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Ewa B. MOLISZEWSKA[1] and Violetta SMIATEK[1]

TOXIC PROPERTIES OF *Alternaria radicina* CULTURE FILTRATES AGAINST CARROT SEEDS AND SEEDLINGS

WŁAŚCIWOŚCI TOKSYCZNE PRZESĄCZY POHODOWLANYCH *Alternaria radicina* W STOSUNKU DO NASION I SIEWEK MARCHWI

Abstract: The carrot seed samples health condition and their germination ability were discussed for the period 1997–2003. For the discussed period, the average infection of seed plots was 9.8 % and the seeds' percentage germination was 68.4 %. These parameters were correlated each other. The culture filtrates of five isolates of *Alternaria radicina* were tested in the laboratory experiments. *A. radicina* isolates were obtained from carrot seeds. Toxic activity of metabolites included in culture filtrates showed different activity against seeds and seedlings depending on the isolate type. Their phytotoxic influence was observed against germination of seeds; more dead seeds and abnormal seedlings were observed under their influence as well as the time of germination was prolonged. The influence on the normally developed and healthy seedlings showed the phytotoxic activity for more concentrated culture filtrates and a positive activity for low concentrated culture filtrates (1 %, 2 %) of isolates 001 and 003. The direct relationships between culture filtrates and their concentrations were not observed.

Keywords: carrot, *Alternaria radicina*, toxins, culture filtrate

Carrot (*Daucus carota* L.) is one of the major vegetable plant. It is grown in many cultivars and varieties because of the roots which are rich source of the β-carotene. Carrot roots can also accumulate some substances harmful for human beings. This can be prevented by use of healthy carrot seeds. Seeds frequently are contaminated by *Alternaria dauci* and *A. radicina* [1–3].

A. radicina is a seed-transmitted pathogen and causes seed rot, decreases seed germination percentage and reduces seedlings survival [4, 5]. Increase in seed infestation/infection by this pathogen results in the reduced germination of carrots [1,

[1] Department of Biotechnology and Molecular Biology, Opole University, ul. kard. B. Kominka 4, 45–035 Opole, Poland, phone/fax 77 401 60 50, email: ewamoli@uni.opole.pl

[2] Regional Station of Plant Protection and Seed Evaluation in Opole, ul. Wrocławska 172, 45–810 Opole, Poland, phone/fax 77 474 46 19, email: fiolaf@op.pl

2]. The black rot of carrot roots is a postharvest disease important during the storage and cultivation carrots for seed-yield. Carrot seeds and debris have been considered to be the critical primary inoculum of the disease in the field [2, 4].

A. radicina produces phytotoxins: radicin (RAD), radicinol (ROH) and *epi*-radicinol (*epi*-ROH). These phytotoxins were found in carrot samples naturally contaminated with *Alternaria* and showing black rot symptoms. Toxic activity of *A. radicina* is expressed on carrot tissues. Radicins are reported as not hazard for consumers but otherwise they express its phytotoxic activity important to obtain good quality of plant material [2].

The aim of this work was to evaluate a toxic activity of five strains of *A. radicina,* isolated from diseased carrot seeds, against carrot seeds and seedlings.

Materials and methods

The natural infestation by *A. radicina* and *A. dauci* of carrot seed samples was measured according to routine methods used for seed evaluation [6]. Beside this the seed samples germination ability was evaluated. Data obtained in 1997–2003 for samples from Bydgoszcz, Opole and Poznan were analyzed.

The five isolates of *A. radicina* were obtained from diseased carrot seeds cv. Krakowia during routine tests in Regional Station of Plant Protection and Seed Evaluation in Opole in 2003 [6]. Isolates were incubated on PDA medium as well as they were stored on PDA slants at +4 ^{o}C.

