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Comparative Study on Effects of Chemical Fungicides and a Biocide on Cotton Seedling Damping-Off Caused by *Rhizoctonia solani*

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ABSTRACT

The present study evaluated the efficacy of the fungicides Moncut and Rizolex-T and the biocide Bio-Control T34 (*Trichoderma asperellum*) on suppressing incidence of cotton seedling damping-off caused by *Rhizoctonia solani* on cotton cultivar Giza 86. The dual treatments of the two fungicides and the biocide also evaluated *in vitro* and *in vivo*. *In vitro* studies showed that Moncut completely inhibited *R. solani* linear growth when it was applied at a rate of 250ppm, while Rizolex-T completely inhibited *R. solani* growth when it was applied at a rate of 125ppm. The biocide inhibited the linear growth of *R. solani* by 59.17%. The biocide growth was not affected by all the applied concentrations of Moncut, while it was partially inhibited by the high concentrations of Rizolex-T. Under greenhouse conditions, the two chemical fungicides suppressed incidence of damping–off, while the biocide was unable to suppress the disease. The use of Moncut before or after the biocide succeeded in reducing the disease incidence. On the other hand, Rizolex-T caused significant reduction in disease incidence only when it was applied before the biocide. The application half or fourth the initial dose of the chemical fungicides before or after the biocide caused significant reduction in the disease incidence. The application of Moncut by the initial dose followed by the biocide caused increases in the activity of polyphenol oxidase (PPO) and phenols and sugars contents of the seedlings. Application half the initial dose of Moncut before or after the biocide caused increases in the previously mentioned variables. The use fourth the initial dose of Moncut caused increases in the same variables when it was followed by the biocide, while the use of this dose after the biocide caused decreases in the same variables.

Keywords: Cotton, *Rhizoctonia solani*, fungicide, biocide, *Trichoderma asperellum*

INTRODUCTION

The importance of the Egyptian cotton (*Gossypium barbadense* L.) as an important export crop is well documented in literature due to its excellent fiber traits.

Several fungi in particular Rhizoctonia solani Kuhn can infect cotton seeds and seedlings causing cotton seedlings damping-off (Mikhail *et al*., 2010).

Cotton seedling damping-off is difficult to control due to the broad host range of this pathogen, and the viability of its resting structure. Commercially acceptable resistant cultivars are unavailable in Egypt, large-scale application of solarization and fumigation to reduce resting structure in soil is expensive and difficult to achieve. Thus, the widespread use of seed-dressing fungicides for controlling the disease has become indispensable under Egyptian condition (Aly *et al*., 2017). While effective fungicides are available (Elsamawaty, 1999), it is becoming increasingly evident that their widespread use is associated with some problems, such as the potential harmful effect on non-target organisms, environmental pollution, and the development of resistant races of the pathogens (Alwathani and Perveen, 2012, Malaj *et al*., 2014 and Queyrel *et al*., 2016).

To overcome all these problems, the application of biological control agents such as *Trichoderma* spp. has become an alternative approach to manage the disease. For example, *T. asperellum* is well-known biological agent against various soil born plant pathogens (De Schutter *et al*., 2002 and Benítez *et al.,* 2004).

Biological control mechanisms by the bicontrol agent involve competition for space and nutrition, volatile and diffusible antibiosis, hydrolytic enzymes production (Navaneetha *et al*., 2015) in addition *Trichoderma* spp. can colonies plant roots leading to induce growth and nutrient adsorption for the plant (Harman *et al*., 2004). Certain isolates of *Trichoderm*a spp. invades the vascular tissue or epidermal cells of plant root, giving rise to the accumulation of signal molecules, which leads to induce systemic resistance (Wasternack *et al*., 2006) and increases in peroxidase (PO), polyphenol oxidase (PPO) activities and total phenols (Christopher *et al*., 2010)

Trichoderma spp. have been successfully used for controlling cotton damping-off caused by *R. solani* (Ahmed *et al*., 2000; Asran *et al*., 2005; Osman *et al*., 2009)

The advantage of using biocontrol agents are that they live in the same ecological niche, where plant pathogen live, thus providing competition reliable for inhibition of many plant disease and that they do not cause environmental contamination (Misaghi and Donndelinger 1990).

The present study was initiated *in vitro* and *in vivo* to evaluate the possibility of using two chemical fungicides (Moncut and Rizolex-T), a biocide (*T. asperellum*) and their dual treatments for controlling cotton seedling damping-off caused by *R. solani*.

