African Crop Science Journal, Vol. 19, No. 3, pp. 197 - 209
ISSN 1021-9730/2011 $4.00

POTENTIAL ANTAGONISTIC FUNGAL SPECIES FROM ETHIOPIA FOR BIOLOGICAL CONTROL OF CHOCOLATE SPOT DISEASE OF FABA BEAN

S. SAHILE, P.K. SAKHUJA[1], C. FININSA[1] and S. AHMED[2]
Department of Plant Science, University of Gondar P. O. Box 196, Gondar, Ethiopia
[1]Department of Plant Science, Haramaya University P. O. Box 138, Dire Dawa, Ethiopia
[2]International Center of Agricultural Research in the Dry Areas, P. O. Box 5466, Aleppo, Syria
Corresponding author: hanasahile@yahoo.com

(Received 14 January, 2011; accepted 10 September, 2011)

ABSTRACT

Chocolate spot disease (*Botrytis fabae* Sard) is one of most yield limiting constraints of faba bean (*Vicia faba*). There is promise in using biological control agents to control chocolate spot diseases, nevertheless, this strategy has not been fully exploited. The objective of this study was to assess the prevalence of different antagonistic fungi on phyloplane of faba bean in Ethiopia and to evaluate their antagonistic potential against the pathogen. A total of 110 isolates of *Trichoderma* species were obtained from faba bean leaves from 12 districts, which were grouped into 18 distinct groups differing in colony and other characters. Similarly, 26 distinct isolates belonging to species of *Penicillium, Aspergillus, Fusarium* and *Phioalophora* were identified from leaves of faba bean. *In vitro* and *in vivo* studies revealed strong antagonistic potential of many isolates. Thirteen isolates of *Trichoderma* produced 4 mm or more inhibition zone and reduced growth of pathogen colony, when grown in dual culture with it. Antagonistic isolates caused lysis of pathogen mycelium more than 6 mm on agar plates. The antagonists significantly reduced pathogen growth in a range of 24.5 to 0.8 mm. The efficacy of the *Trichoderma* isolates ranged from 47.6 to 98% and that of the other fungal isolates ranged from 13.1 to 34.5%. On detached leaves, isolates 6-1T, 18-3T and 87T of *T. ovalisporum* and 52-BT, 108-1T and 108-4T of *T. longibrachiatum* were found to reduce development of chocolate spot on four genotypes of faba bean. The outcome indicates that biocontrol agents, particularly of species *Trichoderma* are prevalent on faba bean leaves and can be further explored and developed into effective mycofungicides for management of chocolate spot disease of faba bean.

Key Words: Aspergillus *Botrytis fabae*, *Trichoderma*, *Vicia fabae*

RÉSUMÉ

Chocolate spot disease (*Botrytis fabae* Sard) is one of most yield limiting constraints of faba bean (*Vicia faba*). There is promise in using biological control agents to control chocolate spot diseases, nevertheless, this strategy has not been fully exploited. The objective of this study was to assess the prevalence of different antagonistic fungi on phyloplane of faba bean in Ethiopia and to evaluate their antagonistic potential against the pathogen. A total of 110 isolates of *Trichoderma* species were obtained from faba bean leaves from 12 districts, which were grouped into 18 distinct groups differing in colony and other characters. Similarly, 26 distinct isolates belonging to species of *Penicillium, Aspergillus, Fusarium* and *Phioalophora* were identified from leaves of faba bean. *In vitro* and *in vivo* studies revealed strong antagonistic potential of many isolates. Thirteen isolates of *Trichoderma* produced 4 mm or more inhibition zone and reduced growth of pathogen colony, when grown in dual culture with it. Antagonistic isolates caused lysis of pathogen mycelium more than 6 mm on agar plates. The antagonists significantly reduced pathogen growth in a range of 24.5 to 0.8 mm. The efficacy of the *Trichoderma* isolates ranged from 47.6 to 98% and that of the other fungal isolates ranged from 13.1 to 34.5%. On detached leaves, isolates 6-1T, 18-3T and 87T of *T. ovalisporum* and 52-BT, 108-1T and 108-4T of *T. longibrachiatum* were found to reduce development of

chocolate spot on four genotypes of faba bean. The outcome indicates that biocontrol agents, particularly of species *Trichoderma* are prevalent on faba bean leaves and can be further explored and developed into effective mycofungicides for management of chocolate spot disease of faba bean.

