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In vivo Assay for Antagonistic Potential of Fungal Isolates against Faba bean (*Vicia faba* L.) Chocolate Spot *(Botrytis fabae* Sard.*)*

Ermias T. Taffa[1,*], Chemeda F. Gurmessa[2] and Samuel Sahile W. Mariam[3]

[1]Sinana Agricultural Research Center, P.O.Box 208, Bale-Robe, [2]School of Plant Sciences, Haramaya University, P.O.Box 138, Dire Dawa, [3]Fuculity of Natural and Computational Science, University of Gondar, P.O.Box 196, Gondar, Ethiopia.

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Abstract

Due to its high protein content, faba bean (*Vicia fabae* L.) leaves harbor many microorganisms besides *Botrytis fabae*. The objective of this study is to explore fungal isolates residing on faba bean leaves and evaluate their antagonistic potential against *B. fabae*. For this matter, 236 leaf samples were collected from different districts of West Hararghe and Bale zones. Out of which 72 fungal species were isolated and evaluated for their *in vivo* biocontrol potential against chocolate spot (*B. fabae* Sard.). The *in vivo* assay was conducted in two stages where detached leaf test and intact leaf test was involved. Significant difference ($p<0.05$) resulted among fungal isolates to affect incubation period (IP) and disease severity (DS) on local and Shalo cultivars on detached leaf. Isolates GO2-3, GB6-3, S16-2, A12-1 and 52-BT resulted incubation period of 3.7 - 4.7 days, where it was 2 days on untreated control of both cultivars. Lower disease severity was recorded from the leaf treated by GO2-3 and S16-2 on local and GO3-2 on Shalo based on 1-4 rating scale. On intact plant, significant difference ($p<0.05$) among fungal isolates was resulted to affect IP, diseases incidence (DI) and DS. Higher IP was recorded from isolates GB6-3 (3.3), S16-2 (3), and GO3-2 (3 days) on local. GO3-2 showed better reduction (66.7 %) of chocolate spot incidence on Obse compared to the control (100%). Isolates S16-2, GO3-2 and GB6-3 resulted lower disease severity (percent severity index) of 35.6-51.1% as compared to control (73.3-84.4%) on the three cultivars.

Keywords: Faba Bean, *Botrytis fabae*, Antagonistic Fungi, Biocontrol, *Trichoderma* Spp.

1. Introduction

Faba bean (*Vicia faba* L.) is a food and feed legume of great socio-economic importance and is one of the earliest domesticated food legumes in the world, probably in the late Neolithic period (Metayer, 2004). Faba bean ranks sixth in production among the legumes grown in the world. China has been the main producing country, followed by Ethiopia, Egypt, Italy, and Morocco (Salunkhe and Kadam, 1989). Even though Ethiopia is the world's second largest producer of faba bean, its share is only 6.96% of world production and 40.5% within Africa (Chopra *et al*., 1989). The average yield of this crop under small-holder farmers ranges from 1.0 to 1.2 t ha^{-1} (Agegnehu *et al.,* 2006), while world average grain yield of faba bean is around 1.8 t ha^{-1} (ICARDA, 2008). In the Ethiopia highlands, faba bean is one of the most important food crops. It is a source of cash to the farmers and foreign currency to the country. The growing importance of faba bean as an export crop in Ethiopia has led to a renewed interest by farmers to increase the area under production (Samuel *et al.,* 2008). However, the productivity of faba bean in Ethiopia is far below its potential due to a number of factors. The biological limitations include inherently low grain yielding potential of the indigenous cultivars and susceptibility to biotic and abiotic stresses (Mussa *et al.,* 2008). Diseases, chocolate spot (*Botrytis fabae* Sard.), rust (*Uromyces Vicia fabae*), and black root rot (*Fusarium solani*) contribute to the low productivity of the crop. Chocolate spot is considered to be the most important and destructive in Ethiopia causing the yield loss of up to 61% on susceptible cultivars (Dereje and Beniwal, 1987).