MATERIALS AND METHODS

Source of fungal pathogen:

Rhizoctonia solani isolate used in this study was obtained from the fungal collection of Cotton and Fiber Crops Dis. Res Sec., Plant Pathology Res. Inst. ARC. Giza, Egypt. The isolate was originally isolated from cotton seedling infected with damping-off.

Chemical fungicides and the biocide used in the present study:

Names, formulations and recommended doses of the chemical fungicides, and the biocide used in the present study are presented in Table 1.

Table 1. Common and trade names, formulations, rates of applications of fungicides and a biocide used in present study

Trade names	Formulations	Active ingredients	Mode of action	Chemical family	Recommended dose
Moncut	25%WP	Flutolanil	Systemic-curative	Phenyl benzamides	2g/kg seeds
Rizolex-T	50%WP	Thiram+ tolclofos-methy	Often protectant Systemic-curative	-Dithiocarbamates -Aromatic hydrocarbons	3g/ kg seeds
Bio-Control T34	12%WP	*Trichoderma asperellum*	Competition, antibiosis, and mycoparasitism	Microbial	2g/kg seeds

1. Laboratory experiments:

Effect of chemical fungicides on the linear growth of *R. solani* :

The antifungal test of the fungicides *in vitro* was determined by using poisoned food technique (Adawy *et al.*, 2018).

Effect of the biocide on linear growth of *R. solani*:

In vitro evaluation of antagonistic effect of Bio-Control T34 (*T. asperellum*) against *R. solani* was carried out using the dual culture technique (Skidmore & Dickinson 1976).

Sensitivity of the biocide to the fungicides:

Sensitivity of the biocide T34 to the fungicides (Moncut and Rizolex-T) was performed by using the poisoned plate technique (Adawy *et al.,* 2018).

2. Greenhouse experiments:

Cotton cultivar Giza 86 was used in all greenhouse experiments under the same conditions of temperature, moisture and soil type. All greenhouse tests conducted in Cotton and Fiber Dept. Plant Path. Inst. ARC. Giza, Egypt. Three replicates and 10 cotton seeds/ pot were used in all greenhouse experiments.

Effect of fungicide, biocide and their interactions on damping-off caused by *R. solani* and on growth parameters of cotton Giza 86:

Inoculum of *R. solani* was prepared according to (Asran *et al*., 2005). Disease incidence, plant height and dry weight were recorded 40 days after sowing. Different treatments were applied as shown in Tables 2 and 3.

Table 2. Treatments of the initial doses of fungicides used in the present study

Treatments	Method of application	Rate of applications
Moncut (M)	Seed dressing	2g/kg seeds
Rizolex-T(R)	Seed dressing	3g/ kg seeds
Bio-Control T34 (T34)	Seed dressing	2g/kg seeds
M followed by T34 after one week	Seed dressing and soil drench, respectively	2g/kg seeds, 2g /L
R followed by T34 after one week	Seed dressing and soil drench, respectively	3g/ kg seeds, 2g/L
T34 followed by M after one week	Seed dressing and soil drench, respectively	2g/kg seeds, 2 g/L
T34 followed by R after one week	Seed dressing and soil drench, respectively	2g/kg seeds, 3 g/L

Table 3. Treatments of half and fourth the initial doses of fungicides used in the present study

Treatments	Method of application	Rate of applications
M followed by T34 after one week	Seed dressing and soil drench, respectively	1 g/kg seeds, 2g / L
R followed by T34 after one week	Seed dressing and soil drench, respectively	1.5g/ kg seeds, 2g/ L
T34 followed by M after one week	Seed dressing and soil drench, respectively	2g/kg seeds, 1g/ L
T 34followed by R after one week	Seed dressing and soil drench, respectively	2g/kg seeds, 1.5 g/ L
M followed by T34 after one week	Seed dressing and soil drench, respectively	0.5 g/kg seeds, 2g /L
R followed by T34 after one week	Seed dressing and soil drench, respectively	0.75g/ kg seeds, 2g/L
T34 followed by M after one week	Seed dressing and soil drench, respectively	2 g/kg seeds, 0.5 g/L
T 34followed by R after one week	Seed dressing and soil drench, respectively	2 g/kg seeds, 0.75 g/L

3. Biochemical studies:

Instrument used in biochemical studies:

Determination of enzymes activities, phenolic compounds and sugars content was carried out by using spectrophotometer model UV-Vis spectronic 601

Preparation of enzyme extracts and the assay:

Fresh plant tissues were extracted in phosphate buffer pH 7 (0.1M)), then centrifuged at 3000 rpm for 15 min. at 4°C, thereafter, the supernatant filtered and collected as an enzyme extract. Enzyme extract was stored at 2-5°C and aliquots of these assayed for enzyme activity (Aluko and Ogbadu, 1986).