Key Words: Aspergillus *Botrytis fabae*, *Trichoderma*, *Vicia fabae*

INTRODUCTION

Faba bean (*Vicia fabae* L.) is one of the most important food legumes due to its high nutritive value both in terms of energy and protein contents (24-30 %) and is an excellent nitrogen fixer (Sahile *et al*., 2008a). Ethiopia is the third largest producers of faba bean in the world, next to china and Egypt (Torres *et al*., 2006). Faba bean is grown on 370,000 hectares in Ethiopia with an annual production of about 450,000 tonnes (ICARDA 2006). Despite its wide cultivation, the average yield of faba bean is quite low in Ethiopia, because of many biotic and abiotic constraints (Sahile *et al*., 2008a).

Chocolate spot is the most important disease of faba bean worldwide and is capable of devastating unprotected crops up to 67% (Bouhassan *et al*., 2004). It appears as reddish brown spots on leaves and under favorable conditions on stems, flowers and pods. Subsequently, these spots grow larger and can even merge into black mass. The disease results in heavy premature defoliation and under warm moist conditions crop lodging may occur. Plant growth and most physiological activities are adversely affected leading to drastic reduction in yield (Khaled *et al*., 1995). This disease is caused by *Botrytis fabae* Sard. and *B. cinerea Pers.Fr*. (Harrison 1988). In Ethiopia chocolate spot is caused by *B. fabae* and occurs almost regularly in most faba bean growing areas (Dangachew, 1967). The disease can reduce faba bean yields up to 67% (Bouhassan *et al*., 2004).

Different management options have been developed to reduce the yield losses in faba bean due to chocolate spot worldwide. These include the use of chemical fungicides, resistant/ tolerant varieties, use of certain cultural practices such as crop residue management and altering planting date (Dereje, 1999; Bretag and Raynes, 2004; Hawthorne, 2004). There is weak genetic resistance in cultivars of faba beans to chocolate spot (Lawes *et al*., 1983) and the most common control strategy is fungicide sprays. However, the negative effects of fungicide use are already becoming apparent. For instance, development of resistance in *B. cinerea* and in *B. fabae* against fungicides has been reported (Parry, 1990). Management options recommended in Ethiopia for this disease are application chlorothalonil or mancozeb and late planting (Dereje, 1993; Sahile *et al*., 2008b) but have not been adopted by the farmers at large. Only one resistant variety, CS20DK was released in Ethiopia 20 years ago, but it did not become popular because of lower yield, and subsequently loss of resistance (Gemechu *et al*., 2006).

Biological control is another option, which has not been fully exploited. It is economical, self-perpetuating and usually free from residual effects and can be an important component of integrated disease management. Faba bean phyloplane harbours many microorganisms of different groups, besides the chocolate spot pathogen because of its high proteins content. Some of them might be antagonistic to *B. fabae.* Sherga (1997) found that out of 270 isolates of *Bacillus* tested, 14% had strong antagonistic effect against chocolate spot pathogen in *vitro*. However, fungal antagonists have not been explored for biological management of this serious disease of this important food legume.

The objective of this study was to identify the potential fungal antagonists to *B. fabae* from Ethiopia, which can be developed into commercial mycofungicides for the integrated management of chocolate spot disease of faba bean.

MATERIALS AND METHODS

Collection of leaf samples. Samples of healthy looking leaves were collected from faba bean plants having disease on other leaves, for exploring the resident antagonistic mycoflora of healthy faba bean leaves. Such samples were

collected from farmers' fields of twelve districts of Amhara Regional State in north western Ethiopia. This region is located between longitude 36 - 40° W to E, and latitudes 11 - 13° 45' south-west to north. Similarly leaves aggressively affected by chocolate spot were collected from faba bean plants from a farmer's field in Kutaber district for isolation of virulent pathogen. All the leaf samples were kept in folds of newspapers in a plant press for 48 hr and, thereafter, kept securely in labelled paper bags till isolation of the microorganisms.

