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The Role of *Trichoderma*, VA Mycorrhiza and Dry Yeast in the Control of Rhizoctonia Disease of Potato (*Solanum tuberosum* L.)

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Abstract: Laboratory and pot experiments were conducted to evaluate the efficacy of *Trichoderma viride*, VA mycorrhiza and dry yeast, separately and in combination, as an integrated strategy of Rhizoctonia disease management in potato crop. The challenge inoculation with *T. viride* caused a significant reduction *in vitro* in the linear growth of *Rhizoctonia solani*, particularly when it was performed closer to the time of pathogen inoculation. With the exception of the number of stems, yield and growth attributes of potato plants infected with *R. solani* were significantly affected. *T. viride* application significantly increased the growth components (i.e., plant height, shoot fresh and dry weights, root fresh and dry weights) and tuber yield (i.e., number and weight of tubers) compared to potato plants inoculated with *R. solani* alone. Moreover, the disease incidence and severity, as stem canker or black scurf on progeny tubers, were also significantly alleviated by *T. viride* inoculation. Similarly, VA mycorrhiza enhanced both the growth and yield measurements of Rhizoctonia-inoculated potato plants and significantly reduced the harmful effects of the disease. The dry yeast appeared to be the least efficacious biocontrol agent to *Rhizoctonia* compared to the other two organisms; yet it also significantly improved the disease situation of the infected plants. The combined effect of *T. viride* and VA mycorrhiza with or without yeast excelled other treatments in alleviation of almost all tested facets of the disease development.

Key words: Biological control; potato stem canker; potato black scurf

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is becoming an important food and cash crop in the Sudan. Black scurf, an economically important fungal disease caused by *Rhizoctonia solani* Khun, was found spreading in commercial commercial potato fields in north and central Sudan along the Nile. The disease is worldwide in distribution and can affect the plant from the initiation of sprout emergence through the harvest of tuber (Verma 1992).

Being a soil-borne pathogen with a very wide host range, *R. solani* is among the most difficult to control and is almost impossible to eradicate from infested field soil, even if drastic disinfestation techniques such as methyl bromide applications are used. However, the biological aspect of *Rhizoctonia* control may constitute an important and promising component of integrated disease management, which warrants investigation under the Sudan conditions.

Rhizoctonia is parasitized by several microorganisms such as species of mycorrhiza, *Pseudomonas*, *Trichoderma*, *Gliocladium* and *Laetisaria* and by several soil myxobacteria and mycophagus nematodes (Azcon and Barea 1996; Mercier and Manker 2005). Generally, highly suppressive soils to *R. solani* contain high populations of *Trichoderma* spp. which may be highly antagonistic mycoparasites, causing exolysis of the hyphae (Chet and Baker 1981). Apparently, several conditions must be met for induction and maintenance of effective soil suppressiveness of *R. solani* (Otten *et al*. 2004).

More promises seem to be offered by the use of mycorrhizal fungi, which colonize the roots of their host plants under natural conditions, in the suppression of many phytopathogenic fungi (Larsen and Jakobsen 1996; Harrier and Watson 2004). Some ectomycorrhiza fungi are actually capable of actively lysing phytopathogenic fungi (Kope and Fortin 1990) and increasing the uptake of phosphorus and other nutrients by plants (Yao *et al.* 2002). If such effects occur, then management of these symbionts becomes a means to achieve biological control of plant pathogens and, perhaps, the biological control benefits of VAM are also transferred by interconnection between plants (Azcon and Barea 1996; Artursson *et al.* 2006).

Hunter *et al*. (2006) also found that peat samples became suppressive when amended with yeast. This could be ascribed to the direct attack of yeast cells on Rhizoctonia hyphae or indirectly by serving as substrates for microparasites.

Thus, the present study was intended to assess the efficacy of the biological agents, *Trichoderma*, VA mycorrhiza and dry yeast, on the control of Rhizoctonia disease of potato.

MATERIALS AND METHODS

The plant material and the pathogen

Medium-size potato tubers (40-50 mm diameter) of cultivar "Alpha" were obtained from Wafra Company, Khartoum North, Sudan. *Rhizoctonia solani* saprophytic activity was assessed as previously described by Papavizas *et al*. (1975). The pathogen identification was carried out based on (a) cultural characteristics by examining the pattern and the colour of culture growth on both top and bottom sides of the plate, and (b) morphological characteristics using lens and microscopic examination of mycelium type, pycnidia and microsclerotia. A pure culture of the pathogen was maintained in McCartney bottles containing potato dextrose agar (PDA) at 28°C for two days, and then stored in a refrigerator (4°C) for further studies.

