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CROP PROTECTION

Plant extracts and essential oils on the control of *Alternaria alternata*, *Alternaria dauci* and on the germination and emergence of carrot seeds (*Daucus carota* L.)

Extratos e óleos essenciais vegetais no controle de *Alternaria alternata*, *Alternaria dauci* e na germinação e emergência de sementes de cenoura (*Daucus carota* L.)

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ABSTRACT

The present study aims to investigate garlic, pepper and coriander plant extract as well as neem and orange peel essential oil effective ness to control ***Alternaria alternata*** *and* ***Alternaria dauci*** *and their efficiency during carrot seeds germination and emergence. A completely randomized design was used in three different experiments. The first experiment evaluated the effect of plant extracts and essential oils on the incidence of* ***A. alternata*** *and* ***A. dauci****. It was done by means of a factorial design applied to five treatments (garlic, pepper, coriander, and neem and orange peel essential oils) at three concentrations (10, 20 and 30%).They also evaluated the controls untreated and with fungicide (Thiram).The second experiment evaluated the effect of the treatment at30% concentration on the germination and emergence of seedlings assessed on trays, and in the third experiment, the presence of* ***A. alternata*** *and* ***A. dauci*** *on the pericarp, endosperm and on the embryo.* ***A. alternata*** *showed higher incidence than* ***A. dauci****. The garlic extract and the orange essential oil showed the potential to control* ***A. dauci*** *and* ***A. alternata****, because their lower concentrations were able to sufficiently reduce the incidence of these fungi and because they do not affect carrot seeds germination and emergence.* ***A. alternata*** *conidia were found on the embryo (8%), pericarp (17%) and endosperm (31%).*

Key words: ***Alternaria*** *spp., seed treatment, alternative control.*

RESUMO

*O presente estudo teve por objetivo verificar a eficiência de extratos vegetais de alho (****Allium sativum*** *L.), pimenta dedo-de-moça (****Capsicum baccatum*** *L.), coentro (****Coriandrum sativum*** *L.), óleos essenciais de nim (****Azadirachta indica*** *A. Juss.) e laranja (****Citrus sinensis*** *L. (Osbeck)) no controle de* ***A. alternata*** *e* ***A. dauci****, bem como, sobre a germinação e emergência de sementes de cenoura. Foram realizados três experimentos com delineamento experimental inteiramente casualizado. No primeiro experimento, avaliou-se o efeito dos extratos e óleos essenciais vegetais sobre a incidência de* ***A. alternata*** *e* ***A. dauci****, em esquema fatorial com cinco tratamentos (alho, pimenta, coentro e óleos essenciais de nim e de laranja), em três concentrações (10, 20 e 30%), acrescido das testemunhas sem tratamento e com fungicida (Thiram). No segundo experimento, avaliou-se o efeito dos tratamentos a 30%, sobre a germinação e emergência de plântulas em bandejas e, no terceiro, a presença de* ***A. alternata*** *e* ***A. dauci*** *no pericarpo, endosperma e embrião. O fungo* ***A. alternata*** *apresentou maior incidência que* ***A. dauci****. O extrato de alho e o óleo essencial de laranja demonstraram potencial para o controle de* ***A. alternata*** *e* ***A. dauci****, pois, na menor concentração, reduziram satisfatoriamente a incidência desses fungos e foram inócuos a germinação e emergência de sementes de cenoura. Conídios de* ***A. alternata*** *foram identificados no embrião (8%), pericarpo (17%) e endosperma (31%).*

Palavras-chave: ***Alternaria*** *spp., tratamento de sementes, controle alternative.*

INTRODUCTION

The sanitary quality of commercial carrot seeds leads to the highest marketable yield with uniform roots, since the microorganisms found on the seeds may affect germination, seedling and the life cycle of the crop. The genus ***Alternaria*** encompasses species that may impair the growing process of the vegetables due to the diseases causes by them. They affect seedlings, leaves, stalks, stems, flowers and fruits. The name given to the diseases caused by ***Alternaria*** spp. changes depending on the crop; in carrot plants, it is called "leaf scorch". Alternariosis is a typically spring-summer time disease which presents highly destructive power under temperatures -from 25 to 32°C- associated

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with 40% relative humidity during the day and 95% at night (TÖFOLI&DOMINGUES, 2004).

