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RHIZOCTONIA SOLANI KÜHN ANASTOMOSIS GROUP 3 AS PATHOGEN OF POTATO AND ITS SENSITIVITY TO SEED-FUNGICIDES

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Abstract

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R. solani (*Thanatephorus cucumeris* (Frank) Donk) isolated from the sclerotia from the potatoes belongs to anastomose group 3 (AG 3), defined as the "potato type". The present study shows the results of research into the efficiency of the seed-fungicides on *R. solani* (AG 3) isolates from various regions (12) of the Czech Republic. In addition to registered seed-fungicides we also tested the unregistered preparation Prestige 290 FS (pencycuron + imidacloprid). Our objective was to test the efficiency of the seed-fungicides on *R. solani* isolates and the sensitivity, or resistance, of the isolates to registered and unregistered seed-fungicides and/or to the active ingredients mancozeb, tolclofos-methyl, pencycuron and carboxin + thiram, and to the unregistered combination of the pencycuron + imidacloprid active ingeriens.

Rhizoctonia solani, anastomosis groups, fungicides

R. solani (*Thanatephorus cucumeris* (Frank) Donk) is a fungus host-specific for potatoes (*Solanum tuberosum* L.); it has a negative affect on uniform emergence, increases the occurrence of gaps in the stand, reduces tuber yields and quality, especially the proportion of very small or oversized tubers (BANVILLE 1989; CARLING et al. 1989; HIDE & HORROCKS 1994). Primarily the mycelium infects the underground parts of the plant, causes crateriform necroses of roots, stolons and stems, and forms sclerotia on the tubers. The sclerotia can then survive in the soil and on the infested tubers (OGOSHI 1987, CARLING et al. 1989). *R. solani* isolated from the sclerotia belongs to anastomose group 3 (AG 3), defined as the "potato type", and the members of which can attack both the aboveground and underground parts of the potato plant.

The present study shows the results of research into the efficiency of the seed-fungicides on *R. solani* (AG 3) isolates from various regions of the Czech Republic. In addition to registered seed-fungicides we also tested the unregistered preparation Prestige 290 FS (pencycuron + imidacloprid). Our objective was to test the efficiency of the seed-fungicides on *R. solani* isolates and the sensitivity, or resistance, of the isolates to registered and unregistered seed-fungicides and/or to the active ingrediens mancozeb, tolclofos-methyl, pencycuron and carboxin + thiram, and to the unregistered combination of the pencycuron + imidacloprid active ingrediens.

MATERIAL AND METHODS

In 2000 and 2001 we collected *R. solani* sclerotia (51 isolates) from untreated tubers of various potato

varieties from different regions of the Czech Republic (12). The sclerotia (ø 2–4 mm) were stored in a dry atmosphere at 18–22 °C. Before use they were disinfected (15 s in 96% alcohol), dried on filter paper and placed onto a potato glucose agar (PGA) containing 300 µg.l^{-1} of chloramphenicol in a Petri dish (PD). After 4 days of cultivation (in darkness, at 24–25 °C) the anastomose groups of the individual isolates were determined.

The sensitivity of *R. solani* to Dithane M 45, Rizolex 50 FL, Monceren 250 FS, Vitavax 200 FF and Prestige 290 FS was monitored under *in vitro* conditions. After cooling the medium (PGA without chloramphenicol) to 50 °C, we added the application liquid containing the required concentration of the active ingrediens (0.0; 0.01; 0.1; 1.0 and 10.0 mg.l^{-1}). After 48 hours we placed a disc (ø 4 mm) with 3-week old mycelium from the edge of the colony into the middle of the PD (ø 100 mm) containing the medium and an addition of the fungicide (1 disc/PD). Sterile distilled water was used for the controls.

The sensitivity test was performed analogically with the cultivation of the fungus (darkness, 22–24 °C). On the fifth day after inoculation of the agar, the diameter of the fungus colony was measured and calculated in two places. Each concentration of the active ingredients and *R. solani* isolates had three replications. The results were statistically assessed using the variance analysis for P = 0.05%.