Peroxidase enzyme assay:

Peroxidase activity was determined according to (Worthington, 1972). The increase in absorbance was determined spectrophotometer at 430 nm every 30 second for 10 reads.

Polyphenol oxidase enzyme assay:

Polyphenol oxidase activity was measured using the method described by (Esterbaner *et al*., 1977). The enzyme activity was measured as the change in absorbance per minute at 495 nm immediately after the addition of catechol solution, which initiated the reaction.

4. Preparation of samples for chemical studies of phenols and sugars contents:

Fresh plant sample (10 g) from each replicate of each treatment was cut into small pieces and immediately macerated into 95% boiling ethanol for 10 min. The macerated were transferred into soxhlet unites containing 75% ethanol as an extraction solvent. The extract process resumed for 12 hrs. Ethanol extracts were filtrated and evaporated until the complete removal of ethanol. The dried residue was dissolved in 5ml isopropanol 50% and kept in freezer till analysis. The extracts were used, later for analysis of phenols and sugars.

Phenol content:

Phenolic compounds (total and free phenols) were determined by the spectrophotometer method as described by (Simons and Ross, 1971). The density of the developed blue color was determined at 520 nm using chatichole as standard.

Sugar content:

Total soluble sugars and reducing sugars were colormerically determined at 540 nm using the picric acid technique as described by (Thomas and Dutcher, 1924). The density of developed color was determined at 540 nm in presence of blank and using glucose as a standard.

5. Statistical analysis:

Data collected were analyzed by MSTAT-C software. The mean differences were compared by the least significant difference (LSD) test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect of chemical fungicides (Moncut and Rizolex-T) on linear growth of *R. solani*:

Moncut and Rizolex-T fungicides reduced linear growth of *R. solani* on Potato Dextrose Agar (PDA) medium compared with control. Efficacy of these fungicides on fungal linear growth was increased by increasing their concentrations. Moncut inhibited *R. solani* linear growth completely (100%) by 250 ppm, while Rizolex-T caused complete inhibition of linear growth of the fungus starting from 125ppm (Table, 4 and Fig, 1). Elshahawy *et al*., (2016) found that Moncut and Rizolex-T were completely inhibited the growth of *R. solani*

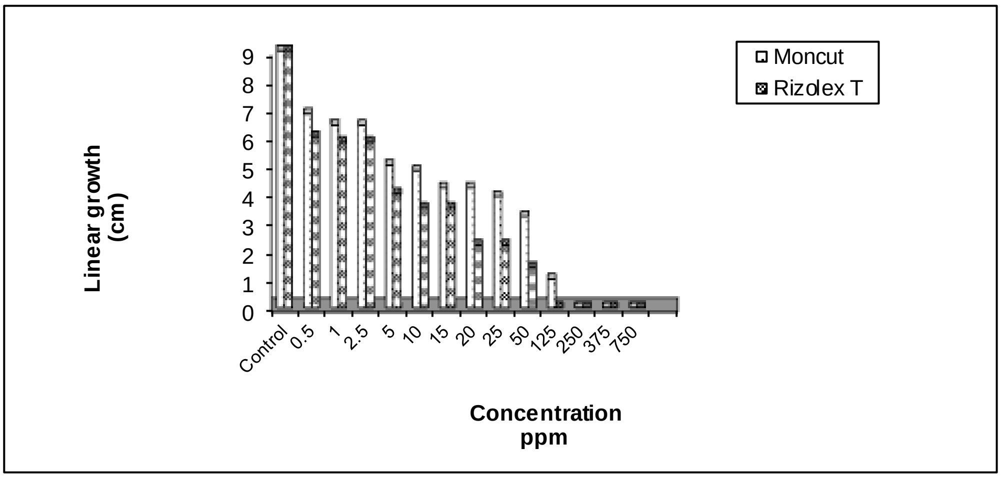

Fig. 1. Effect of Rizolex-T and Moncut on linear growth of *R. solani* on PDA medium

Table 4. Effect of Rizolex-T and Moncut on linear growth of *R. solani* on PDA medium

Concentration (ppm)	Moncut		Rizolex - T	
	Linear growth (cm)	Inhibition (%)	Linear growth (cm)	Inhibition (%)
0.5	6.8[a]	24.44[b]	6	33.33
1	6.4	28.89	5.8	35.56
2.5	6.4	28.89	5.8	35.56
5	5	44.44	4	55.56
10	4.8	46.67	3.5	61.11
15	4.2	53.33	3.5	61.11
20	4.2	53.33	2.2	75.56
25	3.9	56.67	2.2	75.56
50	3.2	64.44	1.4	84.44
125	1	88.89	0	100
250	0	100	0	100
375	0	100	0	100
750	0	100	0	100
Control	9	--	9	--
LSD($P\leq0.05$)	0.093		0.24	