The toxic activity of *A. radicina* strains 001, 003, 004, 005, 006 was investigated in liquid Czapek medium used as culture filtrates after the time of incubation of fungi. The Erlenmayer flasks filled with 250 cm^3 of medium, were inoculated with three 3-mm discs of PDA medium overgrown by the particular isolate and cut from the margin parts of one-week-old colony, then incubated at room temperature for three weeks. Then the culture filtrates were used for toxicogenic activity. The tests for toxic activity of culture filtrate to carrot seeds and seedlings were conducted in plastic boxes lined with sterile blotting paper. The blotting papers in each box were soaked with culture filtrates using concentrations: 100 %, 50 %, 25 %, 12 %, 6 %, 3 %, 2 %, 1 % (v:v) of the raw filtrates. In each box 25 carrot seeds cv. Krakowia were separately sown and incubated in thermostat in eight-hours temperature intervals 30 ^{o}C and 20 ^{o}C. Incubation was made for 21 days, the toxic activity was estimated four times, first time after 7, next after 10, 14 and 21 days. In the control combination, instead of a culture filtrate, sterile distilled water was used. Experiment was done in four replications.

For evaluating the phytotoxic activity of tested strains metabolites against healthy, normally developed seedlings the modified method given by Sulek-Pieta [7] for investigation *Fusarium oxysporum* f. sp. *phaseoli* toxic activity against *Phaseolus vulgaris* L. was used. The healthy and normally developed seedlings were transferred to the solutions of culture filtrates (concentrations the same as in the previous tests as well as the control combination). Their condition and living time were observed comparing with the control. Test was done in four replications.

Results were statistical estimated by the variance method, significant differences between averages were measured according to Duncan's multiple range test ($\alpha = 0.05$).

Results

The infestation of carrot seed samples was significantly correlated with their germination ability. The average infestation of seed samples in the period 1997–2003 was 9.8 % and the germination of seeds was 68.4 %. Average seed germination ranged from 66.0 % to 80.4 % (Table 1).

Table 1

Quality of carrot seeds in three locations illustrated by average infection by *A. radicina*, average seed germination and correlation coefficients between infection and germination of seed samples (significant > 0.306 for $\alpha = 0.05$; significant > 0.432 for $\alpha = 0.01$)

Year	Source of samples	No. of samples	Average infection [%]	Average germination [%]	Correlation coefficient ($\alpha = 0.05$; $\alpha = 0.01$)
1997	Bydgoszcz, Opole	30	22.2	66.8	**–0.6319**
1998	Bydgoszcz, Poznan	48	7.7	68.5	–0.0113
1999	Bydgoszcz, Poznan	51	8.5	71.5	**–0.4426**
2000	Bydgoszcz, Opole, Poznan	69	8.8	66.0	–0.2766
2001	Bydgoszcz, Opole, Poznan	41	10.1	68.9	**–0.4963**
2002	Bydgoszcz, Poznan	41	7.7	66.3	**–0.4675**
2003	Bydgoszcz, Poznan	8	3.8	80.4	**–0.9756**
1997–2003	Bydgoszcz, Opole, Poznan	288	9.8	68.4	**–0.3466**

The test with culture filtrates (CFs) showed that seedlings obtained in CFs environment were smaller and weaker as well as the time of germination was prolonged even to 21 days, although the most germinated seeds were observed in 7th day and next in 10th day of the experiment. In case of CFs of isolates 005 and 006 more germinated seeds were denoted for the concentration 1 % in 7th day. The feature was not observed in 10th day and showed that 1 % concentrated CFs of isolates 005 and 006 could speed up the germination process. The seedlings condition (normal/abnormal) and their health were more significant in accordance with the higher CFs concentrations. Seedling roots were more frequently deformed and shortened than hypocotyls and cotyledons. CFs tested in the experiment showed different phytotoxic properties depending on the origin (fungal isolate) and the concentration level. Seeds treated with not diluted culture filtrates gave only a few normally developed seedlings. For the isolate 003 the lowest phytotoxicty was found in case of CF used in a concentration 25 % – there were 50 % of normally germinated seeds (Table 2, Fig. 2). CF used in low concentrations showed a minute ability to reduction of seed germination although the isolate 003 was the most toxic for carrot seeds comparing with the other ones (Table 2). In most cases, CFs used in the concentration 12 % showed greater ability to reducing seeds germination than

culture filtrates used in conc. 50 % or 25 %. Clean (not diluted) CF caused very high decrease of seed germination or they did not germinated at all in case of isolates 001 and 004 (Table 2). In all test combinations the percentage of seed germination was worse than in the control combination, although in the cases of isolate 004, 005 and 006 it was not statistically different from the control (Table 2).