Currently, there is an urgent need to improve faba bean yield, since this crop remains an important part of Ethiopian diet. Although synthetic chemicals are available as better option, Products from microbes are relatively broad spectrum, bio-efficacious, economical, and environmentally safe and can be ideal candidates for use as bio-pesticides (Macias *et al.,* 1997). Among these, antagonistic microbioagents from soil and/or phylloplane of plants have been reported to show activity against wide array of plant pathogenic fungi (Reddy, 2000). Therefore, controlling *B. fabae* by biocontrol agents seems to be better and preferred than the chemical control (Mahmoud

* Corresponding author. e-mail: ermiastafa@gmail.com.

et al., 2004). Little research, conducted in Ethiopia for the control of *B. fabae*, indicated high potential of local microbial agents (Samuel, 2008). There is a dire need of exploring different areas for suitable and highly effective microbes for management of chocolate spot. Therefore, this work is proposed with the objectives of identifying the potential antagonistic microorganisms associated with the phylloplane of faba bean and evaluates the effect of potential antagonistic microorganisms against chocolate spot.

2. Materials and Methods

2.1. Collection of Faba Bean Leaf Samples

Two hundred forty samples of healthy looking faba bean leaves were collected from faba bean plants showing chocolate spot disease for exploring the resident fungal isolates (Table 1). Ten fields 5–10 km apart from each districts were visited and from each field 3-4 healthy looking plants were randomly selected, and four healthy looking leaves were detached from each plant. Similarly, Faba bean leaves naturally infected by chocolate spot were collected for isolation of *Botrytis fabae*.

Table 1. Faba bean leaf sample collection from major faba bean producing districts of west Hararghe and Bale zones, Oromia.

Zone	District	Altitude (masl)	No. of sample
Bale	Sinana	2361 - 2396	28
	Goro	1981- 2332	28
	Agarfa	2404 - 2501	28
	Goba	2430 - 2606	40
	Gassera	2369-2422	36
West Hararghe	Bedeno	2308-2605	40
	Deder	2401-2737	40

2.2. Isolation of Botrytis fabae and Resident Fungal Isolates from Phylloplane

Botrytis fabae was isolated from faba bean leaves naturally infected by chocolate spot. Leaves were surface disinfected with 1% sodium hypochlorite for 2 min and rinsed in two changes of sterile water, placed on Potato Dextrose Agar (PDA), incubated at 20°C for 7 days (Haggag *et al*., 2006), and purified by repeated sub-culturing. Likewise, antagonistic fungal isolates residing on faba bean leaves were isolated on PDA media. The collected healthy looking leaf samples were washed in two changes of sterile water for 10 minutes each and macerated using mortar and pestle. The suspension was diluted at 10^{-2}, poured on PDA and incubated at 25^{o}C for 7 days. All visible fungal colonies were isolated, purified, coded and stored at 4^{o}C. The fungal isolates which were later found effective were identified.

2.3. Detached Leaf Test

Fifteen antagonistic fungal isolates out of 72 were finally evaluated for antagonistic potential against chocolate spot on detached leaves. Leaves were prepared by detaching apparently healthy looking leaves from faba bean plants grown at open fields of Haramaya University (HU) research site. HU is located at 42^0 30' E longitude and 9^0 26' N latitude elevated at 1980 masl. It receives 780 mm total annual rain fall and minimum and maximum temperature of 1.4^{0}C and 23.4^{0}C, respectively. The faba bean varieties Shalo (EH011-22-1) and Bale local were arranged in Randomized Complete Block Design (RCBD) in this study. Fully expanded leaflets of similar age group were detached from 6 weeks old faba bean plants, from the middle nodes of the two varieties. Leaflets were surface disinfected by 1% sodium hypochlorite for 2 min, and subsequently rinsed with distilled sterile water and allowed to dry on sterile filter paper. Sterile filter paper was put in side the petri dishes and moistened by distilled sterile water. Sterile bent glass rod was put on the filter paper and leaves were put on the glass rod to serve as moist chamber. *B. fabae* and antagonistic fungal isolate spore suspensions were prepared from 10 days old culture. The spore concentration was adjusted to 2.5 x 10^5 spores /ml by using a hemacytometer (Mohammed *et al.*, 1994). One drop (20 µl) of the antagonistic fungal spore suspension was placed near the midrib of the leaves. The Petri plates were incubated at 20 ^{0}C for 36 hrs. Then, a drop of *B. fabae* spore suspension, containing 2.5 x 10^5 spores /ml was added to the midrib, where the drop of the antagonistic fungal spore suspension was placed and incubated at 20 ^{0}C. Plate containing detached leaf inoculated only with *B. fabae* alone was used as control. The study was conducted in three replications arranged in RCBD. The disease development was rated using a 1-4 scale (ICARDA, 1986) where 1 = highly resistant, no infection or very small flecks (1-25% necrosis); 2 = resistant, necrotic flecks with few small lesions (26-50% necrosis), and very poor sporulation; 3 = moderately resistant, medium coalesced lesions (51-75% necrosis) with intermediate sporulation; and 4 = susceptible, large coalesced lesions (76-100% necrosis) with abundant sporulation.

