rest is auto-produced in a controlled manner (by national organisms) or non-controlled (by the farmers).

The level of this sanitary state depends on the quality of multiplied seeds and especially on environmental conditions (Hamza, 1982).

Several diseases (fungi, bacteria and virus) could be transmitted by seeds and caused considerable yield losses. Raymond (1999) reported that the following fungi are transmitted by carrot seeds: *Alternaria dauci*, *Alternaria radicina*, *Cercospora carota*, *Alternaria tenuis* and *Alternaria tenuissima*.

Few reports showed the effect of fungicides on seedborne fungi. Thiram is known able to protect carrot seeds tissues and to remove *Alternaria radicina* seed infection (Biniek and Tylkowska, 1987). Corn (*Zea mays* L.) seed treatment with the fungicide prochloraz was effective to control seed borne transmission and protect against *F. moniliforme* seedling blight. This elimination of seedborne inoculum reduced incidence of kernel rot and avoided the increment in soil inoculum accumulation associated to the introduction of infected seeds (Galperin *et al*., 2003). Through incubation of onion (*Allium cepa* L.) seed samples on potato dextrose agar, some genera such as *Aspergillus*, *Penicillium, Sclerotium*, *Alternaria* and *Fusarium* were identified as seedborne in this crop (El-Nagerabi and Abdall, 2004). Other fungi such as *Fusarium verticillioides* (synonym: *F. moniliforme*), responsible of maize seed rot, can be suppressed and/or controlled at the post infection stage with Plantpro-45 (Yates *et al*., 2003).

To preserve the plant health during the first sensitive stage of their development (germination), seed treatment is usually recommended (Maude, 1978).

Although, the specific and effectiveness product remain the most important question for many seed production companies, few studies reported fungicides that could be able to protect seeds of different vegetables crops.

The aims of this study are to identify the seedborne fungi of some seed vegetables and to test, *in vitro,* the effectiveness of different fungicides on reduction of infestation level.

MATERIALS AND METHODS

Seed material: Three seedlots of vegetable crop were used in this study. Species and cultivars are presented on Table 1.

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Table 1: Different species and cultivars tested

Species	Cultivars
Radish (*Raphanus sativus*)	National
Carrot (*Daucus carota*)	Nantaise demi longue
Pepper (*Capsicum annuum*)	Rouge long ordinaire

Table 2: Fungicides used for the treatment of radish, carrot and pepper seeds

Fungicides	Radish	Carrot	Pepper
Mancozeb		x	x
Maneb	x	x	x
Benomyl	x	x	
Thiophanate methyl	x	x	x

Isolation method: The incubation at 25°C of seeds was made on PDA medium (Potato-Dextrose-Agar) amended with 0.5 mg mL^{-1} of streptomycin sulphate. To determine the potential of fungal transmission, method developed by many authors (Vannacci and Gambogi, 1980; Punja *et al*., 2001; Boughalleb, 2005) was applied.

Seeds were surface-disinfected and washed several times with sterilized water. Four replicates of 100 seeds were placed directly in petri dish PDA medium. These operations were done under aseptic conditions. The plates were incubated at 25°C with an alternate of light and darkness of 12 h. The number of contaminated seeds was counted after 1 to 8 days according to colony development. Contaminated seeds were detected after 1, 5 and 8 days for radish and the carrot, while infestation level of pepper was evaluated after 3, 5 and 7 days. The infection level of each seedlot was estimated according to the following formula:

$$\text{Infection level (\%)} = \frac{\text{Total number of infected seed}}{\text{Total number of tested seeds (Boughalleb, 2005}} \times 100$$

Identification of developed colonies: Incubated seeds were observed daily. Colonies developed around the seeds were transferred to another Petri dish. Key identification of fungal species was used according to Champion (1997).

Test of seeds treatment by fungicides: Based on the phytosanitary index mentioning the recommended fungicides for each pathogenic fungus the following fungicides were tested (Table 2).

Statistical analysis: An analysis of the variance-one-way (ANOVA) was done to assess differences between seedlots. Means were compared according Duncan test at 5% level using the SPSS Software program (SPSS Inc. Headquarters, Chicago, Illinois).