[a] **: mean of three replicates**
[b] **inhibition % = (Control-Treatment)/control) x100)**

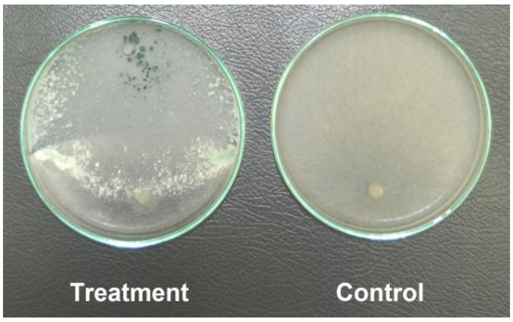

Fig. 2. Effect of the biocide (*T. asperellum)* on linear growth of *R. solani* shows the mycoparasitism of the biocide on the fungus

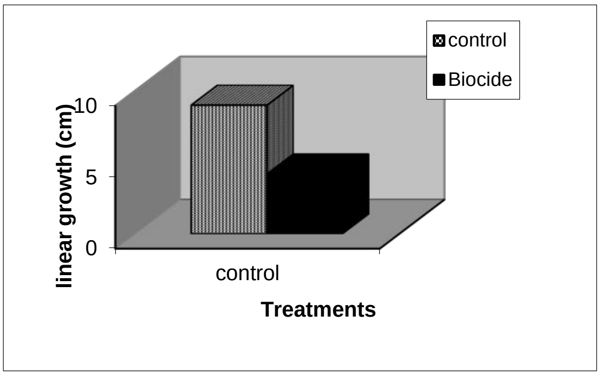

Fig. 3. Effect of biocide on linear growth of *R. solani* on PDA medium

Effect of T34 (*T. asperellum*) on linear growth of *R solani* on PDA medium:

Data in Table (5) and Figs. (2 and 3) revealed that the biocide (T34) caused inhibition of *R. solani* linear growth by 59.17%. Asran, *et al*., (2005) Dar *et al*., (2011) and Elshahawy *et al*., (2016) reported that *R. solani* linear growth was inhibited by *Trichoderma* spp. This inhibitory effect of *T. asperellum* is due to multiple mechanisms of action such as the production of certain enzyme, antibiotics and mycoparasitism (Mukherjee, *et al*., 2012 and Narasimha Murthy, *et al.,* 2013).

Table 5. Effect of biocide on linear growth of *R. solani* on PDA medium

Treatment	Linear growth (cm)	(%) Inhibition
Biocide	4.2	59.17
control	9	0

Effect of Moncut and Rizolex-T on linear growth (cm) of *T. asperellum* on PDA medium:

Data in Table (6) and Fig. (4) revealed that Moncut had no inhibitory effect on linear growth of *T. asperellum*, while Rizolex-T caused significant inhibition of the linear growth of *T. asperellum* starting from 15.0 ppm. The inhibition ranged from 5.56% at 15ppm to 72.22% at 750 ppm. These results mean that *T. asperellum* was tolerant and compatible with Moncut at all tested concentrations, while it was incompatible with Rizolex-T. These results in agreement with Sharma *et al*., (2001), Gampala and Pinnamaneni (2010) and Elshahawy *et al*., (2016). Hameed (2008) reported that Rizolex-T was strongly inhibited the growth of *R. solani* at all concentrations, while it was slighty inhibited the growth of *T. harzianum* compared with *R. solani*. Screening of isoletes of *Trichoderma* sp. against the common fungicides by Sharma and Dureja (2004) indicates the variation in sensitivity towards different groups of fungicides. Tapwal *et al*., (2012) observed the compatibility of *Trichoderma* spp. with some fungicides.