RESULTS AND DISCUSSION

The table shows the results of sensitivity tests of 51 *R. solani* isolates from 12 localities to registered seed-fungicides containing the active ingredients mancozeb, tolclofos-methyl, pencycuron, carboxin + thiram and as yet unregistered pencycuron+imidaclopride-based seed-fungicide. The sensitivity of the various isolates to the seed-fungicides differed considerably.

I: *Sensitivity of the R. solani isolates to mancozeb (Dithane M 45)*

Isolates from	number of isolates	growth of mycelium average (mm) and relatively (%)				
	mg a.i. . l^{-1}	0,00	0,01	0,10	1,00	10,00
Brno-Pisárky	4	61,0 (100)	63,1 (103,4)	33,2 (54,4)	4,0 (6,2)	0,0
Troubsko	3	63,9 (100)	63,9 (105,1)	39,0 (61,0)	12,1 (18,9)	0,0
Lípa	5	55,2 (100)	54,8 (99,3)	37,1 (67,5)	9,6 (17,4)	0,0
Březno	6	51,8 (100)	56,8 (109,6)	23,8 (46,0)	7,6 (14,7)	0,0
Pusté Jakartice	6	58,8 (100)	53,5 (91,0)	34,6 (58,8)	5,4 (9,2)	0,0
Horní Brod	4	65,0 (100)	67,7 (104,2)	46,1 (70,9)	0,0 (0,0)	0,0
Lipník n/Beč.	4	53,3 (100)	56,9 (106,7)	29,8 (55,9)	0,0 (0,0)	0,0
Jičín	3	57,2 (100)	56,4 (98,6)	31,6 (55,2)	8,6 (15,0)	0,0
Turnov	5	64,2 (100)	63,5 (101,9)	40,1 (62,3)	5,5 (8,6)	0,0
Břeclav	5	66,1 (100)	58,6 (96,0)	42,3 (64,0)	2,9 (4,4)	0,0
Ostrava	2	62,3 (100)	67,0 (107,6)	37,7 (60,5)	1,3 (2,1)	0,0
Olomouc	4	64,6 (100)	65,5 (101,4)	29,5 (46,1)	0,0 (0,0)	0,0
average	51	60,3 (100)	60,6 (100,5)	33,7 (56,0)	4,8 (7,9)	0,0

Even a dose of 1.0 mg.l^{-1} of the active ingrediens mancozeb (Dithane M 45) was not sufficient to suppress the growth of all the isolates; growth was inhibited only in isolates from 3 locations, i.e. Horní Brod, Lipník n/Bečvou and Olomouc. When a concentration of 0.01 mg.l^{-1} was applied, the growth of the mycelium of isolates from 7 locations actually slightly increased when compared with the control.

Concentrations of 0.1, 1.0 and 10 mg.l^{-1} of the active ingrediens had a statistically significant effect on the growth of the fungus mycelium. HAUSVATER (2002) reported that preparations containing the active ingredients mancozeb on tuber infestation, i.e. formation of sclerotia, were effective in 33%. A 1% concentration of the active ingredients reduced the growth of mycelia by more than 90% (92.1%), while the effectiveness of a 10% concentration was 100%.