According to MUNIZ & PORTO (1999), ***Alternaria alternata*** is commonly found on carrot seeds. Whenever alone or associated with ***Alternaria dauci*** on the plant, it can reduce the physiological quality of the seed and/or cause seedling to tipping. These species show high survival and distribution potential, since they persist on different seed structures and crop residues. Thus, these structured and residues are the initial inoculum source responsible for the quick spread of these fungi in the field (TÖFOLI & DOMINGUES, 2004). Therefore, the use of healthy and/or fungicide-treated seeds is demanding.

The chemical treatment is applied to vegetable seeds due to its simplicity, efficiency and cost-benefit ratio. Thiram and Captan are fungicide options to carrot seed treatment. However, the chemical method concerns due to intensively manpower demands in vegetable crops from planting to harvesting. Thus, the product contact with potential operators and seeds of users is higher, especially in family farming (MACHADO &SOUZA, 2005). Organic vegetable growers also need disease control alternatives, since chemical fungicides cannot be used in this cropping system.

Plant extracts and essential oils appeared to be efficient to control seed-associated pathogens. They reduce initial inoculum availability and impair resistant strains selection (SCHWAN-ESTRADA et al., 2003). They also show promising results in the control of several fungi species, due to their antimicrobial activity, which is attributed to the phenolic compounds and to the terpenoids. BAKKALI (2008) and KUMAR et al. (2008) emphasized that the cytotoxic property of plant essential oils comes from their lipophilic nature. These oils interact with the lipid layer on the plasma membrane and cross the cell wall.

SOUZA JÚNIOR et al. (2009) found that essential oils from "alecrim-pimenta" (***Lippia sidoides*** Cham.), wild basil (***Ocimum gratissimum*** L.), lemongrass (***Cymbopogon citratus*** Stapf) and "cidrão" (***Lippia citriodora*** Kunth) inhibited the germination and mycelial growth ***Colletotrichum gloeosporioides*** conidia. ROZWALKA et al. (2008) concluded that the partial or total inhibition of ***Glomerella cingulate*** and ***C. gloeosporioides*** mycelial growth *in vitro* showed that most of the studied essential oils and medicinal plants present biologically active compounds with antifungal effect. Thyme, eucalyptus citriodora, citronella and neem oils showed direct effect on ***Phakopsora pachyrhizi***, because they reduce Asian soybean rust severity (MEDICE et al., 2007). The major compounds found in garlic (VIEGAS et al., 2005), coriander (VIEGAS et al., 2005) and pepper extracts (KRAIKRUAN et al., 2008) also had their fungitoxic properties proven to be positive on plants.

Plant essential oils are also useful to treat seeds (BAKKALI, 2008) and prevent the presence of microorganisms on them. The effects of these oils are proven by commercial products from orange essential oils on wheat seeds (STÜLP et al., 2011) and neem essential oils as well as on rice seeds (SILVA et al., 2011). However, studies specifically targeting on the use of natural products to treatment vegetable seeds are still scarce. Therefore, the current study aims to investigate the effectiveness of garlic (***Allium sativum*** L.), red pepper (***Capsicum baccatum*** L.) and coriander plant extracts (***Coriandrum sativum*** L.), and neem (***Azadirachta indica*** A. Juss.) and orange essential oils (***Citrus sinensis*** L. (Osbeck)) to control ***A. alternata*** and ***A. dauci*** as well as their efficiency on carrot seeds germination and emergence.