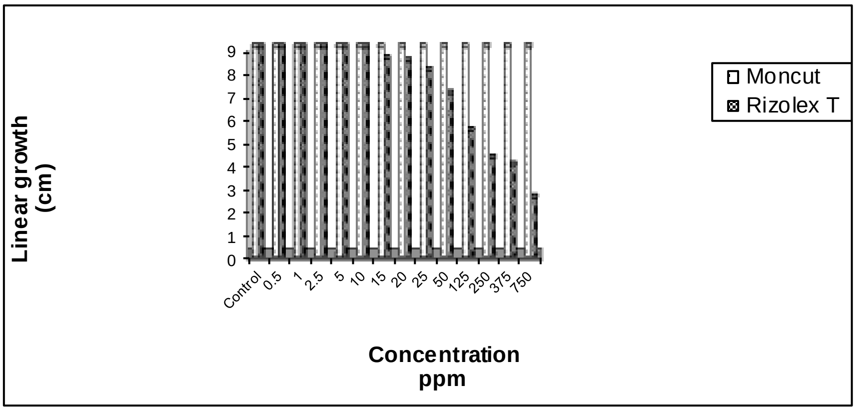

Fig. 4. Effect of Moncut and Rizolex-T on linear growth (cm) of *T. asperellum*

Table 6. Effect of Moncut and Rizolex-T on linear growth (cm) of *T. asperellum*

Concentrations (ppm)	Moncut		Rizolex-T	
	Linear growth (cm)	Inhibition (%)	Linear growth (cm)	Inhibition (%)
0.5	9[a]	0[b]	9	0
1	9	0	9	0
2.5	9	0	9	0
5	9	0	9	0
10	9	0	9	0
15	9	0	8.5	5.56
20	9	0	8.4	6.67
25	9	0	8	11.11
50	9	0	7	22.22
125	9	0	5.4	40
250	9	0	4.2	53.33
375	9	0	3.9	56.67
750	9	0	2.5	72.22
Control	9	--	9	--
LSD(P≤0.05)	N.S.		0.165	

[a] : mean of three replicates
[b] inhibition % = (Control-Treatment)/control) x100)

Effect of initial dose of the fungicides, the biocide and duel treatments on damping-off of cotton seedlings:

Table (7) showed that isolate of *R. solani* was highly pathogenic. It caused 96.67 % disease incidence. Use of Moncut and Rizolex-T as seed-dressing reduced disease incidence by 79.31 and 86.21%, respectively compared with infested control, while use of the biocide (T34) failed to reduce damping-off incidence.

Use of Moncut as seed dressing followed by the biocide when it was added to infested soil as soil drench after one week caused significant reduction in disease incidence by 89.66% compared with infested control.

When cotton seeds treated with the biocide as seed dressing followed by Moncut added as soil drench after one week the reduction in disease incidence was 48.28%.

In case of Rizolex-T, when it was used as seed dressing then the biocide added after one week the disease incidence significantly reduced by 93.1%, while use the biocide first then Rizolex-T was added the reduction in disease incidence was non-significant (20.69%) compared with infested control.

Table 7. Effect of initial doses of fungicides, biocide and dual treatments on damping-off of cotton seedlings cultivar Giza 86

Treatment	Disease incidence (%)	Reduction in disease incidence(%)	Plant height (cm/plant)	Dry weight (mg/plant)
Control	23.33[a]	--	8.53	0.190
Infested control	96.67	--	4.33	0.053
Moncut (M)	20.00	79.31[b]	11.10	0.180
Rizolex-T(R)	13.33	86.21	11.63	0.173
Bio Control T34(T34)	90.00	6.90	10.33	0.090
M followed by T34	10.00	89.66	10.33	0.103
R followed by T34	6.67	93.10	10.17	0.137
T34 followed by M	50.00	48.28	11.53	0.137
T34 followed by R	76.67	20.69	11.50	0.100
L.S.D(≤0.05)	21.23	--	N.S	N.S

[a] : mean of three replicates
[b]Reduction in disease incidence(%)=[((Control Treatment)/control)x100]

These results revealed that use of the biocide first caused decrease in the fungicide efficacy.

Use of the initial dose of the two fungicides significantly decreased the damping-off incidence when were they used individually or followed by the application of the biocide. When the biocide was used first then each of the fungicides was then added the efficacy of Moncut significantly reduced while Rizolex-T failed to reduce the disease incidence significantly. These results may be due to the biocide tolerance of fungicides (Omar 2006 and Chaparro *et al*., 2011) or attributed to the biodegradation effect of the biocide on the fungicides (Sharma *et al*., 2016).

Sharma *et al*., (2016) reported that the use of biocontrol agents in integrated pest management system (IPM) strategies requires the use of effective strains compatible with pesticides which can be well utilized in an IPM and also suitable to reduce the residual effect of pesticides.

The application of the biocide followed by the fungicides (Moncut or Rizolez-T) showed that Moncut was more efficient in controlling the disease than Rizolex-T. These results could be attributing to the high sensitively of the biocide to the antifungal activity of Rizolex-T, while it had low level of sensitivity to the antifungal activity of Moncut.