RESULTS

Fungal species isolated from different seed lots: Three species of *Alternaria* were identified. *A. Tenuissima* was isolated on radish, carrot and pepper seeds while

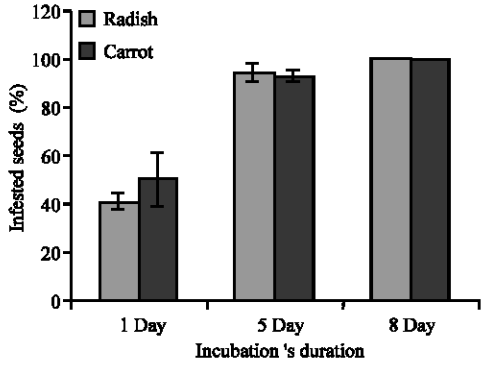

Fig. 1: Percentage of infestation levels of radish and carrot seeds, after 1, 5 and 8 days of incubation at 25°C

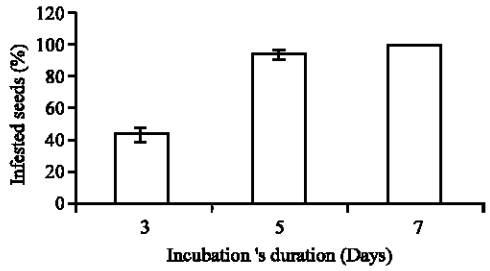

Fig. 2: Evolution of seeds infested levels, for papper after 3, 5 and 7 days of incubation at 25°C

A. dauci and *A. tenuis* were noted on radish and carrot. However, *Epicoccum* sp., *Fusarium oxysporum* and *Botrytis cinerea* were isolated from radish, carrot and pepper seeds, respectively.

Evaluation of infestation level of non-treated seeds: After 3 days of incubation, 41 and 49.75% of infested seeds was noted for radish and carrot, respectively. These levels increased to 94.5 and 93.25% after 5 days and reached 100% of infested seeds for both radish and carrot after 8 days (Fig.1).

For pepper, after three incubation days, the rate of infested seeds is 43 and 94 and 100% after 5 and 7 days, respectively (Fig. 2).

Evaluation of infestation levels of treated seeds and most efficient fungicide determination

Effect of treatment on the infestation level of radish seeds

Treatment by maneb: The treatment with Maneb at 3% dose, showed the best results than those of 3‰ at 5, 8 and 13 days of incubation. However, no significant difference was noted between these two doses at 3 days of incubation. In fact, at 5 days of incubation only 65% of

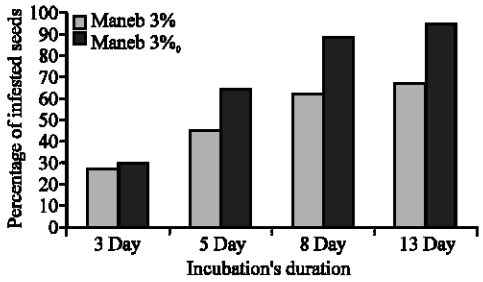

Fig. 3: Percentage of radish infested seeds treated by two of Maneb (3% and 3‰) incubated for 3, 5 and 13 days at 25°C

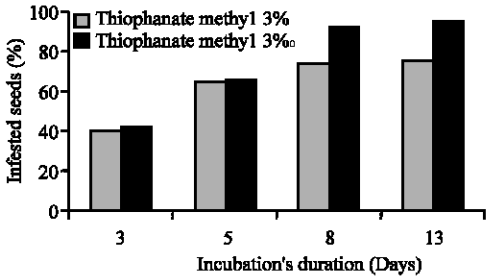

Fig. 4: Percentage of radish infested seeds treated bu two doses of Thiophanate methyl (3% and 3‰) incubated for 3, 5 and 13 days at 25°C

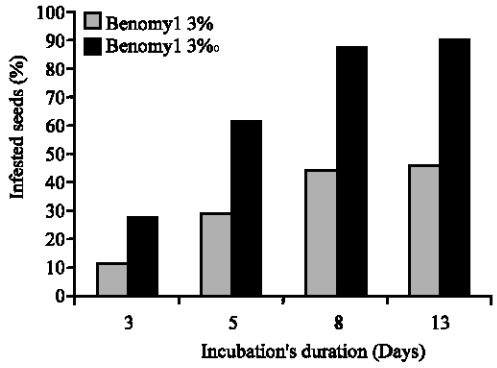

Fig. 5: Percentage of radish infested seeds treated by two doses Benomyl (3% and 3‰) incubated for 3, 5 and 13 days at 25°C

seeds were infested using the dose of 3% against 45% for the dose of 3‰. The similar amplitude was noted at 8 and 13 days of incubation (Fig. 3).