Table 2

Carrot seed germination ability measured after 21 days

Concentration [%]	*Alternaria radicina* isolate				
	001	003	004	005	006
100	0 a*	5 a	0 a	1 a	3 a
50	25 b	28 b	6 ab	16 b	32 b
25	37 c	50 e	42 c	48 cd	47 c
12	41 c	24 b	17 b	42 c	31 b
6	37 c	31 bc	32 c	48 cd	48 c
3	39 c	42 cde	40 c	51 cd	55 cd
2	57 de	34 bcd	42 c	57de	56cd
1	54 d	47 de	65 d	60 de	60 cd
0 (control)	66 e	66 f	66 d	66 e	66 d

* Data indexed by the same letter are statistically not significant in columns.

The condition of seeds and seedlings influenced by culture filtrates was different and depended on the isolate. A minute number of normally developed seedlings were

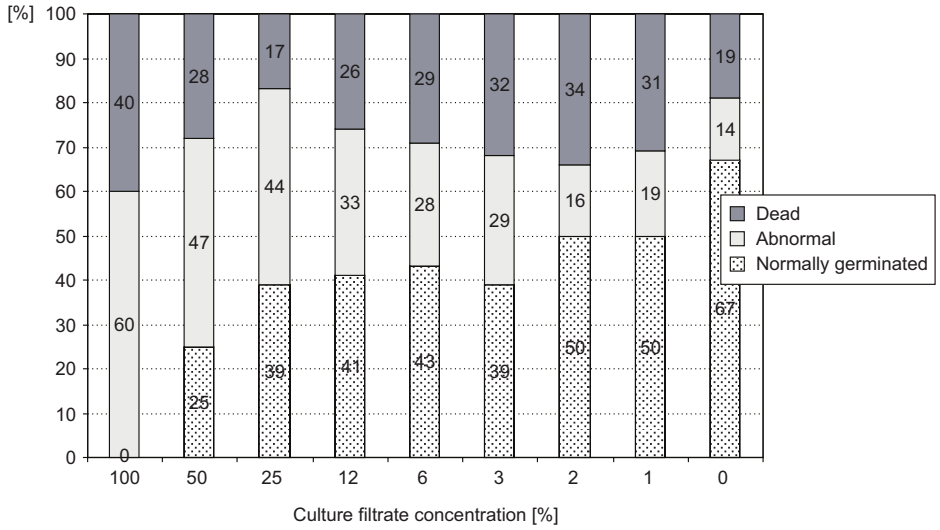

Fig. 1. The influence of culture filtrate of *A. radicina* 001 on the germination process of carrot seeds

observed under pure culture filtrates in case of isolates 003, 005 and 006 (5 %, 1% and 3 %, respectively). The isolate 004 used in conc. 50 % showed very similar activity – only 6 % of normally germinated seeds. For filtrates of 003, 004 and 006 in conc. 12 % we observed higher toxic activity than in case of twice more concentrated CFs (Figs. 1–5). In the combinations with the CFs of isolates 005 and 006 a high number of