Effect of half and fourth initial dose of the fungicides, the biocide, and dual treatments on damping-off of cotton seedlings:

Results in Table (8) showed that using half of initial dose of Moncut or Rizolex-T then adding biocide to infested soil as soil drench after one week caused significant reduction in disease incidence by 50% and 66.66%, respectively, compared with infested control. The same results obtained when the biocide used first as seed dressing then fungicides used as soil drench, where the reduction was 45.84%with both fungicides.

Use of fourth initial dose of Moncut or Rizolex-T followed by the biocide after one week as soil drench caused significant reduction in the disease incidence by 50% and 66.66% respectively. When the biocide added first then any of the two fungicides were added the disease reduced by 54.16 % (with Moncut) and by 50% (with Rizolex-T). These results indicated that the deleterious effects of the fungicides on the biocide decreased with the reduction in the fungicidal dose.

Rhizoctonia solani caused significant reduction in plant height by 45.33% compared with uninfested control. All treatments caused significant increased in plant height except the treatment of seeds with *T. asperilum* then use half or fourth initial dose of Rhizolex-T as soil drench.

Table 8. Effect of half and fourth dose of fungicides, biocide and dual treatment on damping-off of cotton seedlings cultivar Giza 86

Treatment	Disease incidence (%)	Reduced disease incidence (%)	Plant height (cm/plant)	Increase in plant height (%)	Dry weight (mg/plant)
Control	30.00[a]	--	6.53	--	0.153
Infect	80.00	--	3.57	--	0.070
1/2M followed by T34	40.00	50.00[b]	6.20	42.4	0.103
1/4M followed by T34	40.00	50.00	6.20	42.4	0.097
T34 followed by 1/2M	43.33	45.84	6.63	46.2	0.107
T34 followed by 1/4M	36.67	54.16	6.80	47.5	0.120
1/2R followed by T34	26.67	66.66	6.37	44.0	0.090
1/4R followed by T34	26.67	66.66	7.13	49.9	0.113
T34 followed by 1/2R	43.33	45.84	5.17	30.9	0.103
T34 followed by 1/4R	40.00	50.00	5.50	35.1	0.110
L.S.D(≤0.05)	21.94	--	1.67		N.S

[a] : mean of three replicates **[b] Reduction in disease incidence(%)=[((Control-Treatment)/control)x100]**

[c] Increase in plant height (%)=[((Treatment – Control)/control)x 100]

Howell *et al*., (1997) also mentioned that biological and fungicide treatments may provide longer term protection than that provided by fungicide alone, through colonization and protection of the developing root system and reduction of pathogen inoculum by parasitism of pathogen propagules in the soil. They also mentioned that a biological control that can function in combination with fungicide seed treatment to suppress seedling diseases in the field might have value as a commercial product for seedling disease control.

Effect of initial dose of the fungicides , the biocide and dual treatment on oxidative enzymes:

Data in Table (9) revealed that *R. solani* caused significant increase in PO activity in cotton seedlings compared with untreated, while it caused significant decrease in PPO activity compared with untreated control. All treatments caused significant decreases in PO activity compared with infested seedlings except treating cotton seeds with Rizolex-T followed by biocide (T34) where the enzyme activity increased but this increase was not significant. On contrast, all treatments caused significant increases PPO activity compared with infested control.

Effect of initial dose of the fungicides, the biocide and dual treatment on phenolic compounds:

Data in Table (9) showed that the infection caused significant decrease in phenolic compounds. Some treatments caused significant or non significant decreases in phenolic compounds (total and free) such as Rizolex-T, T34, Rizolex-T then biocide and biocide then Rizolex-T. On the other hand, other treatments caused significant or non significant increases in phenolic compounds (total and free) such as Moncut, Moncut then biocide (T34), and biocide then Moncut.

Table 9. Effect of initial dose of fungicides, biocide and dual treatment on oxidative enzymes, phenolics compounds and sugars in cotton seedlings cultivar Giza 86

Treatments	peroxidase activity Δ_{430}	polyphenol oxidase Δ_{495}	Phenolic compounds (total) mg/g fw	Phenolic compounds (free) mg/g fw	sugars (total) mg/g fw	sugars (reducing) mg/g fw
Control	0.627	0.132	7.136	6.643	2.69	2.547
Infested control	1.082	0.031	6.113	5.454	2.105	0.728
Moncut (M)	0.308	0.094	6.281	5.48	1.944	0.662
Rizolex-T(R)	0.053	0.171	4.512	4.182	1.254	0.505
Biocontrol T34(T34)	0.424	0.162	4.644	4.165	1.108	0.516
M followed by T34	0.665	0.138	6.846	6.624	2.489	0.797
R followed by T34	1.098	0.145	5.162	4.814	0.957	0.619
T34 followed by M	0.456	0.101	6.743	6.506	1.603	0.529
T34 followed by R	0.42	0.054	3.623	3.441	0.905	0.482
L.S.D(≤0.05)	0.042	0.007	0.405	0.666	0.099	0.061

Effect of initial dose of the fungicides, the biocide and dual treatment on sugars content:

The disease caused significant decrease in sugars contents (total and reducing) compared with control as shown in Table (9). All treatments caused significant decreases in sugars compared with infested control except treatment with Moncut followed by T34 where the sugars contents significantly increased.