II: *Sensitivity of the R. solani isolates to pencycuron (Monceren 250 FS)*

Isolates from	number of isolates	growth of mycelium average (mm) and relatively (%)				
	mg act.subst. . l^{-1}	0,00	0,01	0,10	1,00	10,00
Brno-Pisárky	4	50,7 (100)	10,8 (21,3)	4,0 (7,9)	0,0	0,0
Troubsko	3	48,3 (100)	13,5 (31,7)	5,7 (9,7)	0,0	0,0
Lípa	5	48,2 (100)	17,5 (30,5)	6,9 (14,1)	0,0	0,0
Březno	6	53,2 (100)	14,7 (25,4)	9,8 (17,3)	0,0	0,0
Pusté Jakartice	6	54,0 (100)	11,4 (26,7)	2,4 (4,4)	0,0	0,0
Horní Brod	4	57,7 (100)	14,6 (25,3)	6,5 (8,0)	0,0	0,0
Lipník N/Beč.	4	44,0 (100)	14,4 (32,7)	3,1 (7,0)	0,0	0,0
Jičín	3	48,4 (100)	13,2 (27,3)	2,5 (4,3)	0,0	0,0
Turnov	5	51,5 (100)	14,3 (23,9)	1,7 (5,0)	0,0	0,0
Břeclav	5	49,3 (100)	9,9 (20,1)	1,9 (3,8)	0,0	0,0
Ostrava	2	52,0 (100)	11,2 (21,5)	1,8 (2,9)	0,0	0,0
Olomouc	4	50,7 (100)	18,3 (37,9)	6,8 (13,4)	0,0	0,0
average	51	50,7 (100)	13,7 (23,6)	4,4 (8,2)	0,0	0,0

A concentration of the active ingrediens pencycuron (Monceren 250 FS) as low as 0.01 $mg.l^{-1}$ reduced mycelium growth by more than 70% and 1.0 $mg.l^{-1}$ inhibited the growth of mycelia of all the isolates. The effect of 1.0 and 10.0 $mg.l^{-1}$ concentrations was statistically highly significant, as growth was inhibited completely. At 0.1 and 0.01 $mg.l^{-1}$ concentrations of the active ingrediens, the diameter of the *R. solani* colony compared to the controls was only 8.7 and 26.9%, respectively, i.e. growth was reduced by 81.3 and 74.1% compared to the controls; this result is statistically highly significant.

III: *Sensitivity of the R. solani isolates to pencycuron + imidacloprid (Prestige 290 FS)*

Isolates from	number of isolates	growth of mycelium average (mm) and relatively (%)				
	mg a i. . l^{-1}	0,00	0,01	0,10	1,00	10,00
Brno-Pisárky	4	49,4 (100)	11,8 (41,9)	1,9 (2,6)	0,0	0,0
Troubsko	3	49,8 (100)	9,3 (53,5)	0,8 (0,6)	0,0	0,0
Lípa	5	54,3 (100)	15,2 (35,7)	1,4 (3,9)	0,0	0,0
Březno	6	58,4 (100)	17,6 (33,2)	0,5 (1,2)	0,0	0,0
Pusté Jakartice	6	50,4 (100)	12,0 (42,0)	3,4 (1,5)	0,0	0,0
Horní Brod	4	57,9 (100)	12,7 (45,6)	0,6 (1,0)	0,0	0,0
Lipník n./Beč.	4	44,6 (100)	12,4 (36,0)	1,2 (3,7)	0,0	0,0
Jičín	3	48,6 (100)	13,5 (36,0)	1,0 (4,9)	0,0	0,0
Turnov	5	55,1 (100)	14,3 (38,5)	2,4 (2,3)	0,0	0,0
Břeclav	5	48,9 (100)	8,1 (6,0)	0,0 (0,0)	0,0	0,0
Ostrava	2	59,2 (100)	7,9 (7,5)	0,0 (0,0)	0,0	0,0
Olomouc	4	55,5 (100)	16,7 (35,5)	0,0 (0,0)	0,0	0,0
average	51	52,7 (100)	12,6 (34,3)	1,2 (1,8)	0,0	0,0

The effect of all concentrations of the active ingredients pencycuron + imidacloprid (Prestige 290 FS) on growth of the fungus mycelium was statistically highly significant. Concentrations of 10.0 and 1.0 mg.l^{-1} caused the mycelium to stop growing; at a concentration of 0.01 mgl^{-1} it reached 23.9% the size of the control and at 0.1 mg.l^{-1} only 2.3%.