MATERIALS AND METHODS

Five batches of cv. Brasilia carrot seeds were evaluated in the Seed Analysis and Plant Pathology Laboratories at State University of Northern Paraná, *Campus* Luiz Meneghel (UENP-CLM), Bandeirantes County/ PR (23º17'S latitude, 50º09'W longitude, 419m altitude). The treatments consisted of plant extracts (garlic, pepper, and coriander) and neem (Neem®) and orange essential oils (Orobor®, mineral-free) at the concentrations of 10, 20 and 30%. They also evaluated absolute (treatment-free) and relative (with thiram fungicide, 300ml100kg^{-1}seed) controls. Seeds were kept immersed in the solutions for 1 minute in the treatments using plant extracts and essential oils. Three seed batches were purchased from a certified company. They were health-treatment free and packed in airtight cartons. Their germination percentage was informed on the labels, namely: 82% in batch 1, 80% in batch 2 and 87% in batch 3. The other two batches were produced in UENP-CLM experimental area with 80% germination in batch 4 and 81.5% in batch 5.

Plant extracts preparation

Plant extracts were prepared by aqueous extract technique using peeled garlic cloves, red pepper with seeds, and green and fresh coriander leaf bundles. The ratios of 10, 20 and 30 grams of fresh material in 100mlof distilled water at the respective concentrations of 10, 20 and 30%, were respected. The milled fresh material was added to the distilled water (which was previously sterilized by autoclaving) when it was at boiling point using a refractory container. The container was sealed and wrapped in Kraft paper to avoid the possible loss of volatile compounds and the light incidence on the extract. Each solution was sieved and individually stored in amber flask and cooling at room temperature.

Experiment 1: the control of ***Alternaria alternata*** and ***Alternaria dauci*** on carrot seeds by the use of plant extracts and essential oils

The seeds from the five batches were previously sterilized by immersion in 1% sodium hypochlorite solution for 1 minute. Next, they were subjected to treatments using garlic, pepper and coriander plant extracts; and neem and orange essential oils at 10, 20 and 30% concentration. Absolute (treatment-free) and relative controls (with Thiram fungicide, 300ml 100kg^{-1}of seed) were used. Eight replicates with 25 seeds each were performed in each treatment.

Then, the treated seeds were distributed into 9cm diameter disposable Petri dishes (25 seeds per plate) on three sterile filter paper sheets soaked in sodium chloride (NaCl) solution, in osmotic potential 1.2 MPa using Van't Hoff formula (SALISBURY & ROSS, 1991) for the calculation. The plates were incubated under white fluorescent light and 12-hour photoperiod, at 25°C, for seven days (BRASIL, 2009). ***Alternaria alternata*** and ***Alternaria dauci*** incidence (%) was determined in stereoscopic microscope. The slides with fungal material on were observed in optical microscope, whenever it was necessary.

Experiment 2: Effect of plant extracts and essential oils on seedling germination, emergence and on tipping caused by ***Alternaria***

The experiment was performed in batches showing higher (batch 5) and lower (batch 1) ***Alternaria*** species incidence. The batches were treated with plant extracts and orange essential oil (OEO) at 30%concentration. The neem essential oil (NEO) treatment was excluded because it showed less effect on ***A. dauci*** and ***A. alternata*** incidence reduction than that of OEO and thiram.

The treated seeds were submitted to germination test and it was carried out using four repetitions with 50 seeds per treatment. The seeds were equidistantly distributed on previously moistened filter paper in distilled water - applied at the ratio of 2.5 times the dry paper weight. The seeds were kept in transparent plastic containers, in BOD growth chamber, at the alternating temperature of 20-30°C, i.e., 20°C for 16 hours (simulating night time) and 30°C for 8 hours (simulating day period). The number of normal seedlings with expanded cotyledon leaves was recorded in the seventh and four tenth days of experiment (BRASIL, 2009).

The seedling emergence test was performed in greenhouse in four replicates with 50 seeds per treatment. The seeds were sown 1cm deep in 200-cellplastic trays filled with the commercial substrate Carolina® -which is specific for vegetable seedling production. The trays were kept in a bow model greenhouse and irrigated in the morning and evening times. The number of emerged seedlings was recorded in the fourteenth day of experiment.

Seedlings tipping 10, 15, 20, 25 and 30 days after emergence were removed from the trays, washed in distilled water and placed in Petri dishes prepared with PDA medium plus antibiotics (200mg L^{-1} streptomycin sulfate). They were kept in BOD for a 12-hour photoperiod, at 25°C, for seven days. After, ***Alternaria*** spp. incidence (%) was evaluated in stereoscopic microscope.

