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Project Research Report

Evaluation of Antifungal Activity of *Tricoderma harzianum* and Fungicides against Some Potato Leaf Spots Pathogens in Greenhouse and Laboratory

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Introduction

The early and late blight diseases of potatoes are found in nearly all areas of the world where they are grown. Early blight, that caused by *Alternaria solani* (Sorauer), formerly called *Macrosporium solani* (Ellis and Martine), is one of the major foliar diseases of potatoes and causes premature defoliation of potatoes, causing black lesion with concentric ring on leaves. Late blight, that caused by *Phytophthora infestans* (De Bary), is the most devastating foliar disease of potatoes, which can kill off a field of potatoes in just a few days when weather is favorable (cool and wet), causing purple black or brown–black lesions at the leaf tip of potato leaves. They also attack potato tubers in the field and storage.

The objectives of this research are :

1. Evaluating effect of *Trichoderma harzianum* and some fungicides on inhibiting the growth of *Alternaria solani* and *Phytophthora infestans* in laboratory conditions.
2. Evaluating of antifungal activity by fungicides and *Trichoderma harzianum* against some potato leaf spots in the greenhouse.
3. Comparing between fungal bio-agent and some fungicides and effects on diseases severity by *Phytophthora infestans* and *Alternaria solani* on potato crops.

1- EXPERIMENTAL RESULTS
Pathogenicity .1

1.1 Symptomology

a) ***Alternaria solani***

Disease symptoms on leaves of plants infected with *A. solani* isolates are as follows**:** Leaf lesions frequently have "bulls-eye" or "target spot" appearance (Figure 1), advanced lesions usually have angular margins because they are limited by leaf veins and as infection progresses, the entire leaf becomes chlorotic, then dies.

b) ***Phytophthora infestans***

Disease symptoms on leaves of plants infected with *P. infestans* isolates**:** infected foliage are initially yellow, becomes water soaked and eventually blackens. Leaf symptoms comprise purple-black or brown-black lesions at the leaf tip (Figure 2), later spreading across the leaf to the stem.

Figure 1. Symptoms on leaves of plants infected with *A. solani* (isolates A1 and A2 by artificial inoculation. V1, cv.Picasso;V2,cv Diamond;V3,cv.Baraka; V4,cv.Vivaldi; and V5, cv.Kondor

A1 isolate

Control V1 Control V2

Control V3 Control V4

Control V5

A2 isolate

Control V1 Control V2

Control V3 Control V4

Control V5

1.2. Greenhouse pathogenicity tests of leaf infection

a) Effect of four isolates of *A. solani* and *P. infestans* on disease reaction

Pathogenicity of four isolates of *A. solani* and *P. infestans* potato leaf blights on five cultivars of potato.

Data in table 1 and figures (3 and 4) showed that all isolates of *P. infestans* and *A. solani* didn't differ significantly in their virulence on five cultivars of potato crop, as means of diseased leaf area given were; 48.83, 57.94, 54.98 and 53.50% given by A1 and A2 of *A. solani* isolates and P1 and P2 of *P. infestans,* respectively. While disease incidence symptoms were not seen on the untreated control of potato cultivars.

b) Effect of potato cultivars tested on disease reaction of four isolates of *A. solani* and *P. infestans* leaf blights

Data in table 1 and figures (3 and 4) also showed the potato cultivars reaction against isolates of *A. solani* and *P. infestans* disease incidence. Percentages of diseased leaf area were maximum (81.38 and 74.17%) given by Picasso and Vivaldi potato cultivars, followed by 56.12% given by Diamond and 39.35% given by Kondor cultivar. The least disease reaction (17.49%) was given by Baraka cultivar.

c) Interaction effect of *A. solani* and *P. infestans* isolates and potato cultivars on disease reaction of leaf blights

Data in table 1 figures (3 and 4) showed also the interaction effect of *A. solani* and *P. infestans* isolates and potato cultivars tested on leaf blights disease reaction. Picasso, Kondor and Diamond didn't show significant difference in disease reaction caused by A1 isolate of *A. solani*, While there was a significant difference between Vivaldi and Baraka in their disease reaction against the same isolate. The four potato cultivars did not react significantly against isolate A2 of *A. solani*, but Baraka cultivar showed the least significant disease reaction caused by the same A2 isolate. Such data may indicate that isolates obtained of *A. solani* are different isolates.

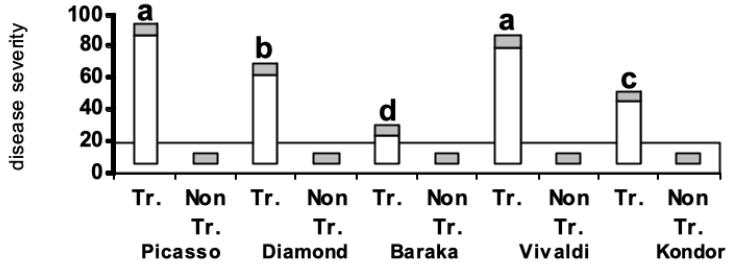
Isolates P1 and P2 of *P. infestans* on Picasso, Diamond and Vivaldi gave the same significance of disease reaction but differed from these given by Kondor and Baraka cultivars, which means that both isolates are having the same characters and probably are having the same biological component.