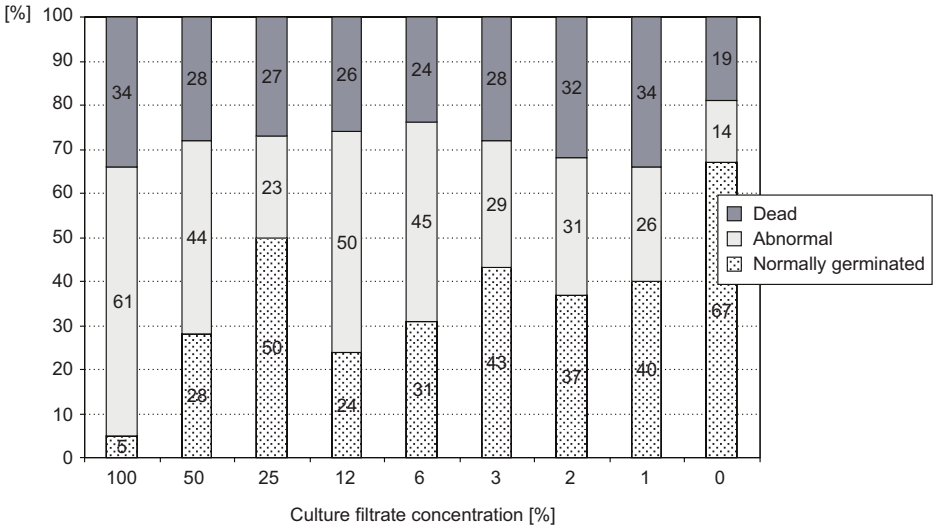

Fig. 2. The influence of culture filtrate of *A. radicina* 003 on the germination process of carrot seeds

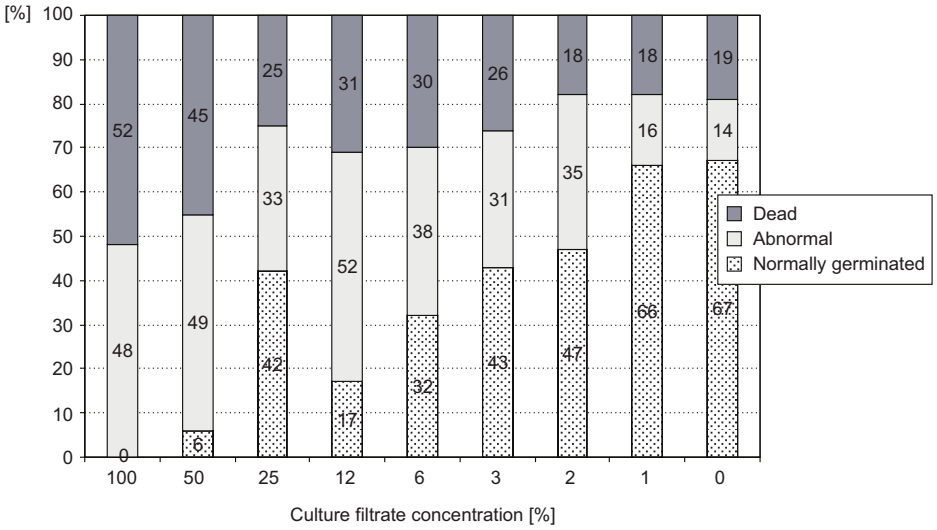

Fig. 3. The influence of culture filtrate of *A. radicina* 004 on the germination process of carrot seeds

abnormally germinated seeds were not observed in conc. 6–3 % to 1 % comparing with the control, but the number of dead seeds was higher (Figs. 4, 5). Higher concentrated CFs (100–12 %) showed the phytotoxic influence directed rather against seedlings than seeds. The lowest concentrated CFs had not significant activity. The effect on seeds ranged from 57 % and 52 % of dead seeds for not diluted CFs of isolate 006 and 004,

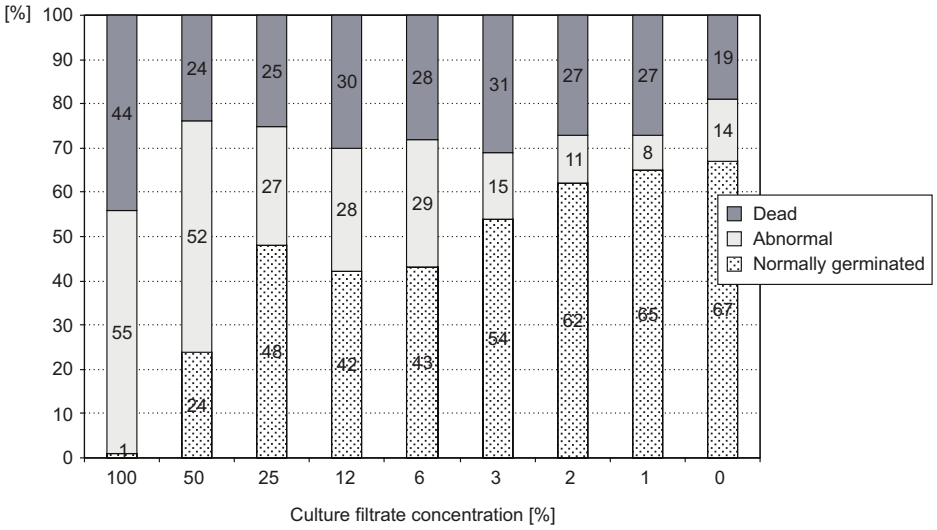

Fig. 4. The influence of culture filtrate of *A. radicina* 005 on the germination process of carrot seeds