Isolation of faba bean resident mycoflora and pathogen. The collected leaves of faba bean were surface sterilised in 1% sodium hypochlorite and subsequently washed three times in sterilised distilled water. From decontaminated leaves 5-mm^2 pieces were cut with sterile scalpel and placed on potato dextrose agar (PDA) in 9 cm diameter culture dishes. These were incubated for one week at 21° C±1 and the fungi emerging from leaf tissues were transferred to PDA and purified. Fungal isolates in pure cultures were coded and transferred to screw-capped culture bottles containing faba bean extract dextrose agar and potato dextrose agar (FBEA/PDA) and stored at 4 °C. The *B. fabae* was isolated from diseased leaves on PDA, purified, identified and stored at 4 °C as stock culture of pathogen.

Cultural characteristics of isolates. The isolates of all fungi were grown on PDA in culture dishes at 21° C ±1 for 96 hr. Characteristics like colony colour and diameter, morphological and sporulation of all the isolates were compared and recorded (Dhingra and Sinclair, 1986). Generic level identification of isolates was done and broadly classified into *Trichoderma* isolates and other fungal isolates. Different isolates within both groups showing similar colony characteristics were again grouped together and one representative isolate of each subgroup was taken for antagonistic potential studies. Cultural characteristics like growth rate, colony colour and diameter and medium reverse colour of all representative isolates of *Trichoderma* and other fungi were studied at 21°C on PDA. Isolates of *Trichoderma* that showed promising antagonistic activity were identified from CAB International Global Plant Clinic, London, UK.

Antagonistic activity in fungal isolates against B. fabae. The antagonistic activity of different fungal isolates from apparently healthy leaves was tested against *B. fabae* firstly *in vitro* and then *in vivo.*

The fungi isolated from the leaves were tested for antibiosis activity to *B. fabae* on PDA in 9 cm Petri dishes. Three petri-dishes were inoculated with 2-mm mycelial disc from the edges of an actively growing colony of *B. fabae* on one side and with a similar sized disc of fungal isolate on the other side and incubated at 21° C ±1. After 72 hr of growth the inhibition zones at the junction of colonies of fungal isolates and *B. fabae* were measured using verner calipper.

In order to test for *lysis of B. fabae colony* 4 ml of *B. fabae* mycelial disc was placed on PDA (15 ml) in 9 cm petri-dish and incubated at 21 °C ± 1. After three days of mycelial growth 2-mm agar disc of the potential fungal isolates of *Trichoderma,* and other fungal isolates from actively growing colonies were placed on the colony of *B. fabae* and incubated at 21°C ± 1. Lysis of *B. fabae* colony was examined periodically under stereomicroscope (50x) and the width of lysed mycelia around the colony of the lytic fungal isolate was measured. The experiments were replicated three times in completely randomized design. Culture plates with *B. fabae* alone were used as the control.

Six *Trichoderma* isolates showing fast growth, significant inhibition zone and lytic activity were identified at CAB International Global Plant Clinic and used in the *in vivo* test of the antagonistic potential. Detached-leaves of four faba bean varieties namely CS20DK (tolerant), EH91011-6-2 (moderately resistant) and EH0013-18 (susceptible check) and one local check were used *in vivo* testing using the aggressive *B. fabae* culture according to the Paul *et al.* (1995) procedure. Leaves without antagonist evidence were used as the control.

Leaves of faba bean of the same age group were sterilised with 70% ethanol and placed in sterile 15 mm petri-dishes having sterilised filter paper moistened with sterile water. A

spore suspension of *B. fabae* (2.5 x 10^5 spores ml^{-1}) was prepared according to Mohammed *et al*. (1994). One drop (1 ml) of the spore suspension was placed near the midrib. The covered petri-dishes serving as moist chamber were incubated at 20 °C. After 24 hours, a drop of potential antagonist having 2.0 x 10^5 cfu ml^{-1} was added to the pathogen at the midrib and incubated at 20 °C again. The experiment was arranged in a complete randomised design.Disease reaction was recorded at 48, 72, 96 and120 hr of inoculation of antagonist using a 1-5 scale for detached leaf test (ICARDA, 1986).

Statistical analysis. Analysis of variance was carried out using SPSS V.12. Measurement data from *in vivo* test using detached leaves severity scales were subjected to SAS (Ver. 8). Mean comparisons were made using the Least Significant Difference test at P<0.05 test.