2.4. Intact Plant Test

Nine fungal isolates which showed promising results in detached leaf tests were further evaluated in intact plant test (greenhouse). Three faba bean varieties, Obse (EH95073-1), Shalo (EH011-22-1) and Bale local were arranged in RCBD using three replications. Seeds were surface disinfected in 1% sodium hypochlorite for 10 min followed by washing in three changes of distilled sterile water. The spore suspension of both *B. fabae* and the fungal isolates were prepared in the same way as in section 3.6 above. Six weeks old faba bean plants grown in greenhouse using 23.5 cm diameter plastic pots (5 plants/pot) field with sand, manure and compost in 1:2:3 ratio were sprayed with 20 ml/plant/pot of each fungal isolates at a concentration of 2.5x10^5 spores/ml (Mohammed *et al.*, 1994). Inoculated pots were covered with moistened plastic bags for 24 hr to increase the relative humidity of the environment to favor the development of sprayed fungi. After 2 days of incubation, plants were inoculated by 20 ml/plant/pot of *B. fabae* spore suspension, containing 2.5x10^5spores/ml. Pots sprayed with 20 ml/plant/pot *B. fabae* spore suspension alone was used as control. Thereafter, each pot was

covered with moist plastic bags for 24 hr to maintain high relative humidity (RH) of the environment. The temperature and the RH of the greenhouse during the study period were in a range of 19-22^{0}C and 88-91%, respectively. The disease development was rated using 1-9 scale, where, 1= No disease symptoms or very small specks; 3= few small discrete lesions; 5= some coalesced lesions with some defoliation; 7= large coalesced sporulating lesions, 50% defoliation and some dead plant; and 9= Extensive lesions on leaves, stems and pods, severe defoliation, heavy sporulation, stem girdling, blackening and death of more than 80% of plants (Bernier *et al*., 1993). The disease data recorded based on scoring scale mentioned above was converted to percentage severity index (PSI) according to Wheeler (1969):

$$PSI = \frac{\text{Sum of Numerical Ratings X 100}}{\text{Number of Plants Scored X Maximum Score on Scale}}$$

2.5. Data Analysis

Data on incubation period (days), disease incidence (%) and severity (%) were analyzed using ANOVA SAS procedure (SAS, 2002), to know the effect of fungal isolates on the growth of the pathogen and development of chocolate spot. Least significant difference (LSD) value was used to separate the treatment means.