Treatment by thiophanate methyl: For 5 days of incubation, 65 and 66% was noted for radish seeds treated by Thiophanate methyl at doses of 3% and 3‰, respectively. From 8 days of incubation, no significant

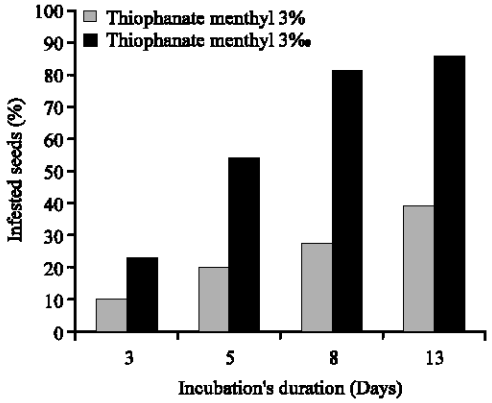

Fig. 6: Percentage of carrot infested seeds treated by two doses of Thiophanate methyl (3% and 3‰) incubated for 3, 5, 8 and 13 days at 25°C

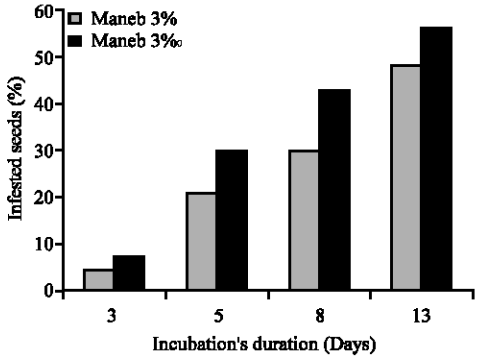

Fig. 7: Percentage of carrot infested seeds treated by two doses of Maneb (3% and 3‰) incubated for 3, 5, 8 and 13 days at 25°C

difference was detected between the infection percentages using 3% or 3‰ dose (74 and 92%, respectively) (Fig. 4).

Treatment by benomyl: Seeds treated with Benomyl revealed the lower percentages of infection and especially for the dose of 3%. After 3 days of incubation, 11 and 28% were detected for doses 3% and 3‰, respectively. After 13 days of incubation, 46 and 90% of infested seeds were noted for these doses, respectively (Fig. 5).

Effect of fungicide treatment on the infestation levels of carrot seeds

Treatment by thiophanate methyl: The difference between the effects of the two doses is especially considerable at 8 and 13 days of incubation. After 13 days of incubation, 86% of contaminated seeds (dose of 3‰) and 39% for 3% (Fig. 6).

Treatment by maneb: Maneb permitted a reduction of the percentage of seeds infested until 56 and 48% using 3‰ and 3% dose, after 13 days, respectively. However, treated seeds showed a seed infestation percent ranged between 30 (3% dose) and 43% (3‰) after 8 days of incubation (Fig. 7).

Treatment by benomyl: The results of seed carrot treated with Benomyl, after 3, 5, 8 and 13 days of incubation detected no difference between the two doses of pest (Fig. 8).

Treatment by mancozeb: For the mancozeb, the dose of 3% gave the best results compared to 3‰ dose. After 3 days of incubation, only 6 and 9% of infestation was found for the dose of 3% and 3‰, respectively. The difference between the percentages of infestation is more important at the 13th day of incubation (Fig. 9).

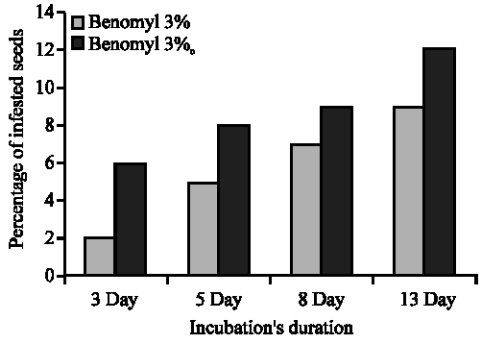

Fig. 8: Percentage of carrot infested seeds treated by two doses of Benomyl (3% and 3‰) incubated for 3, 5, 8 and 13 days at 25°C

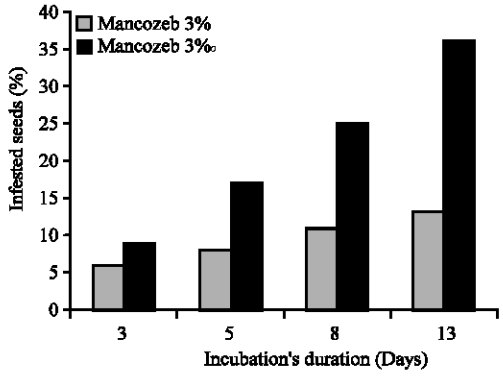

Fig. 9: Percentage of carrot infested seeds treated by two doses of Mancozeb (3% and 3‰) incubated for 3, 5, 8 and 13 days at 25°C

Effect of fungicide treatment on the infestation levels of pepper seeds

Treatment by thiophanate methyl: After 3 days of incubation, the fungal colonies have been observed at a level of 1 and 4% of infested seeds, respectively, for 3% and 3‰. After 12 days, 37% of infested seed were recorded for 3‰ dose, whereas the dose of 3%, seems to be more efficient, showing 7% of seeds infested (Fig. 10).