Table 1. Pathogenicity of *A.solani* (A1and A2) and *P.infestans* (P1 and P2) isolates on leaves of five potato cultivars in greenhouse in percentage

Isolate	Mean disease severity											
	Cultivar											
	Picasso		Diamond		Baraka		Vivaldi		Kondor		Mean	
	Tr.	Non Tr.	Tr.	Non Tr.	Tr.	Non Tr.	Tr.	Non Tr.	Tr.	Non Tr.	Tr.	Non Tr.
A1	78.96 **a**	0.00	25.21 **a**	0.00	16.67 **b**	0.00	72.5 **ab**	0.00	50.83 **a**	0.00	48.83 **a**	0.00
A2	82.49 **a**	0.00	60.83 **a**	0.00	17.49 **b**	0.00	74.16 **a**	0.00	52.49 **a**	0.00	57.49 **a**	0.00
P1	89.69 **a**	0.00	62.19 **ab**	0.00	22.08 **bc**	0.00	69.38 **ab**	0.00	31.56 **b**	0.00	54.98 **a**	0.00
P2	74.38 **ab**	0.00	76.25 **ab**	0.00	13.75 **c**	0.00	80.63 **a**	0.00	22.5 **bc**	0.00	53.5 **a**	0.00
Mean	81.38 **a**	0.00	56.12 **b**	0.00	17.49 **d**	0.00	74.17 **a**	0.00	39.35 **c**	0.00		

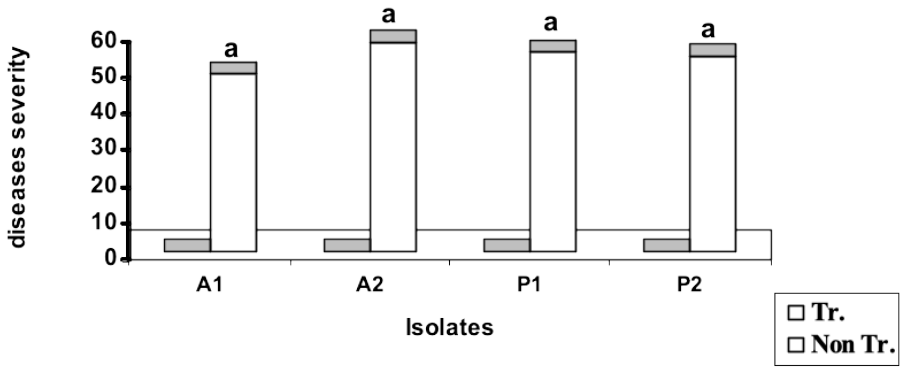
Tr. = cultivar treated with isolate spores
Non Tr. = cultivar non treated with isolate spores
LSD (P=0.05) between isolate means =12.66
LSD (P=0.05) between Cultivar means = 14.16
LSD (P = 0.05) interaction of isolate x cultivar = 56.56
Means followed by the same letters are not significantly different at P = 0.05 (LSD test) .

Figure 3 . Susceptibility of five potato cultivars to foliar infection by A.solani (A1and A2) and P.infestans (P1 and P2) isolates in greenhouse in percentage



Tr. = cultivar treated with isolate spores
Non Tr. = cultivar non treated with isolate spores
Columns followed by the same letters are not significantly different at P= 0.05 (LSD test)

Figure 4. Pathogenicity of *A.solani* (A1 and A2) and *P.infestans* (P1 and P2) isolates on leaves of five potato cultivars in greenhouse in percentage



Tr. = cultivar treated with isolate spores
Non Tr. = cultivar non treated with isolate spores
Columns followed by the same letters are not significantly different at P= 0.05 (LSD

2. The laboratory study

2.1 Effect of three fungicides and *T. harzianum* with five concentrations on the growth of *A. solani* and *P. infestans*

The effect of fungicides and *T. harzianum* on growth of *A. solani* and *P. infestans* (Figures 5, 6 and 7) were tested in Petri dishes at concentrations 0, 5, 10, 50 and 100 µg a.i./ml (designated 1, 2, 3, 4, and 5 respectively) for

fungicides and 0, 40 x 10^3, 76 x 10^3, 152 x 10^3 and 2 x10^5 cell/ml (designated 1, 2, 3, 4 and 5 respectively) for *T. harzianum*.

The data in Table 2 and figure 8 show that there were significant differences among treatments in mycelia growth of *A. solani*.

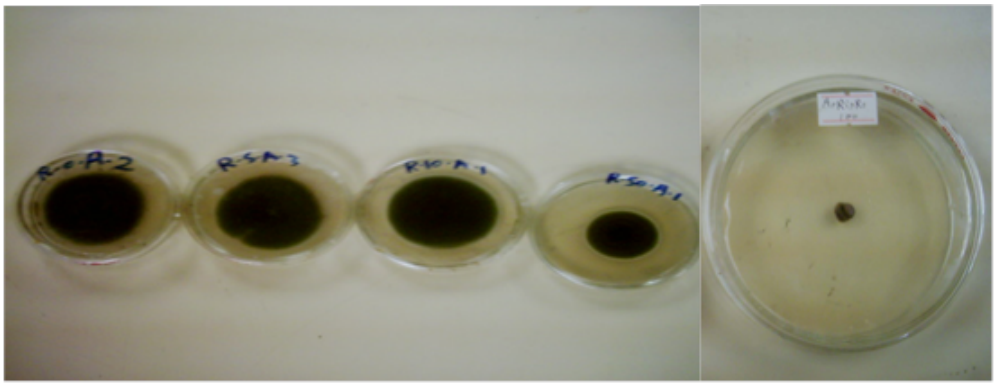
Quadris and *T. harzianum* were the best effect on mycelial growth than the other treatments. Cupravit was the least effect on mycelial growth.