3. Results

3.1. Detached leaf test

Incubation period: Significant difference ($p<0.05$) were obtained among fungal isolates in affecting the incubation period. Out of the total isolates evaluated, five of them were better in increasing the incubation period. Isolates GO2-3 (*T. harzianum*), GB6-3 (*T. harzianum*), S16-2 (*T. polysporum*), A12-1 (*T. oblongisporum*) and 52-BT (*T. longibrachiatum*) prolonged the incubation period to 4.7, 4.7, 4.7, 4 and 4 days on local cultivars and 3.7, 4.3, 4.3, 4 and 4.3 days on Shalo variety, where as incubation period on control was 2 days on both varieties (Table 2). Alison and Mansfield (1984) in their experiment on onion bulb scales and detached leaves for their response to the development of *Botrytis* spp. showed that *B. squamosa* developed spreading lesion within three days of inoculation. *B. allii* and *B. cinerea* also developed spreading lesions within five days of inoculation and *B. fabae* was also produced limited lesions five days after inoculation. In the current study, the first visible symptom of *B. fabae* was observed within two to five days of inoculation depending on the type of fungal antagonists inoculated with the pathogen and within two days of inoculation on control. Bouhassan *et al.* (2004) in their experiment to screen faba bean genotypes to chocolate spot resistance, reported that small lesions characteristic of chocolate spot appeared six to eight hours after inoculation. As they enlarged with time, these small spots fused to form larger lesions, the severity of which varied according to lines. The mean of the scores indicated that the discrimination among the lines was significant three days after inoculation based on the lesion visual score and five days after inoculation based on the lesion diameter.

Table 2. Effect of antagonistic fungal isolates on incubation period (days) of faba bean chocolate spot on two varieties.

Isolate	Fungal species	Local	Shalo [1]
Go2-3	*Trichoderma harzianum*	4.67^{a}	3.67^{ab}
Gb6-3	*Trichoderma harzianum*	4.67^{a}	4.33^{a}
S16-2	*Trichoderma polysporum*	4.67^{a}	4.33^{a}
A12-1	*Trichoderma oblongisporum*	4.00^{ab}	4.00^{a}
52-BT	*Trichoderma longibrachiatum*	4.00^{ab}	4.33^{a}
S11	*Trichoderma hamatum*	3.33^{bc}	3.67^{ab}
117-2T	*Trichoderma longibrachiatum*	3.00^{cd}	2.33^{c}
Go3-2	*Trichoderma gamsi*	3.00^{cd}	3.67^{ab}
Gb25-3	*Trichoderma virens*	2.67^{cd}	2.33^{c}
Gb15-2	*Trichoderma spirale*	2.67^{cde}	2.33^{c}
2An	*Trichoderma koningii*	2.33^{de}	2.67^{bc}
Ga3-2	*Trichoderma longibrachiatum*	2.33^{de}	2.67^{bc}
2A-17	*Trichoderma koningii*	2.00^{e}	2.67^{bc}
Gb25-1	*Trichoderma citrinoviride*	2.00^{e}	2.67^{bc}
Ga3-3	*Trichoderma ovalisporum*	2.00^{e}	2.00^{c}
Control		2.00^{e}	2.00^{c}
LSD		0.90	1.13
CV (%)		17.52	21.81

Means in the same column with the same letter are not statistically different at $p\leq0.05$. [1] Mean incubation period for chocolate spot symptom development (mean of the three replications).

Diseases severity: Significant difference ($P<0.05$) occurred among fungal isolates on both Shalo and local faba bean cultivars in reducing the disease severity. On both varieties the lowest disease severity was recorded from the leaf treated by Go2-3 (*T. harzianum*) with disease score of 1, followed by S16-2 (*T. polysporum*) and Go3-2 (*T. gamsi*) with disease severity of 1 and 1.33 on local and Shalo varieties, respectively. While on control the disease severity was 2 and 2.33 on local and Shalo, respectively (Table 3). Omar *et al.* (1986) in their research to explore the effect of virus infection on developmen of *Botrytis* lesion by detached leaf technique, found that chocolate spot caused by *B. fabae* developed very well on both virus free and virus infected leaves. Lesion development was most rapid and extensive and sporulation most pronounced on the oldest leaf with lession development rate of up to 1.9 mm/day on plants artificially infected with spore concentration of 2 x 10^5 spores/ml. Like wise, they recorded a percent severity index (PSI) of up to 21.1% and 54.2% 3 and 9 days after inoculation, respectively. In the current study more or less comparable results were obtained. Samuel *et al.* (2009) reported that, out of the total 20 *Bacillus* isolates screened most of the isolates reduced development of *B. fabae* on detached faba bean leaves. Most of the isolates limited

chocolate spot expansion to 1-2.5 in 1-5 scoring scale while the development of the disease reached 4.5 on local cultivar based on the same scale.

