International Journal of Farming and Allied Sciences
Available online at www.ijfas.com
©2015 IJFAS Journal-2015-4-4/309-313/ 30 April, 2015
ISSN 2322-4134 ©2015 IJFAS

FUNGAL CULTURES EXTRACT AS A BIO-CONTROL AGENT FOR SUPPRESSION OF PHELIPANCHE RAMOSA L. GERMINATION

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ABSTRACT: This study was conducted to investigate the effects of crude extract, supernatant and pellet of fungal isolates A1, S14, K22, M25 (isolated from tomato fields) and *Trichoderma harzianum* strain on germination of *Phelipanche ramosa* under laboratory conditions. All experiments were conducted at Bio-pesticides and Bio-fertilizers Department, Environment, Natural Resources and Desertification Research Institute (ENDRI), National Centre for Research (NCR), Khartoum, Sudan. Results displayed that the crude extract (pellet and supernatant) of *T. harzianum* and isolate K22 inhibited germination of broomrape by 90 and 83% respectively, as compared to the control. *T. harzianum* and isolate K22 supernatant inhibited germination of broomrape by 100 and 79% respectively as compared to the control. The pellets of the above mentioned microorganisms inhibited germination by 100 to 73%, respectively. From these results it was concluded that fungal crude extract of *T. harzianum* and isolate K22 are the most efficient for biological control of *P. ramosa*.

***Keywords*:** fungi, germination, pellet, *Phelipanche ramosa*, supernatant

INTRODUCTION

The branched broomrape is an obligate root parasite of many economically important dicotyledonous crops such as tomato, tobacco, potato, cabbage, eggplant, carrot, mustard, and sunflower. Its area of distribution is predominantly in the Middle East, Eastern Europe, the Mediterranean basin, Western Asia, East Africa and America (Yoneyama , 2006). Difficulties in broomrape control are due to the production of a large number of seeds that can lay dormant in the soil for many years, only germinating if stimulated by host root exudates. Once a seed is stimulated, it produces a germ tube that grows in the direction of the host plant. So far, no efficient control measures for *Phelipanche* have become available to farmers (Müller-Stöver, 2001). Single methods such as delayed sowing and use of resistant varieties have shown unsatisfactory results. Control of *Orobanche* may be possible by integrating control measures. The integration of biological control with other *Phelipanche* management methods is of increasing research interest. The objective of this study was to evaluate the effects of the potential fungal isolates and *Trichoderma* crude, pellet and supernatant against *Phelipanche* germination under laboratory conditions.

MATERIALS AND METHODS

GR24 stock solution

Stock solution of the stimulant was prepared by dissolving 1mg in 1ml of acetone and completing to volume 100 ml with sterile distilled water. The solution was kept in a fridge at 5°C till used.

Isolation of soil-borne fungi

Soil samples were collected from eight sites in Khartoum state. Six samples were collected from each infested and non-infested tomato field. Sampling was randomly made at a depth of 5-10 cm, at each location and was kept in plastic bags. The spread plate method was used for isolation of the fungi. Briefly, ten grams of each sample were suspended in 90 m l of sterilized distilled water then shaken till completely dispersed. Serial 10 fold dilutions were used. Aliquots (0.2 and 0.3 µl) from dilutions 10-3 and 10-6 were added to Petri dishes containing Potato Dextrose Agar medium (PDA). The dishes, inverted, were incubated in the d ark a t 27°C for 7-9 days. The isolated fungi were preserved in PDA media and kept at 5°C for further studies (Ahmed , 2014).

Medium preparation and sterilization Potato-Dextrose Agar medium (PDA)

The medium was prepared by boiling 200g of sliced potato in 1 liter distilled water until the potato was soft. Twenty gram s o f dextrose and 20g agar powder were added to the medium and the volume was a adjusted to 1 liter then sterilized by autoclaving for 15 minutes at 121°C and left to cool. All fungi were maintained on Potato Dextrose Agar (PDA). Cultures on solid medium were stored a t 5°C until use.

P. ramosa seeds collection, surface disinfection and conditioning

P. ramosa seeds were collected from infested tomato plants in khartoum state, sudan. *Phelipanche seeds* were surface disinfection as described by Rugheim , (2014). Briefly, about 20 surface disinfected *P. romosa* seeds were sprinkled on each of the glass fiber discs in each Petri dish. Then dishes were sealed with parafilm and placed in black polythene bags and incubated at 18°C for 11 days. The discs containing *P. ramosa* seeds were blotted dry on normal filter paper (No. 1) to remove excess water and then transferred to sterile Petri dishes. Each disc was treated with 30 µl of GR24 at 5 and 10 ppm. Then seeds were re-incubated and examined for germination at 7 days later. Germination percentages were then calculated.

