

# Combination between Benzothiadiazole and *Trichoderma Viride* to Control Chocolate Spot Disease of Faba Bean and Their Effects on some Biochemical Characters

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## ABSTRACT

Faba bean is a food plant in many countries in the world. *Botrytis fabae* is a fungus that caused chocolate spot disease in faba bean. In this work, two concentrations of benzothiadiazole (0.3 and 0.5 mM) and suspensions of *Trichoderma viride* prepared in two concentrations ( $1 \times 10^7$  and  $2 \times 10^7$  spore/ml). The concentrations of benzothiadiazole and *Trichoderma viride* used alone and in combination to control chocolate spot disease in faba bean. Application of benzothiadiazole and *Trichoderma viride* reduced chocolate spot disease when used alone or in combination as foliar treatment. Total chlorophyll content and flavonoids content were increased when benzothiadiazole and *Trichoderma viride* used in treated plants compared to the infected control and to the plants treated with Tridex 80 % wp.

**Key Words:** benzothiadiazole, *Trichoderma viride*, chocolate spot disease.

## INTRODUCTION

Teosinte Faba bean is rich in energy and protein content and has a high nutritive value. *Botrytis fabae* is a fungus caused chocolate spot disease in faba bean. This disease appears on leaves, stems, flowers and pods which caused yield losses (Sahile *et al.*, 2010, Knany *et al.*, 2009 and Elsayed *et al.*, 2016).

Benzothiadiazole (BTH) is an organic compound provide good protection to plant against many of plant pathogens (Hafez 2010 and Nianlai *et al.*, 2010).

*Trichoderma* spp. are fungi found in the soil and have antagonistic effect against many of phytopathogenic fungi (Frac 2017). *Trichoderma* spp. used as biopesticides, biofertilizers and soil amendments in commercially marketed (Frac *et al.*, 2017).

The objective of this study is to use benzothiadiazole and *Trichoderma viride* to control chocolate spot disease in faba bean each alone or in combination and compared their effect with the recommended fungicide (Tridex 80 % wp).

## MATERIALS AND METHODS

Benzothiadiazole [benzo-(1, 2, 3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH)] an organic compound used as aqueous solution in two

concentrations, 0.3 and 0.5 mM. Obtained from Elgomharia company.

80 % wp : An organic wettable powder fungicide for the control and prevention of various fungal diseases on crops. Active ingredient Mancozeb. Obtained from Ag chem company.

*Trichoderma viride* and *Botrytis fabae* growing on (PDA) medium in autoclaved plates. *Trichoderma viride* incubated at 25 °C and *Botrytis fabae* incubated at 20 °C. Hemocytometer slide was used to prepare spore suspensions which adjusted to ( $2.5 \times 10^7$  spore/ml) for *Botrytis fabae* and ( $1 \times 10^7$  and  $2 \times 10^7$  spore/ml) for *Trichoderma viride* (Jay *et al.*, 2017). Isolates were provided from department of plant pathology faculty of agriculture Alexandria university.

In each pot five seeds of faba bean were sown, under green house controlled conditions. The experiment was conducted after 45 days from sowing (cv Giza 3). The growing seedlings were sprayed with *T. viride* and benzothiadiazole. Treatments are:

1. Plants sprayed with sterilized water only (control).
2. Plants sprayed with *Botrytis fabae* (untreated infected pathogen).
3. Plants sprayed with *Botrytis fabae* + benzothiadiazole (BTH) at concentration 0.3 mM.
4. Plants sprayed with *Botrytis fabae* + benzothiadiazole (BTH) at concentration 0.5 mM.
5. Plants sprayed with *Botrytis fabae* + *T. viride* at concentration  $1 \times 10^7$  spore/ml.
6. Plants sprayed with *Botrytis fabae* + *T. viride* at concentration  $2 \times 10^7$  spore/ml.
7. Plants sprayed with *Botrytis fabae* + benzothiadiazole / *T. viride* (0.3 mM /  $1 \times 10^7$  spore/ml).
8. Plants sprayed with *Botrytis fabae* + benzothiadiazole / *T. viride* (0.3 mM /  $2 \times 10^7$  spore/ml).
9. Plants sprayed with *Botrytis fabae* + benzothiadiazole / *T. viride* (0.5 mM /  $1 \times 10^7$  spore/ml).

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Received June 10, 2018, Accepted June 27, 2018

10. Plants sprayed with *Botrytis fabae*+ benzothiadiazole / *T. viride* (0.5 mM /  $2 \times 10^7$  spore/ml).

After 50 days of sowing, the parameters were determined. Five pots were used as replicates for each treatment (Harman *et al.*, 2004 and Jay *et al.*, 2017).

Disease severity percentage was measured according to Hanounik (1986).

Total flavonoids were determined according to Hertog *et al.*, (1992) and total chlorophyll content was determined according to method described by Procter (1981) and calculated using the formula reported by Arnon (1949). Data were analyzed according to (Gomez and Gomez 1984).

### RESULTS AND DISCUSSION

Using benzothiadiazole and *Trichoderma viride* alone or in combination reduced chocolate spot disease severity than untreated infected control. Increasing the concentrations of benzothiadiazole and *Trichoderma viride* increased the reduction in disease severity up to 91.68% agree with (Frac *et al.*, 2017). Using benzothiadiazole alone was more effective than using

*Trichoderma viride* alone to control the disease. The highest reduction percentage was when using benzothiadiazole (0.5 mM) and *Trichoderma viride* ( $2 \times 10^7$  spore/ml) in combination up to the reduction percentage when using Tridex 80% wp, as shown in table 1.