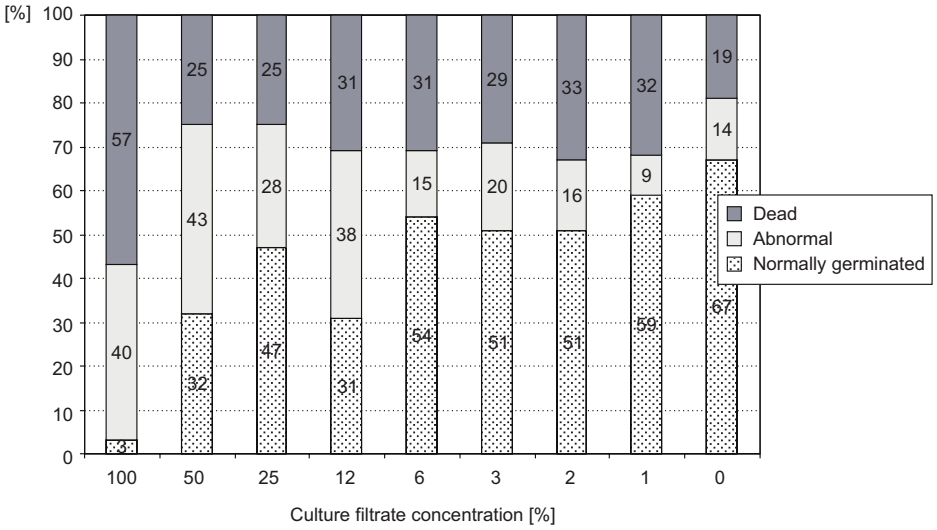

Fig. 5. The influence of culture filtrate of *A. radicina* 006 on the germination process of carrot seeds

respectively to 18 % of dead seeds for CF of isolate 004 in conc. 2 % and 1 % (Figs. 1–5). The results show that fungal metabolites activity is a combination of the influence on the seeds and seedlings condition but they depend on the CFs origin (fungal isolate) and their concentration.

Normally developed carrot seedlings were put on the Petri dishes lined with the blotting paper and soaked with diluted CFs. The influence of tested CFs on seedlings showed that the best results were obtained for low concentrated culture filtrates (2 %, 1 %) comparing with the control. The time of seedlings persisting/living was for one week longer for seedlings treated with low (1 %, 2 %) concentrated CFs of isolates 001 and 003. For filtrates of other isolates the results for highly diluted CF were comparable with the control (Table 3). The isolates 001 and 003 showed the weaker influence on the seedlings than the other isolates. The strongest phytotoxic activity showed the isolate 004 (Table 3).

Table 3

The influence of culture filtrates of *A. radicina* isolates on the time of living [days] of healthy and normally developed carrot seedlings

Culture filtrate concentration [%]	*Alternaria radicina* isolate				
	001	003	004	005	006
100	4.0 a*	4.8 a	2.3 a	3.0 a	3.0 a
50	5.0 a	6.0 a	4.3 b	3.5 a	3.0 a
25	8.3 ab	8.3 ab	3.8 ab	3.8 a	3.8 a
12	8.0 ab	7.5 ab	4.8 b	5.8 ab	3.5 a
6	8.8 ab	10.5 bc	5.5 bc	7.3 bc	5.8 ab
3	10.0 b	14.3 cd	7.0 c	8.8 cd	8.0 bc
2	16.0 c	13.3 cd	9.5 d	9.8 cd	8.5 bc
1	18.5 c	17.3 d	10.25 d	11.0 d	10.8 c
0 (control)	10.5 b	10.5 bc	10.5 d	10.5 d	10.5 c

* Data indexed by the same letter are statistically not significant in columns.