Effects of fungal crude, supernatant and pellet on P. ramosa seeds germination

In all experiments, treatments were arranged in Randomized Complete Design with 4 replicates. Four fungal isolates and *Trichoderma* were selected, based upon a preliminary screening, for their ability to inhibit germination of GR24 induced *P. ramosa* seeds. PD medium was inoculated with 3 discs (collected by core borer 4mm diameter) of each of the fungal obtained from 7 - 9 days old cultures propagated on PDA medium. The flasks were incubated for 10 days at 27 oC, with intermittent hand shaking every three days to maintain better growth. After 10 days, the culture was separated by centrifugation a t 4000 rpm for 10 minutes to obtain the fungal supernatant metabolites and pellet suspension. Five ml of the crude culture (with pellet), supernatant culture (with toxins), pellet culture, un-inoculated liquid PD medium and sterilized distilled water were added each alone to each Petri dish. *P. ramosa* seeds were sprinkled on the glass fiber discs in each Petri dish. The dishes, sealed with Parafilm, were incubated at 28°C for 10 days. *P. ramosa* discs were treated with GR24 at 5 and 10 ppm and re-incubated and examined for germination. Each experimental run included two controls: *P. ramosa* seeds conditioned in sterile distilled water and seeds conditioned in wheat flour medium.

All experiments treatments were arranged in a Randomized Complete Design (RCD) with four replicates. Data on percentages germination was calculated for each disc and transformed to arcsine (Gomez and Gomez, 1984) and subjected to analysis of variance (ANOVA). Means were compared using the least significant difference (LSD) at 5% level.

RESULTS AND DISCUSSION

Effects of fungal isolates and *T. harzianum* strain crude extract applied during conditioning on *P. ramosa* seeds germination in response to GR24

Results in table (1) showed that GR24 applied at concentrations of 10 and 5 ppm to seeds conditioned in water induced germination by 47.80 – 46.95% respectively and when conditioned in un-inoculated broth medium induced germination by 46.80 – 48.23% respectively. The crude extract (pellet and supernatant) of *T. harzianum* gave the highest significant (P≤0.05) inhibition on seed germination of broomrape during conditioning by 94-97% followed by isolate K22 (83-87%) in response to GR24 10 and 5 ppm respectively compared to control. The effects of all other isolates were not significant.

Table 1. Effects of fungal isolates and *T. harzianum* strain applied during conditioning on *P. ramosa* seeds germination in response to GR24

Germination (%)							
	Conditioning media		Fungal isolates and strain				
GR24 (ppm)	W[1]	B[2]	A1	S14	M25	*T. harzianum*	K22
10	47.80b (49.81)	46.80b (48.39)	51.99b (53.39)	53.10b (62.56)	52.10b (59.82)	5.75a (0.00)	8.13a (4.00)
5	46.95b (48.81)	48.23b (45.24)	50.80b (51.49)	49.75 (58.33)	49.87b (51.94)	2.68 (0.00)	3.27a (1.36)
Mean	47.38	47.52	51.40	51.43	50.99	2.88	5.7

[1]Water, [2]PD broth medium. Values without ( ) indicate arcsine transformed data.
Means followed by the same letter(s) are not significantly different according to DMRT at P≤0.05
S.E. for GR24 10 ppm = (± 4.528) S.E GR24 5 ppm = (± 4.691)

Effects of fungal isolates and *T. harzianum* strain crude extract applied after conditioning on *P. ramosa* seeds germination in response to GR24

Results showed that GR24 (10 and 5 ppm) applied to seeds conditioned in water and broth medium induced germination by 53.01 – 48.70% and 54.25 – 49.95%, respectively (Table 2). The crude extract (pellet and supernatant) of *T. harzianum* completely inhibited conditioned seed germination of broomrape in response to 5ppm GR24 concentration and significantly (P≤0.05) inhibited germination by 93.73% in response to 10ppm GR24 concentration compared to control. While treating the conditioned seeds with isolate K22 crude extract at GR24 10 and 5ppm inhibited germination significantly (P≤0.05) by 89.53 – 93.26% respectively.

Table 2. Effects of fungal isolates and *T. harzianum* strain applied after conditioning on *P. ramosa* seeds germination in response to GR24

Germination (%)							
	Conditioning media		Fungal isolates and strain				
GR24 (ppm)	W[1]	B[2]	A1	S14	M25	*T. harzianum*	K22
10	53.01[b] (63.54)	54.25[b] (65.65)	53.72[b] (79.70)	63.30[b] (68.45)	59.83b (73.41)	3.32a (1.32)	5.55a (1.78)
5	48.70[b] (56.02)	49.95[b] (58.56)	52.40[bc] (73.79)	59.25[c] (65.23)	52.11bc (62.14)	0.00 (0.00a)	3.57a (1.32)
Mean	50.86	52.10	53.06	61.28	55.97	1.66	4.56

[1]Water, [2]PD broth medium. Values without ( ) indicate arcsine transformed data.
Means followed by the same letter(s) are not significantly different according to DMRT at P≤0.05
S.E. for GR24 10 ppm = (± 4.635) S.E GR24 5 ppm = (± 3.927)