The fungus did not grow on PDA agar amended with Ridomil Gold MZ and Quadris at fungicide concentration 100 µg a.i./ml (5) while it able to grow in concentrations varying from 5 to 50 µg a.i./ml (2 to 4). The fungus able to grow on PDA agar amended with cupravit and *T. harzianum* at concentrations 100 µg/ml (5) and 2×10^5 cell /ml (5) respectively.

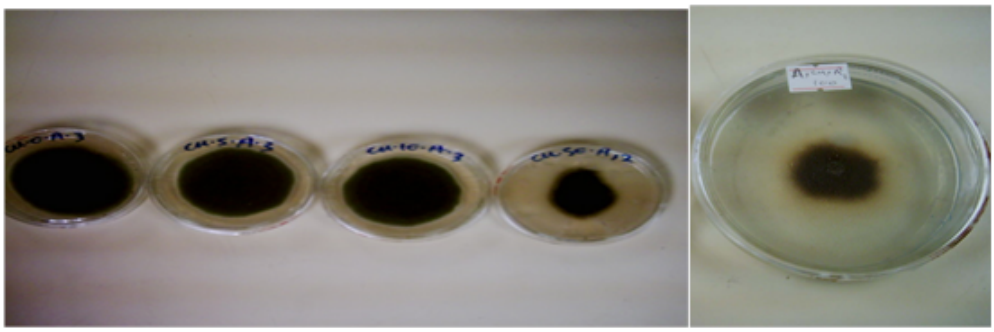
The data in Table 2 and figure 9 show that there were significant differences among treatments in mycelia growth of *P. infestans*.

T. harzianum was the best effect on mycelial growth than the other treatments followed by Quadris and Ridomil Gold MZ, whereas Cupravit was the least effect on mycelial growth. The fungus did not grow on PDA agar amended with Ridomil Gold MZ at concentration 100 µg a.i./ml (5) while it able to grow in concentrations from 5 to 50 µg a.i./ml (2 to 4). The fungus able to grow on PDA agar amended with cupravit, Quadris and *T. harzianum* at concentrations 100 µg a.i./ml (5) and 2×10^5 cell /ml (5) respectively.

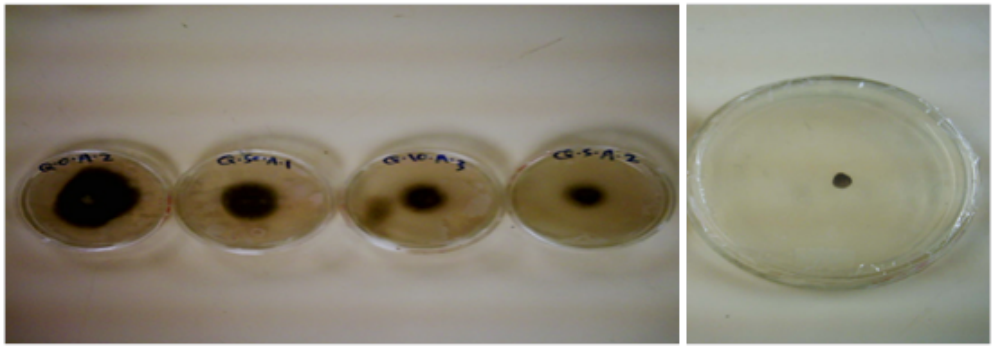
Figure 5. Effect of three fungicides with five concentrations on colony diameter (cm) of *A. solani* after 7 days



Ridomil Gold MZ (0, 5, 10, 50 and 100 µg a.i./ml from left to right)



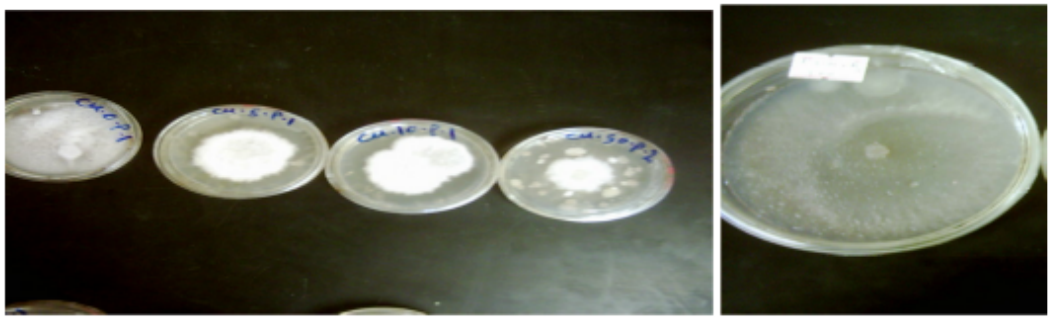
Cupravit (0, 5, 10, 50 and 100 µg a.i./ml from left to right)



Quadris (0, 5, 10, 50 and 100 µg a.i./ml from left to right)

Figure 6. Effect of three fungicides with five concentrations on colony diameter (cm) of *P. infestans* after 7 days

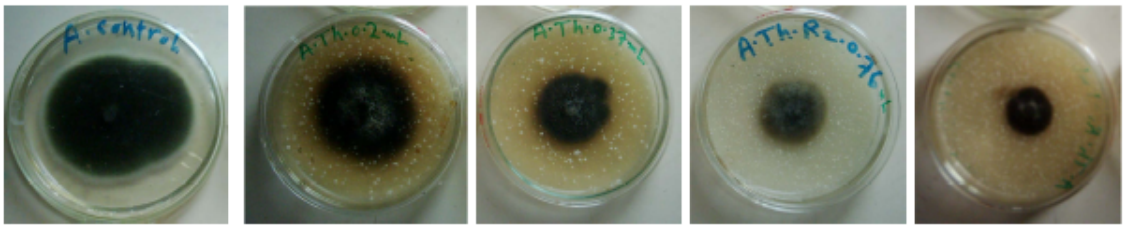
Ridomil Gold MZ (0, 5, 10, 50 and 100 µg a.i./ml from left to right)