Effect of half and fourth initial dose of the fungicides, the biocide and dual treatment on oxidative enzyme:

Data in Table (10) showed that PO activity significantly increased in cotton seedlings grown in infested soil, while PPO activity non significantly decreased compared with non infested soil. All treatments caused significant decreases in PO activity except treatment seeds cotton with half dose of Rhizolex-T followed by biocide T34 where enzyme activity significantly increased compared with infested control and treatment half dose of Moncut followed by the biocide but this increase was not significant. All treatments caused significant increases in PPO compared with infested control.

Effect of half and fourth initial dose of the fungicides, the biocide and dual treatment on phenolic compounds:

The infection by *R. solani* caused significant increase in phenolic compounds in cotton seedlings compared with control as shown in Table (10). Total phenolic compounds significantly increased compared with infested control soil when cotton seeds treated with half dose Moncut then biocide, half dose Rizolex-T then biocide, fourth dose Moncut then biocide, and biocide then half dose Moncut, while it significantly decreased when cotton seeds treated with biocide then half dose Rizolex-T, fourth dose Rizolex-T then biocide, biocide then fourth dose Moncut and biocide then fourth dose Rizolex-T.

Table 10. Effect of half and fourth dose of fungicides, biocide and dual treatment on oxidative enzymes, phenolecs compounds and sugars in cotton seedlings cultivar Giza 86

Treatments	peroxidase activity Δ_{430}	polyphenol oxidase Δ_{495}	Phenolic compounds (total) mg/g fw	Phenolic compounds (free) mg/g fw	sugars (total) mg/g fw	sugars (reducing) mg/g fw
Control	0.344	0.023	3.904	3.187	0.5	0.453
Infested control	0.608	0.022	6.549	5.058	1.513	1.241
½M followed by T34	0.671	0.0447	7.6563	5.248	1.91	1.754
½R followed by T34	0.754	0.031	7.473	5.4027	1.81	1.532
T34followed by ½M	0.354	0.029	7.7857	5.7117	2.143	1.2727
T34 followed by ½R	0.597	0.033	5.5393	4.9317	2.024	1.762
¼M followed by T34	0.511	0.043	7.43	5.4207	2.397	1.54
¼R followed by T34	0.481	0.032	4.9557	3.2543	1.148	0.4813
T34 followed by ¼M	0.461	0.086	5.3127	4.2007	1.4153	0.4523
T34 followed by ¼R	0.580	0.117	4.7853	3.3453	0.8913	0.82
L.S.D(≤0.05)	0.072	0.003	0.315	0.617	0.377	0.043

Free phenolic compounds significantly increased only in one treatment in which cotton seeds treated with biocide were followed by half dose of Moncut, while it significantly decreased compared with infested control in three treatments, which were fourth dose of Rizolex-T followed by biocide, biocide followed by fourth dose of Moncut, and biocide followed by fourth dose Rizolex-T.

Effect of half and fourth the initial dose of the fungicides , the biocide and dual treatment on sugars:

Data in Table (10) revealed that sugars contents (total and reducing) significantly increased as a result of infection with *R. solani*. Total sugars significantly increased as a result of treatments half dose Moncut then biocide, biocide then half dose Moncut, biocide then half dose Rizolex-T and fourth dose Moncut then biocide, while it significantly decreased as a result of treating cotton seed with biocide then fourth dose Rizolex-T.

Some treatments caused significant increase in reduced sugars such as half dose Moncut then biocide, half dose Rizolex-T then biocide, biocide then half dose Rizolex-T and fourth dose Moncut then biocide, while other treatments caused significant decrease in reduced sugars. Treating cotton seeds with biocide then half dose Moncut caused non significant increase in reduced sugars.

Treatment cotton seeds with initial dose of Moncut then the biocide T34 caused increases in PPO activity, and the contents of phenolic and sugars. Use of half the initial dose of Moncut befor or after the biocide T34 caused increasing the activity of PPO and the contents of phenols and sugars. The same result was true in the case of using fourth of the initial dose of Moncut followed by the biocide. These results are in agreement with Arseneault *et al.*, (2014) who mentioned that some biocontrol agent activate plant systemic resistance by increasing activity of antioxidante enzymes and phenolic compounds.