Experiment 3: Finding ***Alternaria*** in seed structures

200 seeds from batch 5 (the one with the higher ***Alternaria*** incidence) were used, and 100 of them were distributed on previously moistened filter paper in distilled water for 24 hours. After, the pericarp was separated from the endosperm using a scalpel (the blade was disinfected after each procedure). The remaining 100 seeds were directly soaked in distilled water for 72 hours in order to soften the pericarp before a bevel cut was performed on the edge next to the embryo. Thus, the embryo could be exposed after a mild local pressure was applied over it. The separated pericarp, endosperm and embryo were placed in Petri dishes containing three sheets of previously moistened filter paper in distilled water. They were then incubated at 25°C for seven days, under a12-hour photoperiod. Evaluations were individually done in all the seed structures using stereoscopic microscope. Results were expressed in percentage.

Experimental design and statistical analysis

A completely randomized design was used in experiments 1 and 2. The fungal incidence percentage of each structure was recorded in the third experiment, no statistical approach was used. Experiment 1 was conducted using the factorial design 5x3 (treatments x concentrations) as well as the absolute and relative controls.

Alternaria dauci showed plant extract and essential oil effects only. Therefore, data presented in table 2 represent the average of the three concentrations per batch, in each treatment. The original data were turnedinto√x+0.5for variance analysis and the averages were grouped by Scott-Knott test (P≤0.05) using the Sisvar® statistical software.

RESULTS AND DISCUSSION

Alternaria alternata showed higher incidence than ***A. dauci*** in seeds without treatment (Tables 1 and 2). It is worth highlighting that batches 4 and 5 presented significant percentage in both pathogens. REIS et al. (2003) reported that ***A. alternata*** was the prevalent pathogen in their research on ***Alternaria*** species transmitted by carrot seeds. These authors recommended the use of a germplasm screening program to find the potential resistance sources of these species, because of their high pathogenicity potential.

The plant extracts and essential oils significantly reduced ***A. alternata*** incidence when it was compared to the untreated control (Table1). Treatments were similar to each other in 84% of the batches, at the three herein used concentrations. Both the neem (NEO) and the orange (OEO) essential oils decreased ***A. alternata*** incidence at all used concentrations and they showed similar performance to that of the witness treated with fungicide. However, OEO reached zero fungal proliferation in 73.3% of the batches.

The treatments had significant effect on ***A. dauci*** (Table 2), possibly due to the lower initial percentage of the fungus in the untreated seeds. ***A. dauci*** incidence significantly decreased after the treatment using plant extracts and essential oils. Again, OEO showed similar reduction to that of the witness treated with fungicide, and garlic had the best performance among plant extracts. STÜLP et al. (2011) assumed that the disease control in wheat seeds resulted from the use of commercial

Table 1 - ***Alternaria alternata*** incidence (%) in cv. 'Brasilia' carrot seed batches after treatments using garlic, coriander and pepper extract and neem (NEO) and orange essential oils (OEO) at 10, 20 and 30% concentration.