IV: *Sensitivity of the R. solani isolates to tolclofos-methyl (Rizolex 50 FL)*

Isolates from	number of isolates	growth of mycelium average (mm) and relatively (%)				
	mg a. i.. . l^{-1}	0	0,01	0,1	1,0	10,0
Brno-Pisárky	4	50,8 (100)	38,5 (75,8)	29,0 (57,1)	0,0	0,0
Troubsko	3	52,2 (100)	42,7 (81,8)	32,0 (61,3)	0,0	0,0
Lípa	5	52,5 (100)	29,9 (56,9)	22,0 (41,9)	0,0	0,0
Březno	6	44,1 (100)	29,1 (66,0)	13,4 (30,4)	0,0	0,0
Pusté Jakartice	6	42,5 (100)	31,2 (73,4)	20,1 (47,3)	0,0	0,0
Horní Brod	4	52,3 (100)	41,1 (78,6)	30,6 (58,5)	0,0	0,0
Lipník N/Beč.	4	43,1 (100)	18,4 (42,7)	11,8 (27,4)	0,0	0,0
Jičín	3	53,6 (100)	46,6 (86,9)	22,5 (42,0)	0,0	0,0
Turnov	5	54,2 (100)	41,9 (77,3)	36,2 (66,8)	0,0	0,0
Břeclav	5	40,4 (100)	23,4 (57,9)	12,7 (31,4)	0,0	0,0
Ostrava	2	39,6 (100)	27,1 (68,4)	19,5 (49,2)	0,0	0,0
Olomouc	4	44,5 (100)	26,4 (59,3)	24,5 (55,1)	0,0	0,0
average	51	43,1 (100)	30,8 (68,8)	22,8 (47,4)	0,0	0,0

V: *Sensitivity of the R. solani isolates to carboxin + thiram (Vitavax 200 FF)*

Isolates from	number of isolates	growth of mycelium average (mm) and relatively (%)				
	mg a. i. . l^{-1}	0	0,01	0,1	1,0	10,0
Brno-Pisárky	4	54,7 (100)	26,2 (47,9)	10,2 (18,6)	0,0	0,0
Troubsko	3	57,3 (100)	22,8 (39,8)	11,0 (19,2)	0,0	0,0
Lípa	5	49,8 (100)	15,6 (31,3)	7,5 (15,1)	0,0	0,0
Březno	6	61,2 (100)	14,2 (23,2)	9,6 (15,7)	0,0	0,0
Pusté Jakartice	6	56,3 (100)	16,4 (29,1)	5,1 (9,0)	0,0	0,0
Horní Brod	4	55,1 (100)	18,1 (32,8)	9,5 (17,2)	0,0	0,0
Lipník N/Beč.	4	47,5 (100)	19,5 (41,0)	10,4 (21,9)	0,0	0,0
Jičín	3	53,2 (100)	23,4 (44,0)	14,2 (26,7)	0,0	0,0
Turnov	5	55,5 (100)	15,2 (27,4)	8,6 (15,5)	0,0	0,0
Břeclav	5	57,6 (100)	17,3 (30,0)	4,0 (6,9)	0,0	0,0
Ostrava	2	46,6 (100)	19,6 (42,1)	11,3 (24,2)	0,0	0,0
Olomouc	4	54,3 (100)	17,0 (31,3)	6,8 (12,5)	0,0	0,0
average	51	54,1 (100)	18,8 (35,0)	9,0 (16,9)	0,0	0,0