Cupravit (0, 5, 10, 50 and 100 µg a.i./ml from left to right)

Quadris (0, 5, 10, 50 and 100 µg a.i./ml from left to right)

Figure 7. Effect of *T. harzianum* with five concentrations on colony diameter (cm) of *A. solani* and *P. infestans* after 7 days



0 , $4x10^3$, $76x10^3$, $152x10^3$ and $2x10^5$ cell /ml from left to right on *A.solani*

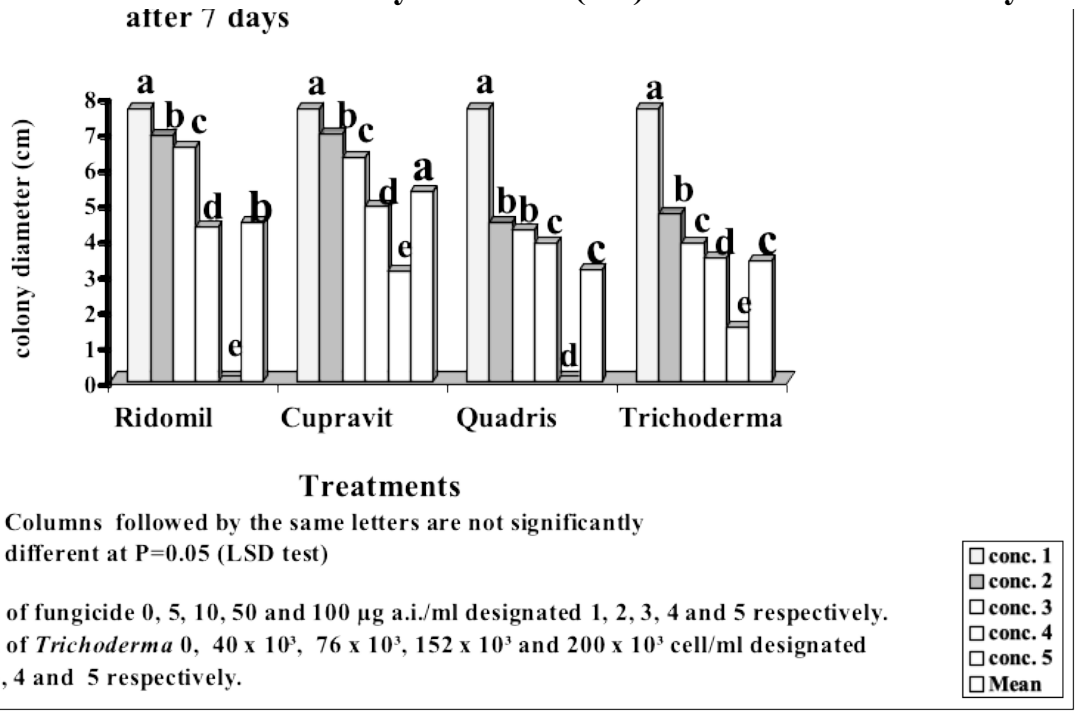
0, $4x10^3$, $76x10^3$, $152x10^3$ and $2x10^5$ cell /ml from left to right on *P.infestans*

Table 2. Effect of three fungicides *and T. harzianum* with five concentrations on colony diameter (cm) of A. solani and *P. infestans* after 7 days

Treatmen	*A . solani*						*P .infestans*					
	Con . Of Fungicide (µg a.i./ml)* and *T.harzianum* (cell/ml)**											
	1	2	3	4	5	Mean	1	2	3	4	5	Mean
Ridomil MZ	7.68 a	6.92 b	6.57 c	4.35 d	0.00 e	4.46 **b**	8.37 a	6.48 b	6.32 b	6 .00 c	0.00 d	4.70 **b**
Cupravit	7.68 a	6.97 b	6.3 c	4.91 d	3.08 e	5.32 **a**	8.37 a	7.17 b	7.00 b	7.00 b	7.02 b	7.05 **a**
Quadris	7.68 a	4.45 b	4.25 b	3.9 c	0.00 d	3.15 **c**	8.37 a	5.63 b	5.53 b	5.08 c	1.00 d	4.31 **c**
T.harzianum	7.68 a	4.73 b	3.88 c	3.48 d	1.5 e	3.39 **c**	8.37 a	4.82 b	4.18 c	2.93 d	2.90 d	3.71 **d**

LSD (P=0.05) between treatment means = 0.3
LSD (P=0.05) between concentration means = 0.3
Means followed by the same letters are not significantly different at P=0.05 (LSD test).
*Concentrations of fungicide 0, 5, 10, 50 and 100 µg a.i./ml designated 1, 2, 3, 4 and 5 respectively.
**Concentrations of *T.harzianum* 0, 40 x 10^3, 76 x 10^3, 152 x 10^3 and 2 x 10^5 cell/ml designated