Table 3. *In vivo* effect of fungal isolates on faba bean chocolate spot severity using detached leaf technique on two varieties.

Isolate	Fungal species	Local	Shalo [1]
S16-2	*Trichoderma polysporum*	1.00^{c}	1.33^{bc}
2An	*Trichoderma koningii*	1.00^{c}	1.67^{abc}
Go3-2	*Trichoderma gamsi*	1.00^{c}	1.33^{bc}
Go2-3	*Trichoderma harzianum*	1.00^{c}	1.00^{bc}
A12-1	*Trichoderma oblongisporum*	1.00^{c}	1.33^{bc}
Gb6-3	*Trichoderma harzianum*	1.33^{bc}	1.33^{bc}
2A-17	*Trichoderma koningii*	1.33^{bc}	1.00^{c}
S11	*Trichoderma hamatum*	1.33^{bc}	1.33^{bc}
52-BT	*Trichoderma longibrachiatum*	1.33^{bc}	1.33^{bc}
Ga3-2	*Trichoderma longibrachiatum*	1.67^{ab}	2.00^{ab}
Ga3-3	*Trichoderma ovalisporum*	1.67^{ab}	2.00^{ab}
Gb25-3	*Trichoderma virens*	1.67^{ab}	2.00^{ab}
117-2T	*Trichoderma longibrachiatum*	2.00^{a}	2.00^{ab}
Gb25-1	*Trichoderma citrinoviride*	2.00^{a}	2.00^{ab}
Gb15-2	*Trichoderma spirale*	2.00^{a}	2.00^{ab}
Control		2.00^{a}	2.33^{a}
LSD		0.64	0.68
CV (%)		26.19	25.12

Figures in the same column with the same letter are not statistically different at $p \leq 0.05$. [1] Mean disease severity based on 1-4 rating scale for detached leaf test (ICARDA, 1986) where 1 = highly resistant, no infection or very small flecks (1-25 % necrosis); 2 = resistant, necrotic flecks with few small lesions (26-50 % necrosis), and very poor sporulation; 3 = moderately resistant, medium coalesced lesions (51-75 % necrosis) with intermediate sporulation; and 4 = susceptible, Large coalesced lesions (76-100 % necrosis) with abundant sporulation.

3.2. Intact plant test

Incubation period: The bioagents varied in their potential to increase the incubation period of the chocolate spot in greenhouse. On local cultivar, significant difference ($p<0.05$) resulted among fungal isolates in their potential to increase the incubation period of *B. fabae*. Out of the total isolates evaluated, three of them were better in increasing the incubation period of chocolate spot. Isolates GB6-3 (*T. harzianum*), S16-2 (*T. polysporum*), and GO3-2 (*T. gamsi*) prolonged the incubation period to 3.33, 3 and 3 days, respectively (Table 4). The difference between bioagents in increasing the incubation period on Shalo and Obse varieties was not statistically significant. The incubation period on control pot was 2 (two) days after inoculation (Table 4).

Table 4. *In vivo* effect of antagonistic fungal isolates on incubation period (days) of faba bean chocolate spot in greenhouse condition on three varieties.

Isolate	Fungal species	Local	Shalo	Obse[1]
GB6-3	*Trichoderma harzianum*	3.33^{a}	3.33	3.33
GO3-2	*Trichoderma gamsi*	3.00^{b}	3.33	3.00
S16-2	*Trichoderma polysporum*	3.00^{b}	3.00	3.00
2A-17	*Trichoderma koningii*	2.00^{c}	3.33	3.67
52-BT	*Trichoderma longibrachiatum*	2.00^{c}	3.33	3.33
GO2-3	*Trichoderma harzianum*	2.00^{c}	3.00	3.33
A12-1	*Trichoderma oblongisporum*	2.00^{c}	3.00	3.00
S11	*Trichoderma hamatum*	2.00^{c}	3.00	3.00
2An	*Trichoderma koningii*	2.00^{c}	3.00	3.33
Control		2.00^{c}	3.00	3.00
CV (%)		7.82	11.65	12.76
LSD		0.31	NS	NS

Means in the same column designated with the same letter are not statistically different at $p \leq 0.05$. NS-not significant

[1] is Mean incubation period for chocolate spot on local, Shalo and Obse varieties from the three replications.