Treatment by mancozeb: Three days after the incubation, the rate of seeds infested is 2% (3‰ dose) and zero for the dose of 3%. Twelve days of incubation showed that the percentage of contaminated seeds increases to 29% at the dose 3‰ and 5% in the case of 3% dose (Fig. 11).

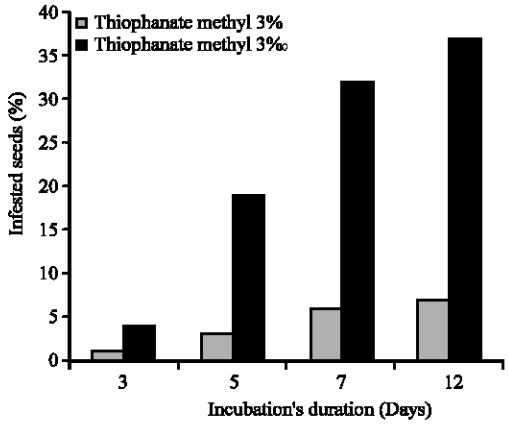

Fig. 10: Percentage of pepper infested seeds treated by two doses thiophanate methyl (3% and 3‰) incubated for 3, 5, 7 and 12 days at 25°C

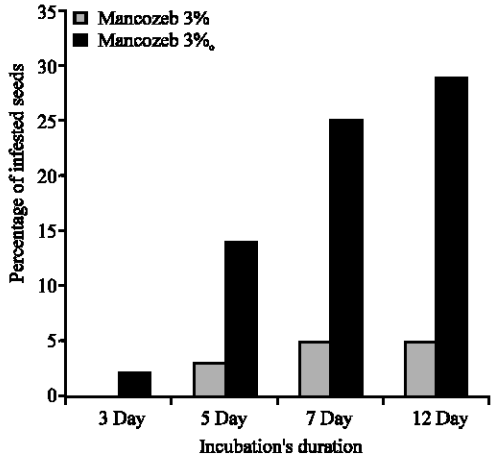

Fig. 11: Percentage of pepper infested seeds treated by two doses of Mancozeb (3% and 3‰) incubated for 3, 5, 7 and 12 days at 25°C

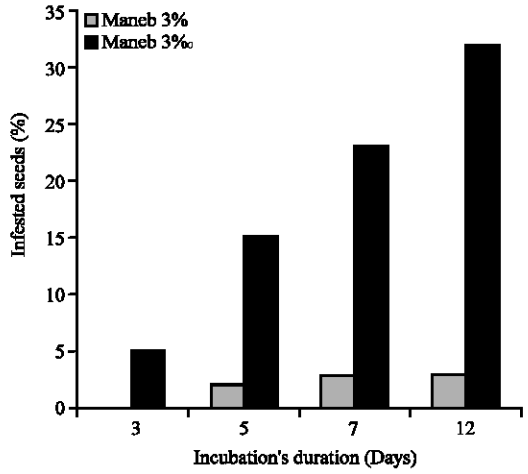

Fig. 12: Perentage of pepper infested seeds treated by two doses of Maneb (3% and 3‰) incubated for 3, 5, 7 and 12 days at 25°C

Treatment by maneb: The percentages of infested seeds after 3 days of incubation for treated seeds with Maneb at 3% and 3‰ doses are 0 and 5%, respectively. After 12 days of incubation, the level was 32% (3‰) and 3% for dose of 3% (Fig. 12).

Maneb revealed most efficient for the dose of 3%, for 7 days of incubation at 25°C level of contamination was 100% for non-treated pepper seeds, whereas for those treated, only 3% of contaminated seeds was recorded.

DISCUSSION

Results presented in this study showed that seeds of radish, carrot and pepper are infested by some fungi such as *Alternaria tenuissima*, *A. dauci*, *A. tenuis*, *Fusarium oxysporum* and *Epicocum*. It appears also that fungicides treatment could reduce infestation level of these seedborne fungi. Similar findings were reported by Raymond (1999) who demonstrated that *A. dauci*, *A. radicinam. A. tenuis* and *A. tenuissima* were transmitted by carrot seeds. Few reports showed the effect of fungicides on seedborne fungi. Biniek and Tylkowska (1987) mentioned that Thiram is able to protect carrot seeds tissues and to remove *A. radicina* seed infection. Recently, Galperin *et al.* (2003) identified *F. moniliforme* from corn seeds that could be controlled by prochloraz treatment. Fungi transmitted by seeds could be controlled by fungicide treatment at reasonable dose. However, this research should be reconducted using other active matter and doses to enlarge the game of fungicide able to reduce seedborne fungi.

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