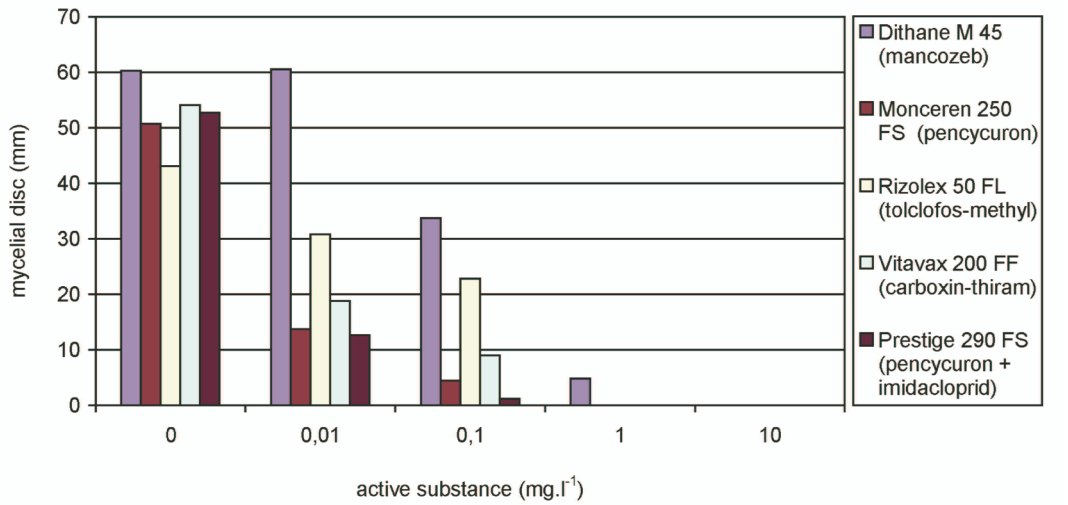
Concentrations of 1.0 and 10 ml.l^{-1} of the active ingrediens carboxin + thiram (Vitavax 200 FF) and tolclofos-methyl (Rizolex 50 FL) had a statistically highly significant effect because they completely stopped the growth of mycelia of the *R. solani* fungus. Statistically significant differences were also discovered between concentrations of 0.1 and 0.01 ml.l^{-1} of the active ingrediens carboxin + thiram, which reduced growth by 83.4 and 65.3%, respectively, and of tolclofos-methyl where the reduction was respectively by 28.5 and 47.1%.

VI: *Influence of seed-fungicides on radial growth R. solani in vitro*

Concentration of acctive ingredients mg.l^{-1}	diametr of colony *R. solani* (mm/%)									
	seed-fungicid - active ingredients									
	Dithane M 45 (mancozeb)		Monceren 250 FS (pencycuron)		Rizolex 50 FL (tolclofos-methyl)		Vitavax 200 FF (carboxin + thiram)		Prestige 290 FS (pencycuron + imidacloprid)	
0,00	60,3	(100,0)	50,7	(100,0)	43,1	(100,0)	54,1	(100,0)	52,7	(100,0)
0,01	60,6	(100,5)	13,7	(26,9)	30,8	(71,5)	18,8	(34,7)	12,6	(23,9)
0,10	33,7	(55,9)	4,4	(8,7)	22,8	(52,9)	9,0	(16,6)	1,2	(2,3)
1,00	4,8	(7,9)	0,0	(0,0)	0,0	(0,0)	0,0	(0,0)	0,0	(0,0)
10,00	0,0	(0,0)	0,0	(0,0)	0,0	(0,0)	0,0	(0,0)	0,0	(0,0)

The concentrations of 1.0 and 10.0 mg.l^{-1} had a statistically highly significant effect on the growth of *R. solani* mycelia, since no further growth was monitored. Concentrations of 0.1 and 0.01 mg.l^{-1} had a statistically significant effect on the growth of mycelia of the fungus, which reduced growth by 51.9 and 30.3%, respectively, compared to the control. Concentrations of 1.0 and 10.0 mg.l^{-1} statistically highly significantly influenced the growth of fungus mycelia; growth was completely suppressed. The effects of 0.1 and 0.01 mg.l^{-1} concentrations were statistically significant, reducing the growth of mycelia by 83.4 and 67.1%, respectively, compared to the control.

Comparisons of the effect of the preparations on growth of mycelia of the *R. solani* fungus showed statistically highly significant differences between the active ingrediens mancozeb (Dithane M 45) and active ingredients of the other seed-fungicides. A 10.0 mg.l^{-1} concentration of all the preparations completely inhibited growth of *R. solani* mycelia; the concentration of 1.0 mg.l^{-1} had the same effect, with the exception of mancozeb (Dithane M 45). The combination of the active ingrediens pencycuron + imidacloprid and of pencycuron alone showed the highest efficiency; mycelium growth was reduced by more than 90% (97.7% and 91.3%, respectively) and a concentration of 0.01 mg.l^{-1} of both ingredients reduced growth by more than 70%.