Rhaiem *et al.* (2002) reported chocolate spot disease symptoms on the leaves and stems of faba bean three days after inoculation, and 7 days after inoculation the susceptible check was already fully infected. On another experiment, El-Hendawy *et al.* (2010) reported the occurrence of chocolate spot lesion 24 hr after artificial inoculation *of B. fabae* in greenhouse. They found disease severity of more than 10, 25 and 65% at 24, 48 and 72 hr after inoculation, respectively.

Disease incidence: Significant difference ($P<0.05$) were observed among the antagonistic fungal isolates evaluated on all the three varieties in reducing chocolate spot incidence. Out of the tested isolates, three of them were effective in reducing the disease incidence, GB6-3 (*T. harzianum*), S16-2 (*T. polysporum*) GO3-2 (*T. gamsi*) showed better reduction 61.67, 59 and 66.67% of chocolate spot incidence on Obse as compared to control (100%) on all varieties, respectively. On plants treated with the rest isolates high percentage of disease incidence (79–100%) was recorded regardless of varieties. However 100% disease incidence was recorded from few pots of local and Shalo varieties treated with some bioagents and on control of all the three varieties (Table 5). High frequency of 100% chocolate spot incidence was recorded from Shalo and no 100 % disease incidence was recorded on Obse except from the control 12 days after inoculation. A number of antagonistic fungal isolates are observed to affect the incidence of plant diseases. Different

researchers reported the potential of the antagonists to reduce disease incidence. A report indicated that gladiolus corms dipped in the culture of *Trichoderma* and four species of *Gliocladium*, not only reduced the disease incidence but also supported better sprouting and yield of corms (Kohl *et al*., 1997; Tesfaye, 1998). The action of antagonistic fungal isolates is not limited to its effect after the symptom development, some antagonists showed their potential effect on spore germination. Elda and Kapt (1999) reported that isolates of *T. harzianum* produced protease in liquid culture medium and on the surface of Bean leaves and reduction in *B. cinerea* germination, and germ tube length.

Disease Severity: Significant difference was obtained among antagonistic fungal isolates (*P<0.05*) of different species for their potential to reduce Chocolate spot severity (Table 6). Out of the tested antagonistic fungal isolates, three isolates showed better performance in suppressing the disease expansion on the three varieties almost equally. Isolates S16-2 (*T. polysporum*), GO3-2 (*T. gamsi*) and GB6-3 (*T. harzianum*) are the best performing fungal isolates which were effective of all the tested isolates which showed percent lesion reduction of 39.47, 39.47 and 42.10%, on local, 57.89, 53.50 and 44.73% on Shalo, 47.47, 48.44 and 42.42% on Obse, respectively over the control from which the highest PSI was recorded. From control pots, PSI of 84.44%, 84.44% and 73.33% were recorded on local, Shalo and Obse varieties, respectively. PSI recorded from leaves treated by the three effective fungal bioagents were 48.89%, 46.67% and 42.22% from GB-6-3%, 51.11%, 39.26% and 37.78% from GO3-2 and 51.11%, 35.56% and 38.52% from S16-2 on local, Shalo and Obse varieties, respectively (Table 6). In the activity of biological control, micro-organisms action is not limited to direct influence on the target diseases, in addition to their direct effect they also enhance the resistance of the plants. A report by Benítez *et al.* (2004) indicates that *Trichoderma* strains are known to promote plant growth and plant defensive.

Table 5. *In vivo* effect of antagonistic fungal isolates on faba bean chocolate spot incidence (%) in greenhouse on three varieties.