Lot	Garlic	Coriander	Pepper	NEO	OEO	----Controls----	
						N/T	Thiram
----10%----							
1	3.5 Bb[1]	8.5 Bb	8.5 Bb	4.0 Bb	0.0 Ab	29.0 Ca	0.0 Ab
2	3.0 Bb	3.0 Bb	5.5 Bb	3.0 Bb	0.0 Ab	45.5 Ba	0.0 Ab
3	3.0 Bc	3.5 Bc	13.0 Bb	3.0 Bc	0.0 Ac	29.5 Ca	0.0 Ac
4	10.5 Bb	11.0 Bb	13.5 Bb	9.0 Bb	0.0 Ac	86.0 Aa	0.0 Ac
5	51.5 Ab	40.5 Ac	35.5 Ac	30.0 Ad	3.5 Ae	89.5 Aa	0.0 Ae
	14.3 c.	13.3 c	15.2 b	9.8 d	0.7 e	55.9 a	0.0 e
CV (%)	49.2						
----20%----							
1	20.0 Ba	8.5 Cb	6.0 Cb	7.5 Bb	0.0 Ab	29.0 Ca	0.0 Ab
2	19.5 Bb	4.5 Cc	3.0 Cc	2.0 Bc	0.0 Ac	45.5 Ba	0.0 Ac
3	19.5 Bb	4.5 Cc	8.0 Cc	3.5 Bc	0.0 Ac	29.5 Ca	0.0 Ac
4	20.0 Bb	19.5 Bb	18.5 Bb	7.0 Bc	0.5 Ac	86.0 Aa	0.0 Ac
5	67.5 Ab	72.5 Ab	72.5 Ab	44.0 Ac	0.5 Ad	89.5 Aa	0.0 Ad
	29.3 b.	21.9 c	21.6 c	12.8 d	0.2 e	55.9 a	0.0 e
CV (%)	46.1						
----30%----							
1	0.0 Bb	5.0 Cb	3.0 Bb	5.0 Bb	0.0 Ab	29.0 Ca	0.0 Ab
2	2.5 Bb	4.5 Cb	2.0 Bb	2.0 Bb	0.0 Ab	45.5 Ba	0.0 Ab
3	1.5 Bb	6.5 Cb	1.5 Bb	2.5 Bb	0.0 Ab	29.5 Ca	0.0 Ab
4	5.5 Bc	18.5 Bb	6.5 Bc	6.5 Bc	0.0 Ac	86.0 Aa	0.0 Ac
5	14.5 Ae	71.0 Ab	25.5 Ad	45.0 Ac	0.5 Af	89.5 Aa	0.0 Af
	4.8 e.	21.1 b	7.7 d	12.2 c	0.1 f	55.9 a	0.0 f
CV (%)	53.9.						

[1]Means followed by the same letter - uppercase in the column and lower case on the line -do not differ from each other according to Scott-Knott test at 5%; CV=coefficient of variation; N/T=No treatment.

Table 2 - ***Alternaria dauci*** incidence (%) in cv. 'Brasilia' carrot seed batches after treatments using garlic, coriander and pepper extract and neem (NEO) and orange essential oils (OEO).

Lot	Garlic	Coriander	Pepper	NEO	OEO	-----------------Controls-----------------	
						N/T	Thiram
1	0.00 Ab[1]	0.00 Cb	0.33 Bb	0.33 Bb	0.00 Ab	9.50 Ba	0.00 Ab
2	0.33 Ab	0.00 Cb	0.00 Bb	0.00 Bb	0.00 Ab	10.50 Ba	0.00 Ab
3	0.33 Ab	0.00 Cb	0.00 Bb	0.00 Bb	0.00 Ab	5.50 Ca	0.00 Ab
4	0.50 Ab	2.00 Bb	1.00 Bb	0.83 Bb	0.17 Ab	14.50 Aa	0.00 Ab
5	1.83 Ad	6.66 Ac	8.50 Ac	10.50 Ab	0.00 Ae	16.50 Aa	0.00 Ae
Mean	0.60 c	1.73 b	1.97 b	2.33 b	0.03 d	11.30 a	0.00 d
CV (%)	40.7						

[1]Means followed by the same letter - uppercase in the column and lower case on the line -do not differ from each other according to Scott-Knott test at 5%; CV =coefficient of variation; N/T=No treatment.

product based on orange essential oil. According to GUIMARÃES et al. (2011), limonene–which is the major OEO constituent–presented antimicrobial activity in ***A. alternata*** mycelial growth, *in vitro*. According to MARÓSTICA JÚNIOR & PASTORE (2007), such result is related to decreased oxidative phosphorylation process rate sin cells.

Allicin – which is found *in natura* in garlic cloves-presents toxic effect on plant pathogen control. It inhibits mycelial growth and spores germination (SCHWAN-ESTRADA et al., 2003). GIRARDI et al. (2009) studied the antifungal potential of garlic in zinnia seeds and they found significant ***Alternaria*** spp. incidence reduction.