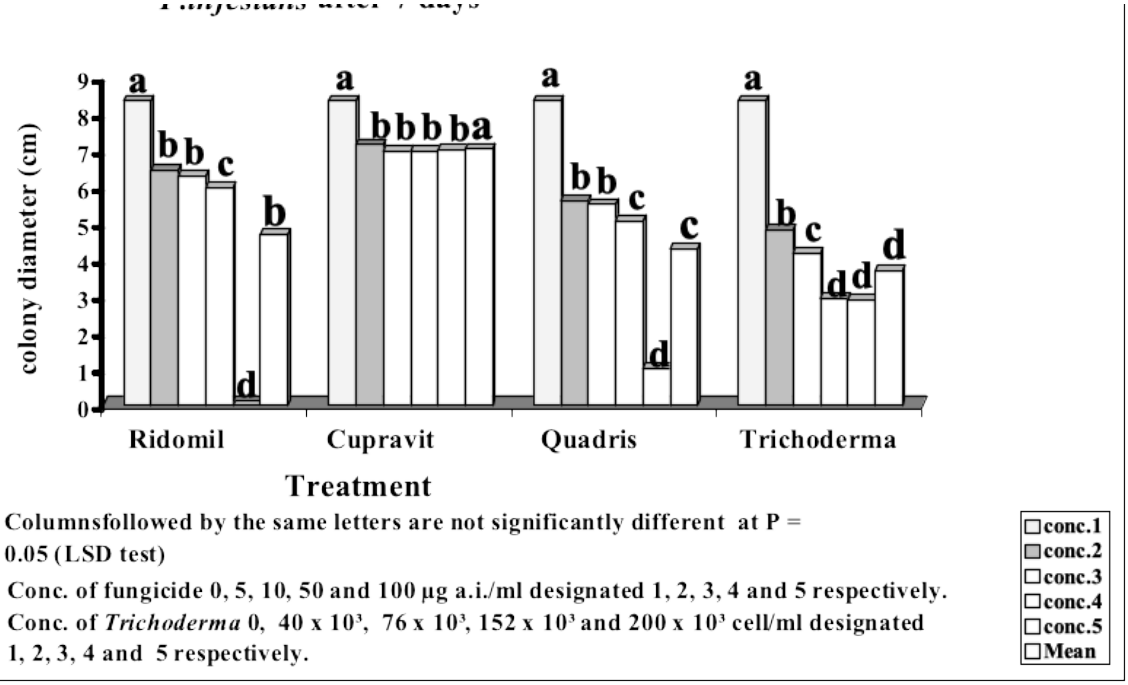
Figure 8. Effect of three fungicides *and T. harzianum* with five concentrations on colony diameter (cm) of *A. solani* after 7 days



Columns followed by the same letters are not significantly different at P=0.05 (LSD test)

Conc. of fungicide 0, 5, 10, 50 and 100 µg a.i./ml designated 1, 2, 3, 4 and 5 respectively.
Conc. of *Trichoderma* 0, 40 x 10^3, 76 x 10^3, 152 x 10^3 and 200 x 10^3 cell/ml designated 1, 2, 3, 4 and 5 respectively.

Figure 9. Effect of three fungicides *and T. harzianum* with five concentrations on colony diameter (cm) of *P. infestans* after 7 days



2.2 Effect of three fungicides and *T. harzianum* with five concentrations on spore germination of *A. solani* and *P. infestans*

The effect of three fungicides and *T. harzianum* on germination of spores of *A. solani* and *P. infestans* were tested. Table 3 and Figure 10 show that there were significant differences in spore germination of *A. solani* among treatments. Quadris or Ridomil Gold MZ gave the lowest germination percentage respectively while cupravit and *T. harzianum* gave the highest germination percentage. Effective of concentrations of treatments on germination of spores were decreased significantly when increasing of the concentrations from 5 to

100 µg a.i./ml (2 to 5), whereas concentration 100 µg a.i./ml **(**5**)** of Quadris gave the lowest germination. Table 3 and Figure 11 show that there were significant differences in spore germination of *P. infestans* among treatments.

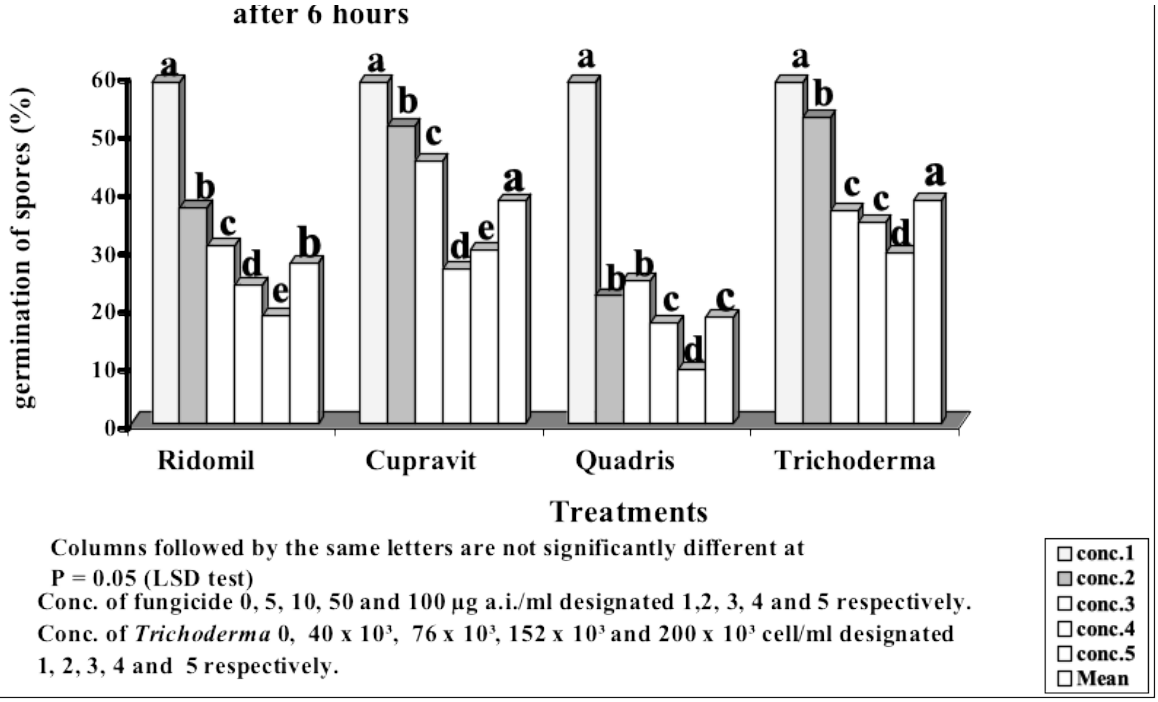
Ridomil Gold MZ successfully inhibited spore germination of *P. infestans* while Cupravit, *T. harzianum* and Quadris gave the highest germination percentage respectively. The effects of concentrations of treatments on germination of spores were decreased **s**ignificantly when increasing of the concentrations from 5 to 100 µg a.i./ml(2 to 5). Ridomil Gold MZ inhibited spore germination at all concentrations.