Days after inoculation	Isolate	Fungal species	Shalo	Local	Obse [1]
4	GB6-3	*Trichoderma harzianum*	12.67^{d}	30.33^{b}	5.33^{e}
	S16-2	*Trichoderma polysporum*	20.67^{c}	67.33^{a}	8.00^{e}
	GO3-2	*Trichoderma gamsi*	24.33^{c}	56.00^{ab}	13.33^{d}
	GO2-3	*Trichoderma harzianum*	39.33^{b}	29.00^{b}	27.67^{bc}
	2A-17	*Trichoderma koningii*	40.33^{b}	46.00^{ab}	29.67^{b}
	52-BT	*Trichoderma longibrachiatum*	40.67^{b}	42.00^{ab}	24.33^{c}
	A12-1	*Trichoderma oblongisporum*	43.00^{b}	64.67^{a}	29.33^{b}
	S11	*Trichoderma hamatum*	43.33^{b}	62.33^{ab}	28.67^{bc}
	2An	*Trichoderma koningii*	44.00^{b}	53.17^{ab}	27.67^{bc}
	Control		53.67^{a}	64.33^{a}	45.67^{a}
	LSD		33.7	7.1	4.6
	CV(%)		11.45	38.38	1.20
8	GB6-3	*Trichoderma harzianum*	53.00^{d}	60.00^{ab}	32.00^{d}
	S16-2	*Trichoderma polysporum*	61.67^{c}	92.17^{a}	30.67^{d}
	GO3-2	*Trichoderma gamsi*	57.33^{cd}	83.33^{a}	34.67^{d}
	GO2-3	*Trichoderma harzianum*	79.33^{b}	46.61^{b}	58.67^{b}
	2A-17	*Trichoderma koningii*	81.00^{ab}	77.33^{ab}	60.00^{b}
	52-BT	*Trichoderma longibrachiatum*	78.97^{b}	67.67^{ab}	51.67^{c}
	A12-1	*Trichoderma oblongisporum*	83.33^{ab}	89.00^{a}	61.67^{b}
	S11	*Trichoderma hamatum*	81.00^{ab}	90.67^{a}	61.33^{b}
	2An	*Trichoderma koningii*	81.33^{ab}	81.15^{ab}	57.67^{bc}
	Control		86.56^{a}	91.00^{a}	82.00^{a}
	LSD		36	6.8	6.2
	CV(%)		5.40	27.17	6.83
12	GB6-3	*Trichoderma harzianum*	84.00^{c}	87.33^{ab}	61.67^{de}
	S16-2	*Trichoderma polysporum*	91.67^{b}	100.00^{a}	59.00^{e}
	GO3-2	*Trichoderma gamsi*	91.00^{b}	96.67^{ab}	66.67^{d}
	GO2-3	*Trichoderma harzianum*	98.00^{a}	79.67^{b}	82.00^{bc}
	2A-17	*Trichoderma koningii*	100.00^{a}	93.67^{ab}	81.33^{bc}
	52-BT	*Trichoderma longibrachiatum*	97.00^{a}	87.33^{ab}	75.67^{c}
	A12-1	*Trichoderma oblongisporum*	100.00^{a}	100.00^{a}	83.67^{b}
	S11	*Trichoderma hamatum*	100.00^{a}	100.00^{a}	80.67^{bc}
	2An	*Trichoderma koningii*	100.00^{a}	97.00^{ab}	80.67^{bc}
	Control		100.00^{a}	100.00^{a}	100.00^{a}
	LSD		17.9	3.3	7.4
	CV(%)		2.04	11.17	5.63

Means in the same column with the same letter are not statistically different at $p \leq 0.05$,

Mean diseases incidence of chocolate spot on the three varieties from the three replications

Table 6. *In vivo* effect antagonistic fungal isolates on faba bean chocolate spot percent severity index in greenhouse on three varieties.