Pathogen inoculation

A pure culture of *R. solani* was grown on PDA for seven days at 28^{o}C. Slurry of the culture was made by adding distilled water (10 x), and then 100 ml aliquots of this slurry were mixed with the top 5-cm soil in plastic bags (designated as infested soil) and immediately irrigated with water to help good fungal growth. Two days later, potato tubers were planted (one whole tuber/bag) in the centre of each plastic bag at a depth of 5 cm.

Trichoderma, dry yeast and mycorrhiza

The biological agent *Trichoderma viride* was obtained from the Faculty of Agriculture, Department of Crop Protection, University of Khartoum. The procedures of preparation and inoculation of *T. viride* were similar to those of *R. solani*. The yeast used in this study was Saf. Instant. S.I. Lesaffre, available as dried yeast, and was obtained from the local market. The method used for isolation of VA mycorrhiza was the wet sieving and

decanting technique multiplied and maintained in open pot cultures of Sudan grass (Garawya) (*Sorghum bicolor* var. *Sudanense*) grown in sterilized sand/silt (1:1 w/w) soil mixture (Mahdi and Atabani 1992).

***In vitro* biological control**

Discs (7 mm in diameter) from both *R. solani* and *T. viride* were prepared from their respective actively growing PDA culture. The growth of *R. solani* was challenged by placing one pathogen disc at the centre of the PDA plate (9 cm in diameter), while four *T. viride* discs were equidistantly placed at the periphery of the plate. The challenge inoculation with *T. viride* was performed on the same day, 2 days and 4 days post-inoculation of *R. solani*. Four replications were used per treatment. The plates were incubated at 28°C, and the observations were recorded after 24 and 48 hours of *Trichoderma* inoculation, particularly on the presence of the inhibition zone and the effect on radial growth of *R. solani*.

The pot experiment

This experiment was conducted to evaluate the effect of *T. viride*, mycorrhiza and dry yeast separately and in combination on Rhizoctonia disease control. Plastic bags (30 cm in diameter) were used for planting, and ten small holes per bag were made at the bottom to drain excess irrigation water. Each plastic bag was filled with 5.0 kg of sterilized silt soil (heated at 160°C for 3 hours). One intact potato tuber (40-50 mm in diameter) was planted per plastic bag. The experiment consisted of eight treatments carried out in Rhizoctonia-infested and non-infested soils. The treatments were (i) untreated control, (ii) *T. viride*, (iii) mycorrhiza, (iv) dry yeast, (v) *T. viride* + mycorrhiza, (vi) *T. viride* + dry yeast, (vii) mycorrhiza + dry yeast, and (viii) *T. viride* + mycorrhiza + dry yeast.

The experimental design used was a complete randomized block design with three replicates. The pots were irrigated at regular intervals in order to maintain the moisture at approximately 75% of the field capacity.

The parameters measured were plant height, total number of stems, fresh weights of shoot and root which were determined immediately after harvest; whereas the shoot and root dry weights were determined after drying plant samples in an oven at 65°C for 48 hours.

The disease incidence was determined by recording the proportion (%) of infected stems (stem canker) out of the total emerged stems, six weeks after planting. Black scurf incidence of progeny tubers was recorded 10 weeks after planting by determining the proportion (%) of tubers bearing sclerotia. The severity of lesions developed on infected stems was evaluated, six weeks after planting, based on a scale of 1-5 as follows:

1 = single lesion, ≤ 25 mm in length
2 = single lesion 26 to 50 mm long or composite lesions totaling ≤ 50 mm
3 = composite lesions totaling > 50 mm but not girdling the stem
4 = lesion (s) size ≤ 25 mm in length but girdling the stem
5 = lesion (s) size ≥ 25 mm in length and girdling the stem

The severity factor was multiplied by the percentage of infected stems and divided by 5 to provide the final figure for the stem lesion rating. Similarly, the severity of sclerotia formation on progeny tubers was calculated at harvest.