Discussion

The phytotoxic activity of radicines is related with carrot tissues. The carrot plant is biennial and pathogens correlated with carrot seeds originate from the carrot plants develop in their second year of cultivation. Because of this the health condition of taproots used in growing plants for seeds is very important. *A. radicina* existing on the taproots can develop on the plants in the second year of growing and then infect seeds. Fungicides used in the controlling root pathogens prevent only those ones free of pathogens but do not those originally infected [1, 8]. Avoiding infection of taproots is important both for commercially produced carrots and for seed production. Contamination of seed plots by *A. radicina* achieved even a 99 % of them at level ranging

from 0.5 % to 82.5 % for Polish samples in the period 1984–1988 and equally for samples from other countries but in infection rates to 35 % [1]. The investigated carrot seed plots were average infected 9.8 % in the period 1997–2003, and the highest rate of infection was 22.2 % (Table 1). These results show that the seeds quality in the experiment was better than quality of seed lots in 1984–1988. This is important in carrot cultivation because use of *A. radicina*-free seed is the first and perhaps most important step toward the integrated management of black rot of carrot. *A. radicina* was found also on seeds treated with thiram and/or iprodione at levels of 0.2 % to 14 % [1], so this highlights that contamination of carrot seeds is a continuing problem. The importance of contamination was shown in germination tests. *A. radicina* isolates and their metabolites (radicins) showed negative influence on the seed percentage germination and their quality as well as their time of germination. In our tests the germination was prolonged to even 21 days although the typical time of carrot seed germination is 7 to 12 days [1]. A risk of damping-off increases if the time of germination is prolonged and if seed samples are infected by pathogens and results in reduced stands and yield. Tests showed also that radicins can influence on the seedlings. They reduced a number of normally developed plants, and the number of abnormal seedlings was higher under their influence as well as the living time of normally developed plants was reduced. The low concentrated toxins were not a hazard for seedlings, we observed even that they could live for longer time than in the control. One of the reason was the non-specific mode of action of radicins and probably remnant of medium diluted together with metabolites, but naturally grown seedlings use nutrients from soil-solution as well. The mode of action of culture filtrates of *A. radicina* observed in our investigation showed its non-specific features [9] and the differentiation between fungal isolates in the ability of producing the phytotoxic components.

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WŁAŚCIWOŚCI TOKSYCZNE PRZESĄCZY POHODOWLANYCH *Alternaria radicina* W STOSUNKU DO NASION I SIEWEK MARCHWI

[1] Katedra Biotechnologii i Biologii Molekularnej
Uniwersytet Opolski
[2] Wojewódzki Inspektorat Ochrony Roślin i Nasiennictwa w Opolu

Abstrakt: Porównano zdrowotność oraz zdolność kiełkowania partii nasion marchwi pochodzących z trzech rejonów Polski w latach 1997–2003. Średnie porażenie nasion w omawianym okresie wynosiło 9,8 %, a średnia zdolność kiełkowania – 68,4 %. Obydwa parametry były ze sobą skorelowane. W doświadczeniach laboratoryjnych zbadano wpływ przesączy pohodowlanych pięciu izolatów *Alternaria radicina* uzyskanych z nasion marchwi. Metabolity zawarte w przesączach pohodowlanych wykazywały działanie fitotoksyczne zarówno na nasiona marchwi, jak i na jej siewki. Obserwowano zwiększoną śmiertelność nasion, rozwój siewek anormalnych oraz przedłużenie kiełkowania. Roztwory przesączy pohodowlanych wpływały także negatywnie na żywotność zdrowych i normalnie rozwiniętych siewek marchwi, jednak filtraty izolatów 001 i 003 w małych stężeniach (1 %, 2 %) wykazywały się zdolnością do przedłużania życia siewek w porównaniu do kontroli. Nie obserwowano bezpośredniej zależności działania badanych przesączy od ich stężenia.

Słowa kluczowe: marchew, *Alternaria radicina*, toksyny, przesącz pohodowlany