Isolate	Fungal species	Local	Shalo	Obse[1]
GB6-3	*Trichoderma harzianum*	48.89^{c}	46.67^{d}	42.22^{c}
GO3-2	*Trichoderma gamsi*	51.11^{c}	39.26^{e}	37.78^{d}
S16-2	*Trichoderma polysporum*	51.11^{c}	35.56^{e}	38.52^{d}
52-BT	*Trichoderma longibrachiatum*	75.56^{b}	59.26^{c}	61.48^{b}
GO2-3	*Trichoderma harzianum*	78.52^{ab}	80.74^{ab}	61.48^{b}
A12-1	*Trichoderma oblongisporum*	82.22^{a}	80.00^{ab}	60.74^{b}
2An	*Trichoderma koningii*	82.22^{a}	83.70^{a}	61.48^{b}
S11	*Trichoderma hamatum*	82.96^{a}	78.52^{b}	60.74^{b}
2A-17	*Trichoderma koningii*	82.96^{a}	81.48^{ab}	61.48^{b}
Control		84.44^{a}	84.44^{a}	73.33^{a}
LSD		6.66	4.79	1.83
CV(%)		5.43	4.20	1.91

Means in the same column designated with the same letter are not statistically different at $p \leq 0.05$. [1] Mean PSI of chocolate spot on three faba bean varieties of three replications.

4. Discussion

In Ethiopia, this type of study is at its infant stage but, little research conducted has indicated high potential of local microbial agents (Samuel *et al.*, 2009). Fifteen fungal species from phylloplane of faba bean leaves were tested *in vivo*, against Chocolate spot in detached leaf test. Out of which nine isolates were promoted to intact plant test in greenhouse. Finally, three of them were found to be effective against chocolate spot. Samuel *et al.* (2009) tested a number of *Bacillus* and *Trichoderma* species and found the result complementing to ours. Similarly, Lo and Lin (2002) reported the potential of *Trichoderma* spp. to enhance plant growth in addition to its disease control potential. The isolates had different potential of controlling the disease on varieties having different level of resistance against the disease Samuel *et al.* (2009), the finding from this study have also confirmed that the resistance level of the varieties have direct influence on the efficacy of isolates. In Egypt, damping off disease incidence was highly reduced by application of *Trichoderma* species. Very low disease incidence of 9-19% and 2.5-7.5% was recorded with application of *Trichoderma* spp. compared to control where 48.5 and 55.8% was recorded at pre- and post-emergence stages, respectively (Abd-El-Khair *et al.*, 2010). Finding from other study revealed that seed inoculation and foliar spray of *Trichoderma* spp. significantly reduced incidence and severity of chocolate spot to the level it can be comparable with fungicides (Saber *et al.*, 2009). Similar result was found in our study, where different *Trichoderma* spp. was tested and three of them showed better performance in reducing chocolate spot incidence. *T. gamsi* gave the highest disease incidence reduction of 66.67%, followed by *T. harzianum* (61.67%) and *T. polysporum* (59%), where 100% incidence was recorded on control which is in agreement with the above finding. Biocontrol agents differ in their disease control potential when applied individually or in combination. Poornima, (2011) reported that *Trichoderma* spp. and *Pseudomonas* spp. showed 62.6% and 36.1% disease control, respectively when applied individually, but with application of *Trichoderma* spp. + *Pseudomonas* spp. the disease control was 50.6%. In the current study, three individually applied isolates; *T. harzianum*, *T. gamsi* and *T. polysporum* lowered disease severity to 42.22-51.11% as compared to the control (73.73-84.44%) on the three varieties. These isolates also affected the incubation period of chocolate spot. Haggag *et al.* (2006), reported that *Talaromyces flavus* and *Trichoderma harzianum* reduced brown spot disease severity on Faba bean. According to this study, lower disease severity of 1.2-6.4% and 2.4-12.5%, was recorded from plants treated with T. *flavus* and *T. harzianum*, respectively, while disease severity on control pot was 32.6%, 50 days after planting under artificial inoculation condition in greenhouse.

5. Conclusion

This study was undertaken to investigate the biocontrol potential on local micro-biota of fungi. A number of fungal isolates was tested against chocolate spot both on detached leaf and intact leaf tests. Almost all of the tested isolates showed biocontrol potential against chocolate spot with varying degrees. But, finally three fungal isolates (*Trichoderma* spp.) was found effective on detached leaf and intact plant tests. Even if found good result from this study, we recommend further study for the expansion and commercialization of these isolates.

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