The total number of tubers harvested were counted and weighed for each treatment, and the means were recorded as a final tuber yield.

RESULTS

In vitro biological control

A conspicuous inhibition zone between the pathogen and *Trichoderma viride* was observed within 24 hours of inoculation of the latter. Then, it became undetectable and followed by over growth of *T. viride* on Rhizoctonia colony. It was evident that *T. viride* sporulated more profusely on *Rhizoctonia solani* culture and that the growth of *R. solani* was significantly retarded *in vitro* by *T. viride* activities. The growth rate of *R. solani* increased significantly with the delay of *T. viride* inoculation. The diameter of *R. solani* linear growth increased from 1.08 cm when the pathogen and *T. viride* were inoculated on the same day to 2.12 and 5.03 cm when the biocontrol agent inoculation was delayed 2 and 4 days, respectively, after the pathogen inoculation. In comparison with the control (8.16 cm), the reduction was greatest when the inoculation of *T. viride* was performed on the same day, while it was least when inoculation was carried out four days later relative to that of *R. solani*.

The pot experiment

Although the total number of stems per pot was not significantly affected by the different treatments, including inoculation with *R. solani*, it was substantially increased in response to application of *T. viride*, mycorrhiza and their combinations with or without yeast (Table1). Treatment with dry yeast alone gave no improvement in the number of stems per pot. On the other hand, plant height was significantly reduced by *R. solani* infection, and the effect was nullified by addition of *T. viride*, mycorrhiza or their combinations with or without yeast. Application of dry yeast alone gave a significant height improvement over the effect of the pathogen inoculation, but was still significantly shorter than all other treatments (Table 1).

Inoculation of *R. solani* significantly ($P<0.05$) reduced the shoot fresh and dry weights of potato plants compared to the nontreated control (Table 2). VAM + *T. viride*, in the presence or absence of dry yeast, produced the best shoot fresh and dry weights compared to other treatments. The effect of mycorrhiza, *Trichoderma* and dry yeast on the root fresh and dry weights (Table 3), in the presence or absence of the pathogen, showed similar patterns to their respective effects on shoot fresh and dry weights (Table 2).

The incidence and severity of stem canker were significantly ($P<0.05$) increased by *R. solani* inoculation compared to the uninoculated control plants, when assessed 6 weeks after planting (Table 4). In addition, sprout blight and profound root system damage were also observed. All biological control treatments significantly reduced the canker development, but dry yeast was significantly the least effective treatment (Table 4). The black scurf development (i.e., incidence and severity of sclerotia), assessed on progeny tubers 10 weeks after planting, followed the same trend observed for stem canker development (Table 5).

The final tuber yield, in terms of number and weight of progeny tubers per pot, was also reduced significantly by the pathogen. However, both parameters were enhanced significantly by the biological amendments under test. Dry yeast was again the least effective treatment (Table 6).

Table 1. Effect of *Rhizoctonia solani* and antagonists on number of stems and height of potato plants

Treatment	No. of stems/pot			Plant height (cm)		
	Non-infested soil	Infested soil	Mean	Non-infested soil	Infested soil	Mean
Control (untreated)	6.33a	3.33a	4.63cd	57.83d	13.66g	35.75c
Trichoderma viride	6.66a	6.33a	6.50b	67.83a	63.33abc	65.58a
VA Mycorrhiza (VAM)	7.66a	6.00a	6.83ab	67.66a	66.16ab	66.91a
Dry yeast	4.33a	3.33a	3.83d	41.83e	34.50f	38.16c
T. viride +VAM	8.33a	7.66a	8.00a	68.66a	65.33ab	67.00a
T. viride + Yeast	5.66a	6.33a	6.00bc	58.33cd	57.66d	58.25b
VAM + Yeast	6.33a	5.66a	6.00bc	61.33bcd	61.00bcd	61.16b
VAM + *T. viride* + Yeast	8.00a	6.66a	7.33ab	67.16a	65.66ab	66.41a
Mean	6.66a	5.66b		61.39a	53.41 b	
LSD 5 % Treatments (T) = 1.27				LSD 5 % Treatments (T) = 3.45		
LSD 5 % Infestation (I) = 0.63				LSD 5 % Infestation (I) = 1.72		
LSD 5 % (T) x (I) = 4.94				LSD 5 % (T) x (I) = 4.94		

Means followed by the same letter(s) do not differ significantly at P= 0.05 level, according to Duncan's Multiple Range Test.