Table 3. Effect of three fungicides and *T. harzianum* with five concentrations on germination of spore of *A. solani* and *P. infestans* after 6 hours

Treatment	*A . solani*						*P . infestans*					
	Con. of Fungicide (µg a.i./ ml) * and *T.harzianum* (cell/ml)**											
	1	2	3	4	5	Mean	1	2	3	4	5	Mean
Ridomil MZ	58.7 **a**	37.3 **b**	30.7 **c**	24.0 **d**	18.7 **e**	27.68 **b**	43.3 **a**	0.0 **b**	0.00 **b**	0.00 **b**	0.00 **b**	0.000 **d**
Cupravit	58.7 **a**	51.3 **b**	45.3 **c**	26.7 **d**	30.0 **e**	38.33 **a**	43.3 **a**	37.0 **b**	30.0 **c**	28.0 **c**	19.0 **d**	28.50 **a**
Quadris	58.7 **a**	22.0 **b**	24.7 **b**	17.3 **c**	9.33 **d**	18.33 **c**	43.3 **a**	26.7 **b**	18.7 **c**	10.7 **d**	6.00 **e**	15.53 **c**
T.harzianum	58.7 **a**	52.7 **b**	36.7 **c**	34.7 **c**	29.3 **d**	38.35 **a**	43.3 **a**	29.3 **b**	21.3 **c**	12.0 **d**	12.0 **d**	18.65 **b**

LSD (P=0.05) between treatment means = 2.93
LSD (P=0.05) between concentration means = 3.28
Means followed by the same letters are not significantly different at P=0.05 (LSD test).
*Concentrations of fungicide 0, 5, 10, 50 and 100 µg a.i./ml designated 1, 2, 3, 4 and 5 respectively.
**Concentrations of *T.harzianum* 0, 40 x 10^3, 76 x 10^3, 152 x 10^3 and 2 x 10^5 cell/ml designated 1, 2, 3, 4 and 5 respectively.

Figure 10. Effect of three fungicides and *T. harzianum* with five concentrations on germination of spore of *A. solani* after 6 hours

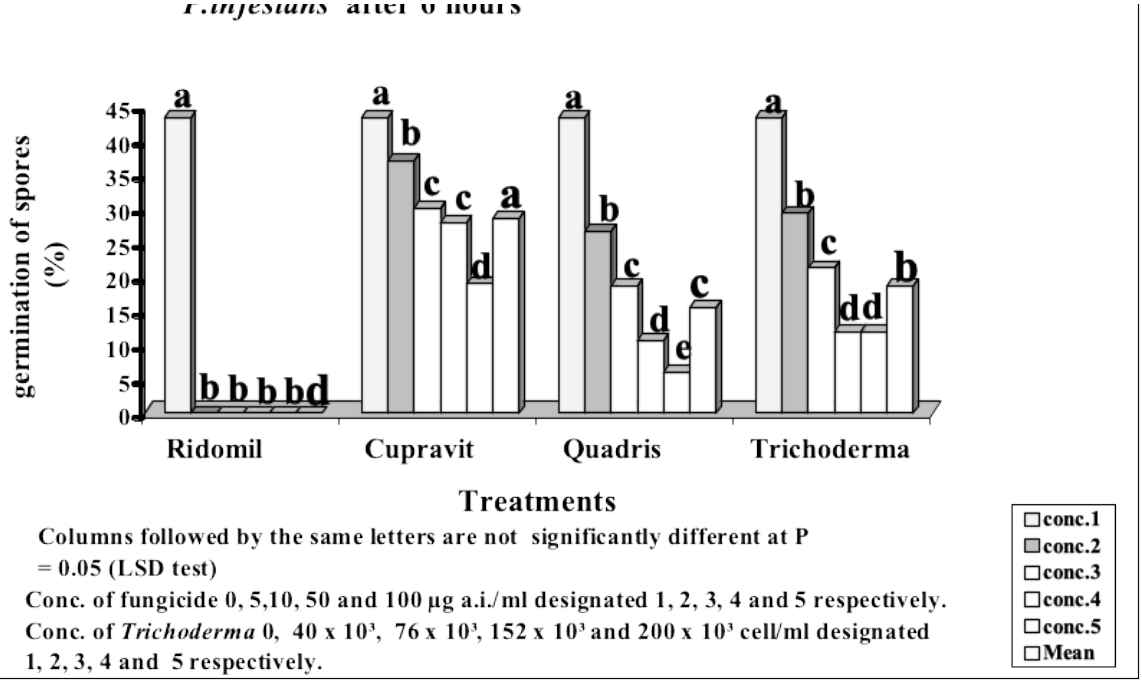


Columns followed by the same letters are not significantly different at P = 0.05 (LSD test)

Conc. of fungicide 0, 5, 10, 50 and 100 µg a.i./ml designated 1,2, 3, 4 and 5 respectively.