Batch 1 showed higher germination values in treatments using plant extracts than those using fungicide (Table 3). However, the 82% germination showed by the batch 1 pack was not confirmed by the germination test (maximum of 76.5%) or by the seedling emergence test (maximum of 44.5%). Batch 5 -except for the OEO treatment-, kept the mean germination higher than the 75% recommended for carrot seed commercialization (MAPA, 2012) -the untreated seeds were included in this calculation-, and it indicates that pathogens were not detrimental to seeds germination. The mean seedling emergence in batch 5 exceeded that of batch 1 in all treatments, especially in the garlic and pepper extract ones.

The current study showed OEO effectiveness to control ***A. alternata*** and ***A. dauci***, despite the lowest average found in the germination and emergence of plants subjected to this treatment in batch 5. It confirmed the bioactive potential of plants or parts of plants in other plant's germination. A detailed further study involving different doses, cultivars and batches must be done to check the interference of the genetic and physiological quality of the seeds in the final result. According to CARNEIRO (2003), phytotoxicity responses depend on the plant species, on its age and on the structural development stage in which the essential oil is applied.

Seedling tipping averages remained below 4.3% in the untreated control but they were significant and close to 10% in coriander in the two batches (1 and 5), and pepper only in batch 5 (Table 3). The ***A. alternata*** conidia were found on seedlings with tipping symptoms. Regarding the fungus location, these conidia were found on the embryo (8%), pericarp (17%) and endosperm (31%). However, MUNIZ & PORTO (1999) found higher percentage of this species on the external side of the carrot seeds.

None of the two ***Alternaria*** species decreased the physiological quality of the seeds. Even the batch with the highest incidence of them showed good seedling germination and emergence performance. According to TRIGO et al. (1997), ***A. alternata*** directly affects the physiological quality of the seeds. MUNIZ & PORTO (1999) reported that the association among the above-mentioned species, the carrot seeds and the plants is not restricted to saprophytism, and it may cause seedling tipping at greater or lesser degree, depending on the type of umbel generating the seeds. However, REIS et al. (2006) concluded that the presence of ***A. alternata*** and ***A. dauci*** did not affect the germination and vigor of coriander seeds. PEDROSO (2009) also reported that tests carried out in a greenhouse showed no losses in carrot seedlings due to the presence of these pathogens. It is worth emphasizing that the internal temperature and humidity in the greenhouse may have been un favorable to the development of ***Alternaria*** species, because the ideal environmental conditions are essential so that the pathogens associated with seeds become active.

Table 3 - Mean germination, emergency and seedling tipping in cv. 'Brasilia' carrot seed batches, after treatment with garlic, coriander and pepper extracts and orange essential oil (OEO) at 30% concentration.

Lot	Garlic	Coriander	Pepper	OEO	--------------Controls------------		CV (%)
					N/T	Thiram	
---Germination---							
1	76.5 A[1]	76.5 A	71.0 A	71.0 A	68.0 B	62.0 B	7.0
5	83.5 A	80.0 A	83.0 A	65.5 B	81.5 A	82.0 A	7.6
---Seedling emergence---							
1	27.5 A	44.5 A	36.0 A	39.0 A	42.5 A	39.5 A	23.5
5	77.5 A	59.5 B	83.5 A	54.5 B	65.0 B	52.5 B	20.2
---Seedling tipping---							
1	0.2 B	9.7 A	2.0 B	0.0 B	1.7 B	1.7 B	50.8
5	0.2 C	8.5 A	6.5 A	0.5 C	4.2 B	1.2 C	53.7

[1]Means followed by the same letter - uppercase on the line -do not differ from each other according to Scott-Knott test at 5%; CV =coefficient of variation; N/T=No treatment.

The presence of ***A. alternata*** and ***A. dauci*** should be avoided even when they do not damage the quality of the carrot seeds. According to TÖFOLI & DOMINGUES (2004), the ***Alternaria*** spp. conidia are able to survive along with seeds on infected culture remains, intermediate hosts and on agricultural equipment, they can remain alive for a year under such conditions.

CONCLUSION

The garlic extract and the orange essential oil showed the potential to control ***A. alternata*** and ***A. dauci***, since their lower concentrations were able to satisfactorily reduce the incidence of these fungi. They were harmless to carrot seed germination and emergence.

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