Table 2. Effect of *Rhizoctonia solani* and antagonists on shoot fresh and dry weights of potato plants

Treatment	Shoot fresh weight (g)			Shoot dry weight (g)		
	Non-infested soil	infested soil	Mean	Non-infested soil	infested soil	Mean
Control (untreated)	198.05e	013.09g	105.57f	14.36a	00.57g	07.43d
Trichoderma viride	207.81b	203.98bcd	205.89c	16.35bc	15.76cd	16.05b
VA Mycorrhiza (VAM)	207.97b	206.16bc	207.06bc	16.52bc	15.37cd	15..94a
Dry yeast	201.01de	162.39f	181.70e	13.30e	06.78f	09.54c
T. viride +VAM	215.78a	215.85a	125.82a	18.10a	17.86a	17.98a
T. viride + Yeast	202.34cde	199.75de	201.04d	15.97c	15.75cd	15.86b
VAM + Yeast	200.43de	207.08bc	206.87c	16.23bc	15.44cd	15.83b
VAM + *T. viride* + Yeast	213.40a	213.30a	210.24b	18.65a	17.48ab	18.79a
Mean	205.85a	177.70b		16.06a	13.12b	
LSD 5 % Treatments (T) = 3.2				LSD 5 % Treatments (T) = 0.89		
LSD 5 % Infestation (I) = 1.59				LSD 5 % Infestation (I) = 0.44		
LSD 5 % (T) x (I) = 4.52				LSD 5 % (T) x (I) = 1.26		

Means followed by the same letter(s) do not differ significantly at P=0.05, according to Duncan's Multiple Range Test.

Table 3. Effect of *Rhizoctonia solani* and antagonists on root fresh and dry weights of potato plants

Treatment	Root fresh weight (g)			Root dry weight (g)		
	Non-infested soil	Infested soil	Mean	Non-infested soil	Infested soil	Mean
Control (untreated)	11.87cd	01.42f	06.64d	1.29cd	0.07e	0.68f
Trichoderma viride	13.09bc	12.42bcd	12.75b	1.83ab	1.56bc	1.70cd
VA Mycorrhiza (VAM)	13.62b	12.34bcd	12.98b	1.85ab	1.33cd	1.59de
Dry yeast	11.11b	06.28c	08.69c	1.74ab	1.07d	1.40e
T. viride +VAM	15.52a	15.15a	15.33a	2.02a	1.86ab	1.94ab
T. viride + Yeast	12.99bc	12.46bcd	12.73b	1.78ab	1.72ab	1.75bcd
VAM + Yeast	12.74bc	12.59bcd	12.66b	1.88ab	1.80ab	1.84abc
VAM + *T. viride* +Yeast	15.78a	14.97a	15.38a	2.04a	1.96a	2.00a
Mean	13.34 a	10.95b		1.80a	1.42b	
LSD 5 % Treatments (T) = 0.95				LSD 5 % Treatments (T) = 0.19		
LSD 5 % Infestation (I) = 0.47				LSD 5 % Infestation (I) = 0.09		
LSD 5 % (T) x (I) = 1.35				LSD 5 % (T) x (I) = 0.27		

Means followed by the same letter(s) do not differ significantly at P=0.05, according to Duncan's Multiple Range Test.

Table 4. Effect of *Rhizoctonia solani* and antagonists on canker development

Treatment	Disease incidence (%)			Disease severity		
	Non-infested soil	Infested soil	Mean	Non-infested soil	Infested soil	Mean
Control (untreated)	1.027c	87.71a	44.37a	4.42c	66.63a	35.52a
Trichoderma viride.	1.040c	01.08c	01.06c	1.04c	01.14c	01.09c
VA Mycorrhiza (VAM)	0.930c	01.08c	01.03c	0.93c	01.14c	01.03c
Dry yeast	1.360c	34.76b	18.06c	1.36c	17.10b	09.23b
T. viride +VAM	0.930c	00.93c	00.93c	0.93c	00.93c	00.93c
T. viride + Yeast	1.190c	01.09c	01.14c	1.19c	01.09c	01.14c
VAM + Yeast	1.080	01.18c	01.13c	1.14c	01.18c	01.16c
VAM + *T. viride* +Yeast	0.930c	01.09c	01.01c	0.93c	01.09c	01.01c
Mean	1.06b	5.66b		` 1.49b	11.29 a	
LSD 5 % Treatments (T) = 2.92				LSD 5 % Treatments (T) = 3.36		
LSD 5 % Infestation (I) = 1.54				LSD 5 % Infestation (I) = 1.67		
LSD 5 % (T) x (I) = 4.37				LSD 5 % (T) x (I) = 4.75		