Conc. of *Trichoderma* 0, 40 x 10^3, 76 x 10^3, 152 x 10^3 and 200 x 10^3 cell/ml designated 1, 2, 3, 4 and 5 respectively.

Figure 11. Effect of three fungicides and *T. harzianum* with five concentrations on germination of spore of *P. infestans* after 6 hours



Columns followed by the same letters are not significantly different at P = 0.05 (LSD test)

Conc. of fungicide 0, 5,10, 50 and 100 µg a.i./ml designated 1, 2, 3, 4 and 5 respectively.

Conc. of *Trichoderma* 0, 40 x 10^3, 76 x 10^3, 152 x 10^3 and 200 x 10^3 cell/ml designated 1, 2, 3, 4 and 5 respectively.

2.3 Effect of three fungicides and *T. harzianum* with five concentrations on length of germ tubes of *A. solani* and *P. infestans*

The effect of three fungicides and *T. harzianum* with five concentrations on length of germ tubes of *A. solani* and *P. infestans* were tested.

The data in Table 4 and figure 12 show that there were significant differences in length of germ tubes of *A. solani* due to effect of treatments. Ridomil Gold MZ and Quadris gave the best effect on length of germ tubes, they were decreased germ tube lengths to level less than that obtained with Cupravit or *T. harzianum*.

The effect of concentrations of treatments on germ tube lengths were decreased significantly when increasing of the concentrations from 5 to 100 µg a.i./ml (2 to

5). All fungicides and *T. harzianum* were similar with inhibition of germ tube lengths of *A. solani* at concentrations 10 and 50 µg a.i./ml (3 and 4) except Ridomil Gold MZ was at concentrations 50 and 100 µg a.i./ml (4 and 5). The highest inhibition was at concentration 100 µg a.i./ml (5) for all fungicides and 2×10^5 cell /ml (5) for *T. harzianum* while Ridomil Gold MZ was at concentrations 50 and 100 µg a.i./ml (4 and 5).

Table 4 and figure 13 show that there were significant differences in length of germ tubes of *P. infestans* due to effect of treatments. Ridomil Gold MZ successfully inhibited germ tubes while cupravit, *T. harzianum* and Quadris gave the highest germ tube lengths respectively. The effect of concentrations of treatments on germ tube lengths were decreased significantly when increasing of the concentrations from 5 to 100 µg a.i./ml (2 to 5). Ridomil Gold MZ inhibited germ tube lengths at all concentrations**.**

Table 4. Effect of three fungicides and *T. harzianum* with five concentrations on length of germ (µm) tubes of *A. solani* and *P. infestans* after 6 hours

Treatment	*A . Solani*						*P .infestans*					
	Con. of Fungicide (µg a.i./ ml)* and *T.harzianum* (cell /ml) **											
	1	2	3	4	5	Mean	1	2	3	4	5	Mean
Ridomil MZ	86.3 a	83.3 a	67.0 b	55.7 c	46.3 c	63.08 b	703 a	0.00 b	0.00 b	0.00 b	0.00 b	0.00 d
Cupravit	86.3 a	95.0 a	74.3 b	68.7 b	56.7 c	73.68 a	703 a	513 b	227 c	199 d	153 e	273 b
Quadris	86.3 a	64.0 b	70.0 b	59.3 b	39.7 c	58.25 b	703 a	111 b	110 b	70.5 c	41.7 d	83.3 c
Trichoderma	86.3 a	96.7 a	86.0 ab	84.0 b	62.7 c	82.35 a	703 a	161 b	134 c	108 d	71.0 e	118.5 a

LSD (P=0.05) between treatment means = 9.5
LSD (P=0.05) between concentration means = 11
Means followed by the same letters are not significantly different at P=0.05 (LSD test).
*Concentrations of fungicide 0, 5, 10, 50 and 100 µg a.i./ml designated 1, 2, 3, 4 and 5 respectively.
**Concentrations of *T.harzianum* 0, 40 x 10^3, 76 x 10^3, 152 x 10^3 and 2 x 10^5 cell/ml designated 1, 2, 3, 4

Figure 12. Effect of three fungicides and *T. harzianum* with five concentrations on length of germ (µm) tubes of *A. solani* after 6 hours