Peroxidase play the most important role in the plant biochemical defense against microbial pathogens because it is involved substrate oxidation, cell wall lignifications, photosynthesis, respiration and growth regulation (Srivastava, 1987). Polyphenol oxidase, a copper containing antioxidant enzyme, oxidizes phenolics to highly toxic quinines which are apparently toxic to pathogens and thereby contribute to disease resistance and it has been implicated as functioning in the defense mechanism against plant pathogen (Das *et al.*, 2004). Narasimha Murthy *et al.*, (2013) reported that *T. asperellum* have multiple mechanisms of action, including co-parasitism via production of β, 1-3-glucanases, antibiotics, competition, solubilization of inorganic plant nutrient, induced resistance and inactivation of the pathogen enzymes involved in the infection process.

Ragab *et al.*, (2015) found increasing in reduced sugars in bean plant treated with bioagent due to increase in biological activity. The increase in biological activity needs reduced sugar to be used in energy production.

The results of the present study suggest that the biocide can be used with reduced dose of selected fungicides for the control of seedling disease caused by *R. solani*. This conclusion is in agreement with Latore *et al*., (1997) who mentioned that antagonistic activity by the biocontrol agent might be effective if it is integrated with other control practice and may result in acceptable levels of disease control with reduce level of chemical fungicides.

In conclusion, the results of the present study indicated that the lowest disease incidence could be obtained by the application of the initial dose or less of Moncut. However, the applications of these results under field conditions require more experiments by using more *Trichoderma* isolates and more fungicides with a variety of cultivars.

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دراسة مقارنة على بعض المبيدات الفطرية ومبيد حيوي من حيث تأثيرها على موت بادرات القطن المتسبب عن فطر ريزوكتونيا سولانى

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في هذه الدراسة تم تقييم فاعلية كل من المبيدين الكمياويين المونكت والريزولكس تى والمبيد الحيوى تى 34 (التريكوديرما اسبيريللوم) كذلك المعاملات المزدوجه لكل من المبيدين مع المبيد الحيوى على تثبيط مرض موت بادرات القطن صنف جيزه 86 المتسبب عن فطر ريزوكتونيا سولانى. اظهرت الدراسه المعمليه ان مبيد المونكت ادى الى تثبيط كامل لنمو فطر ريزوكتونيا سولانى عند تركيز 250جزء فى المليون فى حين ان مبيد الريزولكس تى ادى الى تثبيط كامل لنمو الريزوكتونيا سولانى ولكن عند تركيز 125 جزء فى المليون. عند اختبار المبيد الحيوى على فطر الريزكتونيا سولانى وجد ان المبيد الحيوى ادى الى تثبيط نمو فطر الريزوكتونيا سولانى فى المعمل بنسبة 59.17%. عند اختبار حساسية المبيد الحيوى لكل من المونكت والريزولكس تى اظهرت النتائج ان نمو التريكوديرما لم يتاثر بجميع تركيزات المونكت فى حين ان تركيزات الريزولكس تى العاليه ادت الى تثبيط جزئى لنمو التريكوديرما. اظهرت اختبارات الصوبه نجاح كل من المبيدين الكيماويين فى تثبيط حدوث مرض موت البادرات فى حين فشل المبيد الحيوى فى ذلك. استعمال المونكت مسبوقا او متبوعا بالمبيد الحيوى أدى الى تثبيط معنوى لحدوث المرض فى حين ان الريزولكس تى ادى الى انخفاض معنوى فى حدوث المرض فقط عند استعماله قبل المبيد الحيوى. استعمال نصف او ربع الجرعه المبدئية من المبيدين الكيماويين سواء بعد او قبل المبيد الحيوى أدى الى تثبيط معنوى لحدوث المرض. استعمال المونكت بالجرعه المبدئية متبوعا باضافة المبيد الحيوى أدى الى زياده فى كل من نشاط انزيم البولى فينول أوكسيديز ومحتوى الفينولات والسكريات فى البادرات. استعمال نصف الجرعة المبدئية من المونكت متبوعا او مسبوقا بالمبيد الحيوي أدى الى زياده فى نفس المتغيرات السابق ذكرها. اما عند استعمال ربع الجرعه المبدئيه من المونكت متبوعا بالمبيد الحيوى فقد حدثت زياده فى المتغيرات السابقه فى حين ان استعمال هذه الجرعه مسبوقا بالمبيد الحيوى ادى الى انخفاض فى هذه المتغيرات.