Means followed by the same letter do not differ significantly at P=0.05, according to Duncan's Multiple Range Test.

Table 5. Effect of *Rhizoctonia solani* and antagonists on black scurf development on progeny tubers

Treatment	Disease incidence (%)			Disease severity		
	Non-infested soil	Infested soil	Mean	Non-infested soil	Infested soil	Mean
Control (untreated)	0.79c	68.71a	34.75a	0.79c	58.47a	29.63a
Trichoderma viride.	0.81c	0.73c	0.77c	0.81c	0.73c	0.77c
VA Mycorrhiza (VAM)	0.65c	0.28c	3.46c	0.65c	3.03c	1.84c
Dry yeast	0.81c	31.08b	15.94b	0.81c	12.94b	6.87b
T. viride +VAM	0.65c	0.73c	0.69c	0.65c	0.73c	0.69c
T. viride + Yeast	0.73c	0.81c	0.77c	0.73c	0.81c	0.77c
VAM + Yeast	0.79c	0.81c	0.80c	0.79c	0.81c	0.80c
VAM + *T. viride* +Yeast	0.81c	0.81c	0.81c	0.81c	0.81c	0.81c
Mean	0.75b	13.74a		0.76b	9.79a	
LSD 5 % Treatments (T) = 6.60				LSD 5 % Treatments (T) = 4.05		
LSD 5 % Infestation (I) = 4.80				LSD 5 % Infestation (I) = 2.02		
LSD 5 % (T) x (I) = 13.59				LSD 5 % (T) x (I) = 5.72		

Means followed by the same letter do not differ significantly at P=0.05, according to Duncan's Multiple Range test.

Table 6. Effect of *Rhizoctonia solani* and antagonists on tuber number and tuber weights of potato

Treatment	Disease incidence (%)			Disease severity		
	Non-infested soil	Infested soil	Mean	Non-infested soil	Infested soil	Mean
Control (untreated)	10.66 a	03.33b	07.77b	116.93e	004.41g	060.67d
Trichoderma viride.	11.33 a	11.00a	11.16a	134.01bcd	129.83d	131.92b
VA Mycorrhiza (VAM)	11.66 a	11.66a	11.66a	141.19b	131.89cd	136.54b
Dry yeast	9.66 a	05.55b	07.33b	131.18cd	092.18f	111.68c
T. viride +VAM	12.66 a	11.66a	12.16a	167.25a	164.90a	166.08a
T. viride + Yeast	11.00 a	11.33a	10.66a	137.80bc	130.48cd	134.14b
VAM + Yeast	10.33 a	10.33a	10.66a	137.37bcd	132.52cd	134.94b
VAM + *T. viride* +Yeast	12.00 a	10.66a	11.33a	168.13a	164.71a	166.42a
Mean	0.75b	13.74a		141.73a	118.86b	
LSD 5 % Treatments (T) = 2.13				LSD 5 % Treatments (T) = 4.84		
LSD 5 % Infestation (I) = 1.06				LSD 5 % Infestation (I) = 2.42		
LSD 5 % (T) x (I) = 3.01				LSD 5 % (T) x (I) = 6.85		

Means followed by the same letter(s) do not differ significantly at P= 0.05, according to Duncan's Multiple Range test.

DISCUSSION

The infection of potato plant with *Rhizoctonia solani* caused profound damage in all components of growth and yield, except the plant population where the effect was insignificant. The direct damage on sprouts, stems, roots, stolons and progeny tubers indicated that *R. solani* could be a serious pathogen during all growth stages of the plant and hence a potential threat to potato production. Similar facets of disease development and their consequences on growth and final tuber yield were reported by several investigators (Read *et al.* 1989; Yao *et al.* 2002).