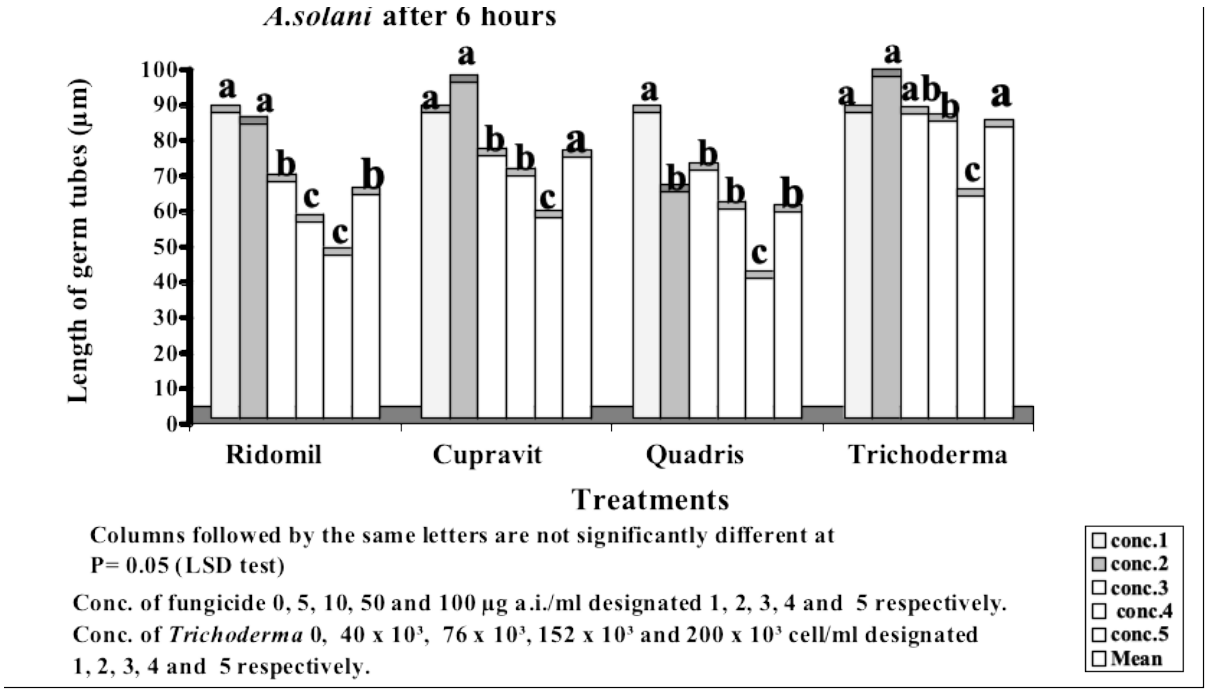
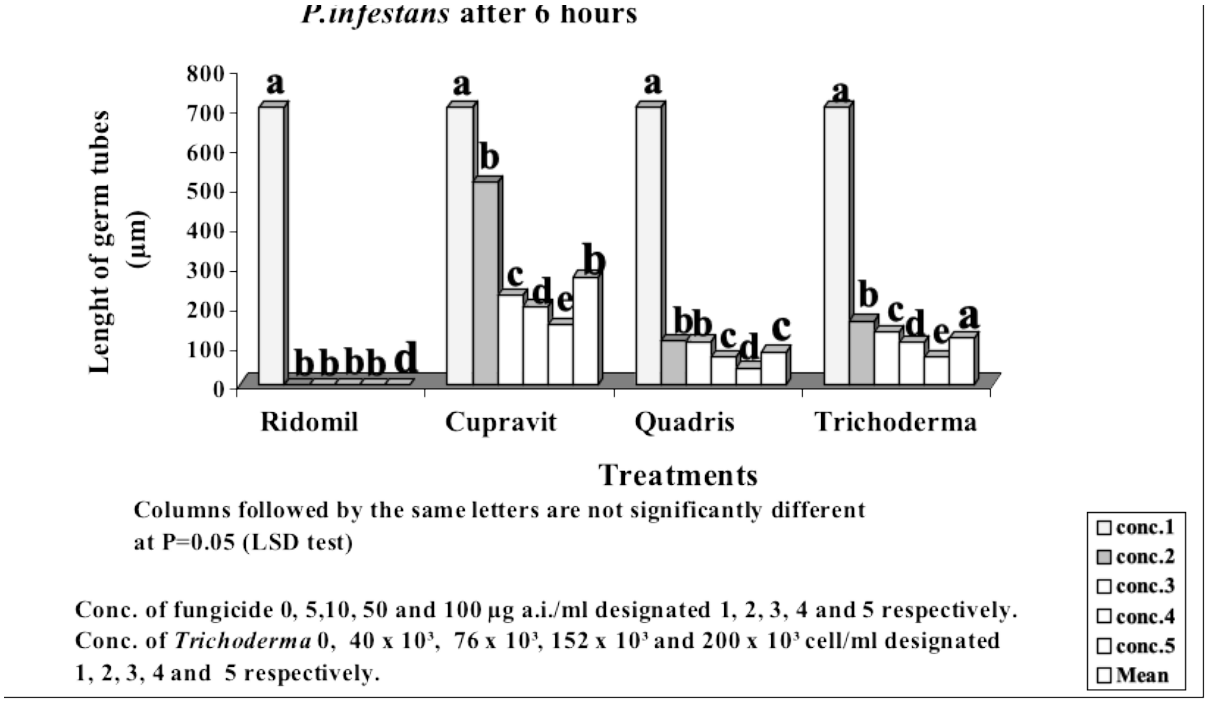


Figure 13. Effect of three fungicides and *T. harzianum* with five concentrations on length of germ (µm) tubes of *P. infestans* after 6 hours



Recommendations

1. Cultivars resistance against early blight and late blight caused by *A. solani* and *P. infestan* such as Baraka cultivar is recommended.
2. There should be a protection spray program using fungicides such as Quadris and Ridomil.
3. Monitoring future resistance against fungicide used.
4. The use of biological control *T. harzianum* needs to be investigated thoroughly against late blight.