Sensitivity of the R. solani (AG 3) isolates to seed-fungicides

The sensitivity of the respective isolates to the tested preparations differs considerably; this is obviously associated with the variability of the *R. solani* population and represents the natural range of reactions within the *R. solani* populations.

Despite the fact that the effectiveness of most of the preparations was high, we must not forget that field conditions are influenced by a number of virtually uncontrollable natural and also technical factors and, last but not least, by human activities, which have a negative effect on the effectiveness of the preparations.

Preparations for dry treatment (mancozeb 80%, tolclofos-methyl 10%) do not ensure uniform treatment of the tubers and the effects of the seed-fungicides were usually weaker or very uneven (HAUSVATER 2002). Protection focused on crop rotations and organic supplementation will increase the natural defensive system.

Although we were able to study only a limited number of *R. solani* isolates, and considering their variability (mycelium growth, sensitivity to seed-fungicide), our conclusions indicate that we monitored no resistance of *R. solani* to the active ingredients of the studied seed-fungicide.

The treatment of the potato planting stock as a protection against *R. solani* is quite common in countries abroad. Carbendazim and benzimidazol-based seed-fungicides were replaced by pencycuron or by a combination of pencycuron + imidacloprid. STACHEWITCZ and BURTH (1990) reported that after treatment with pencycuron the infestation of the stolons and underground parts of the stems was weaker, the effect against tuber deformation and formation of sclerotia was very good, but did not always ensure higher yields (COOKE L. R. & LITTLE G. 1993). Chemical protection against *R. solani* has not been sufficiently explored and opinions on this issue differ. POWELSON et al. (1993) indicated that the application of fungicides at planting do not always provide better protection than using healthy planting stock.

SOUHRN

Rhizoctonia solani Kühn AG 3 jako patogen bramboru a jeho citlivost k mořidlům

V letech 2000 a 2001 byla sesbírána sklerocia z napadených hlíz bramboru z 12 stanovišť z různých oblastí České republiky. Jednotlivé izoláty ze sklerocií byly determinovány jako mnohojaderné *Rhizoctonia* sp. anastomózové skupiny 3 (AG 3), která je definována jako „potato type".

U 51 izolátu byla sledována citlivost *R. solani* vůči registrovaným mořidlům s účinnými látkami mancozeb, pencycuron, tolclofos-methyl, carboxin + thiram a dosud neregistrovanému mořidlu na bázi pencycuronu + imidaclopridu.

Při srovnání účinnosti přípravků na růst mycelia houby *R. solani* byl zjištěn statisticky vysoce významný rozdíl mezi účinnou látkou mancozeb a účinnými látkami ostatních mořidel. U všech přípravků koncentrace 10,0 mg.l^{-1} zcela potlačila růst mycelia *R. solani*, stejnou účinnost vykázala i koncentrace 1,0 mg.l^{-1} kromě mancozebu. Nejvyšší účinnost vykázala účinná látka pencycuron, resp. pencycuron + imidacloprid, u nichž redukce růstu mycelia byla vyšší než 90 % (91,3 % a 97,7 %). U obou účinných látek i při koncentraci 0,01 mg.l^{-1} byla účinnost vyšší než 70 %. Mezi izoláty byly zřetelné rozdíly v citlivosti vůči testovaným přípravkům jak mezi jednotlivými populacemi *R. solani*, tak uvnitř populací.

Při kvalitním ošetření lze počítat s účinností mořidel 60–80 % a se zvýšením výnosu 5–7 %. Podle zahraničních údajů však aplikace fungicidů při výsadbě proti *R. solani* nezajistí lepší výsledky než výsadba zdravé sadby. Fungicidní ošetření sadby také nezajistí stoprocentní ochranu, pokud je půda houbou silně infestována. Vzhledem k požadavkům trhu však je nezbytné zajistit, aby hlízy byly bez sklerocií, tzn. vysazovat pouze zdravou a mořenou sadbu.

Rhizoctonia solani, anastomózové skupiny, mořidla

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