The efficacy of *Trichoderma viride*, as a biocontrol agent, can be inferred from the challenge inoculation test conducted *in vitro* and in the soil. The early detection of the inhibition zone in the culture plate between the antagonist and the pathogen is an indication of antibiosis activity of the antagonist, while the subsequent profused sporulation of *T. viride* and its ability to over grow the pathogen in culture may indicate its ability to directly parasitize the pathogen. The hypotheses of antibiosis, direct parasitism and other mechanism of antagonism have previously been implicated for similar systems (Beagle and Papavizas 1985; Azcon and Barea 1996; Mercier and Manker 2005).

This investigation indicated that the addition of VAM enhanced growth and development of the plant, and may alleviate or significantly reduce the harmful effect of potato stem canker and its black scurf stage. Mycorrhiza inoculation significantly improved almost all measured parameters, i.e., plant height, shoot fresh and dry weights, root fresh and dry weights and tuber yield. According to Artursson *et al.* (2006), VAM can increase growth and phosphorus content of many crops. On the other hand, VAM significantly decreased disease incidence and severity compared to *Rhizoctonia*-infected plants. This could be due to the direct antagonistic effect of VAM on the pathogen and/or the improvement of the growth conditions of the host plant. Generally, application of VAM improved the plant growth by its direct effect on improving the internal status of the crop or indirectly by reducing the harmful effect of the pathogen.

Addition of the dry yeast to *Rhizoctonia*-inoculated potato plants significantly increased all parameters measured compared to the control and significantly reduced disease incidence and severity. According to Hunter *et al*. (2006), peat samples became suppressive when amended with yeast. This could be attributed to the direct attack of the viable yeast cells on the hyphae of *R. solani*, or could be interpreted to indicate that antagonistic microflora develop in response to activity by living entities and that the latter may provide substrates for micro parasites, turning soil suppressive. Generally, it could be concluded that *T. viride*, VA mycorrhiza and dry yeast are promising microorganisms for the control of Rhizoctonia disease of potato under Sudan conditions.

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موجز البحث : أجريت تجارب معملية وفي أصص لتقييم كفاءة فطر التراكوديرما ڤيردي (*Trichoderma viride*) والمايكورايزا (*VA mycorrhiza*) والخميرة الجافة ، كل على حدة ومجتمعين ، كأستراتيجية متكاملة لإدارة مرض رايزوكتونيا البطاطس. أدى حقن فطرالترايكوديرما ڤيردي في بيئة صناعية في نفس الطبق مع المسبب المرضي رايزوكتونيا سولاني إلى إنخفاض معنوي في نمو الأخير ، خاصة عندما تم في وقت اقرب إلى تلقيح المسبب المرضي . تأثرت قياسات النمو والإنتاجية لنباتات البطاطس المصابة بفطر الرايزوكتونيا بشدة ماعدا عدد السيقان الذي لم يتأثر معنوياً . أدت المعاملة بفطر التراكوديرما ڤيردي الى زيادة معنوية في عناصر النمو (طول النبات ، والوزن الرطب والجاف لكل من النمو الخضرى والجذرى ، والإنتاجية من الدرنات (عدداً ووزناً) مقارنة بنباتات البطاطس المعداه بفطر الرايزوكتونيا فقط . بالإضافة الى ذلك فان نسبة الإصابة بالمرض وشدته ، ممثلة في تقرح الساق أو فى وشاح أسود على الدرنات المنتجة ، فقد خف أيضاً بدرجة معنوية بتلقيح الترايكوديرما ڤيردي . كذلك أدت المعاملة

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بالمايكورايزا إلى زيادة في نمو وإنتاجية البطاطس التى تمت عدواها بفطر الرايزوكتونيا مع انخفاض معنوي في التأثير الضار للمرض. ويبدو أن الخميرة الجافة هي الأقل كفاءة كعامل مكافحة بيولوجى ضد الرايروكتونيا مقارنة بالعاملين الأحيائيين الآخرين، ومع ذلك فإنها أدت أيضا إلى تحسين معنوي في الحالة المرضية للنباتات. وقد فاق التأثير المزدوج للترايكوديرما ڤيردي والمايكورايزا، مع أو بدون الخميرة الجافة، المعاملات الأخرى فى تخفيف كل أوجه تكشف المرض التي أختبرت تقريباً.