Increased flavonoid content were obtained when using benzothiadiazole and *Trichoderma viride* alone or in combination than untreated infected plants (Table 2). The flavonoid content significantly increased when using benzothiadiazole (0.5 mM) and *Trichoderma viride* ( $2 \times 10^7$  spore/ml) in combination close to Tridex 80% wp (1F) treatment agree with (Jay *et al.*, 2017).

Increased total chlorophyll content were obtained when using benzothiadiazole and *Trichoderma viride* alone or in combination than untreated infected plants agree with (Philip *et al.*, 2017). The total chlorophyll content significantly increased when using benzothiadiazole (0.5 mM) and *Trichoderma viride* ( $2 \times 10^7$  spore/ml) in combination up to Tridex 80% wp (1F) treatment (Table 3).

**Table 1. Fungicidal activity of benzothiadiazole and *T. viride* alone or in combination on disease severity and reduction percentage of disease compared to Tridex 80% wp (mancozeb)**

Treatment	Concentration	<i>B.fabae</i>	
		Disease Severity %	Reduction %
Benzothiadiazole(BTH)	0.3 mM	21.11	56.06
	0.5 mM	11.75	75.55
<i>T.viride</i>	$1 \times 10^7$ spore/ml	33.12	31.06
	$2 \times 10^7$ spore/ml	27.14	43.51
<i>T.viride</i> + BTH	$1 \times 10^7$ spore/ml+0.3mM	15	68.78
	$2 \times 10^7$ spore/ml+0.3mM	11.10	76.90
	$1 \times 10^7$ spore/ml+0.5mM	7.5	84.39
	$2 \times 10^7$ spore/ml+0.5mM	4	91.68
Tridex 80% wp (mancozeb)	0.5 F	17.9	62.74
	1 F	3	93.76
	1.5 F	2	95.84
Untreated infected control	-	48.04	-
L.S.D at 0.05		3.12	

**Table 2. Effect of using benzothiadiazole and *Trichoderma viride* alone or in combination on flavonoids content in faba bean leaves (mg/gm fresh weight) compared to Tridex 80% wp (mancozeb)**

Treatment	Concentration	Flavonoids content(mg/g fresh weight)
		<i>B.fabae</i>
Benzothiadiazole(BTH)	0.5 mM	1.48
<i>T.viride</i>	$2 \times 10^7$ spore/ml	1.24
<i>T.viride</i> + BTH	$2 \times 10^7$ spore/ml+0.5mM	2.10
Tridex 80% wp (mancozeb)	1 F	2.30
Infected control plants	-	1.10
Healthy control plants	-	2.9
L.S.D at 0.05		0.06

**Table 3 .Effect of using benzothiadiazole and *Trichoderma viride* alone or in combination on total chlorophyll content in faba bean leaves ( mg/gm fresh weight ) compared to Tridex 80 % wp (mancozeb)**

Treatment	Concentration	<i>B.fabae</i>
		Total chlorophyll
Benzothiadiazole(BTH)	0.5 mM	7.44
<i>T.viride</i>	$2 \times 10^7$ spore/ml	6.82
<i>T.viride</i> + BTH	$2 \times 10^7$ spore/ml+0.5mM	7.70
Tridex 80 % wp (mancozeb)	1 F	7.90
Infected control Plants	-	5.66
Healthy Control Plants	-	8.62
L.S.D at 0.05		0.16

In conclusion, results showed that using *Trichoderma viride* and benzothiadiazole in combination was better than using each alone than untreated infected plants respectively in reducing chocolate spot disease , increasing total chlorophyll content and increasing flavonoids content in faba bean leaves . Using *Trichoderma viride* and benzothiadiazole in combination more effective against this disease and may be used as alternative to classical fungicides Tridex 80 % wp (mancozeb) .

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## الملخص العربي

### كفاءة خلط البنزوثيرايدازول و فطر التريكوديرما فردى ضد مرض البقعة الشيكولاتية (التبقع البنى) فى الفول و تأثير ذلك على بعض الصفات البيوكيميائية

محمد عادل عبد العال

على الاوراق ادى لحدوث اختزال كبير لمرض البقعة الشيكولاتية ( التبقع البنى ) فى النباتات المعاملة عن النباتات الغير معاملة . مستويات الفلافينويد فى أوراق النباتات المعاملة ارتفعت الى حد كبير بعد 24 ساعة من المعاملة . النباتات المعاملة حدث بها زيادة ملحوظة فى المحتوى الكلى للكلوروفيل عن النباتات الغير معاملة.

مرض البقعة الشيكولاتية ( التبقع البنى ) مرض مهم جدا يصيب نبات الفول فى مناطق عديدة بالعالم . تركيزات مختلفة من مركب البنزوثيرايدازول (0.3) و 0.5 مللى مولر ( و فطر تريكوديرما فردى  $1 \times 10^7$  ) و  $2 \times 10^7$  جرثومة /مل) استخدمت منفردة أو فى خليط و ذلك لدراسة تأثيرها ضد مرض البقعة الشيكولاتية ( التبقع البنى ) فى الفول الذى يسببه فطر بوتريتس فابى تطبيق المركبات المختبرة بالرش