Effects of fungal isolates and *T. harzianum* strain pellet applied during conditioning on *P. ramosa* seeds germination in response to GR24

GR24 (10 and 5 ppm) applied to seeds conditioned in water and broth medium induced germination by 57.57 – 61.73% respectively (Table 3). The pellet of *T. harzianum* strain broth culture completely inhibited germination at

both GR24 concentrations. Treating the seeds with the pellet of the broth culture of isolate K22 inhibited germination significantly (P≤0.05) by 73.61 – 68.68 % in response to GR24 at 10 and 5 ppm, respectively. The pellet of the broth culture of isolate S14 inhibited germination significantly (P≤0.05) by 42.11% at GR24 (10ppm) and by 46.69 at GR24 (5ppm) compared to control. While the pellet of the broth culture of isolates A1 and M25 have no significant effect on broomrape seed germination.

Table 3. Effects of fungal isolates and *T. harzianum* strain pellet applied during conditioning on *P. ramosa* seeds germination in response to GR24

Germination (%)							
	Conditioning media		Fungal isolates and strain				
GR24 (ppm)	W[1]	B[2]	A1	S14	M25	*T. harzianum*	K22
10	60.07^{d} (77.80)	61.73^{d} (76.52)	59.92^{d} (74.67)	37.05^{c} (45.23)	59.14^{d} (73.58)	0.00^{a} (0.00)	16.25^{b} (10.31)
5	57.57^{d} (70.80)	57.80^{d} (71.46)	57.58^{d} (70.96)	34.12^{c} (43.45)	53.96^{d} (65.25)	0.00^{a} (0.00)	19.33^{b} (11.52)
Mean	59.84	61.28	60.59	48.94	58.63	0.00	12.24

[1]Water, [2]PD broth medium. Values without ( ) indicate arcsine transformed data.
Means followed by the same letter(s) are not significantly different according to DMRT at P≤0.05
S.E. for GR24 10 ppm = (± 3.193) S.E GR24 5 ppm = (± 4.436)

Effects of fungal isolates and *T. harzianum* strain supernatant applied during conditioning on *P. ramosa* seeds germination in response to GR24

The results in table (4) showed that there were no significant differences in germination between the seeds conditioned in water and broth medium at both GR24 concentrations. The supernatant of *T. harzianum* strain completely inhibited germination at both GR24 concentrations compared to control. Treating the seeds with the supernatant of the broth culture of isolate K22 inhibited germination significantly (P≤0.05) by 78.98 - 82.16 % in response to GR24 at 10 and 5 ppm, respectively compared to control. While the supernatant of all other isolates insignificantly affected broomrape germination compared to control.

Table 4. Effects of fungal isolates and strain supernatant applied during conditioning on *P. ramosa* seeds germination in response to GR24

Germination (%)							
	Conditioning media		Fungal isolates and strain				
GR24 (ppm)	W[1]	B[2]	A1	S14	M25	*T. harzianum*	K22
10	61.23^{cd} (73.34)	63.67^{d} (75.89)	64.54^{d} (78.87)	50.29^{c} (60.44)	59.86^{cd} (70.52)	0.00^{a} (0.00)	13.24^{b} (7.36)
5	58.45^{c} (70.34)	58.89^{c} (71.21)	56.63^{c} (69.92)	47.58^{c} (59.23)	54.59^{c} (60.26)	0.00^{a} (0.00)	11.24^{b} (9.48)
Mean	59.84	61.28	60.59	48.94	58.63	0.00	12.24

[1]Water, [2]PD broth medium. Values without ( ) indicate arcsine transformed data.
Means followed by the same letter(s) are not significantly different according to DMRT at P≤0.05
S.E. for GR24 10 ppm = (± 3.193) S.E GR24 5 ppm = (± 4.436)

There are many problems in the field application of soil-applied mycoherbicides. Initially, inoculum pelleting and other technologies have been developed (Amsellem , 2001). Results of this study revealed that *T. harzianum* strain and isolate K22 gave the highest reduction in *Phelipanche ramosa* seeds germination irrespective to conditioning status in response to GR24. These results are in line with Jain and Jacobsohn (1989), who reported that fungi produces toxic materials or change soil pH which negatively affected germination of *P. ramosa*, and agreed with the results of Abuo zeid , (2004); Boari and Vurro (2009) who found that some fungal isolates inhibited *P. ramosa* germination. Cohen , (2002) and Nemat Alla , (2008) reported that fungal toxins suppressed *P. ramosa*. Zonno and Vurro (2002) showed that some toxins produced by fungi of the genus *Fusarium* were able to inhibit germination of *Striga* seeds and proposed their practical use in integrated strategies of parasitic plant management. This study indicated that *T. harzianum* and isolate K22 may be used to control *P. ramosa.* In vitro germination of *O. crenata*

seeds was signicantly decreased by 80% after the inoculation with *Ulocladium botrytis* (Müller-Stöver and Kroschel, 2005).

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