RESULTS

Isolation of resident mycoflora. From 1044 leaves collected from 12 districts, *Trichoderma* was isolated from 110 samples, showing 10.5% frequency of occurrence (Table 1). *Trichoderma* species was found to occur on faba bean leaves in all the districts; however, distribution of these isolates was found to vary among the districts. Highest number (13) of isolates was obtained from Kutaber district, followed by Hulet Eju Ensae. The elevation of the sampled areas varied between 1900 and 3319 meters above sea level.

Fungi other than *Trichoderma* species also appeared in isolations made from the 1044 leaves. Predominant fungi species observed on faba bean leaves were *Penicillium, Aspergillus* and *Fusarium.* Species of *Penicillium* were highly frequent and occurred on leaf samples from several areas. *Aspergillus niger* was isolated from 7 districts namely Debark, Desei Zuria, Kutaber, Farta, Wogera, Yilmana Densa and Hulet Eju Ensae while; *A. flavus* occurred in Kutaber, Lay Gaynt and Gonder Zuria districts. Similarly, *Fusarium* sp. occurred in Ambasel Tehuledrae, Gonder Zuria and Kutaber districts. *Phailophora* sp. was found to occur on faba bean leaves in Kutaber districts only (Table 1).

Cultural characteristics of isolates of *Trichoderma* species and other fungi. Cultivation of fungal isolates on PDA under similar conditions showed that some isolates of the same genus resembled each other in colony characteristics, mycelium and sporulation characters while differed from other such groups of the same genus (Table 2). Eighteen distinct groups of *Trichoderma* were found to occur within its total 110 isolates from faba bean leaves. Further studies on PDA with one representative isolate of each group showed that colony diameters of 18 *Trichoderma* isolates varied from 39.4 to 61.55 mm after 96 hr of growth. Isolates 18-2T, 18-3T, 51b-T, 52-2T, 87T, 108-1T, 108-3T, 108-4T, 114-3T, 117-2T, 118T, 120-2T and 140-2T had fairly high growth rates. Isolates 51-bT, 108-3T, 108-4T, 117-3T and 118T grew as suppressed colonies; while all others had raised aerial growth. Isolates 6-1T, 14-bT, 63T, 87T and 118T had white to green colour; while 51bT and 52-2T had grey to yellow colour (Table 2). All other isolates of *Trichoderma* were green in colour. There were distinct differences in media reverse colour of isolates, which varied from white, yellow, yellowish green to green. Species of *Penicillium* exhibited relatively slow growth rates with colony diameter ranging from 27.1- 31.2 mm in 96 hr of growth on PDA in comparison to *A. niger* and *A. flavus* with colony diameter of 32.2 - 48.4 and 33.2 – 37.2 mm, respectively. *Phailophora* sp. attained 41.3 mm colony diameter in the same period (Table 3).

Antibiosis activity in fungal isolates. Dual culture studies on PDA for evaluation of antibiosis activity of *Trichoderma* species and other fungi revealed that all inhibited growth of *B. fabae* by degrees and exhibited inhibition zone at the junction with the pathogen. Out of 18 *Trichoderma* isolates tested, 13 isolates viz., 6-1T, 14bT, 18-3T, 52-2T, 87T, 108-1T, 108-3T, 108-4T, 117-2T, 118T, 120-2T, 122-1T and 140-2T produced 4 mm or higher inhibition zone (Table 4). Isolates of *Trichoderma* species reduced the growth of *B. fabae* colony by

TABLE 1. Natural occurrence of *Trichoderma* species on faba bean leaves in the districts of northwestern Ethiopia

District	Altitude range (m.a.s.l)	No. of *Trichoderma* isolates obtained	Potential antagonistic *Trichoderma* isolates	Potential antagonistic other fungal isolates	% potential *Trichoderma* isolates within each districts
Yilmana Densa	1980-2405	9	6-1T	10-p2(P. sp), 2(A. n)	5.6
Hulet Eju Ensae	2275-2670	11	14bT, 18-2T, 18-3T,	14(P. sp), 18-1(A. n)	16.7
Gonder Zuria	1969-2463	8	-	29(P. sp), 25(A. f), 24(F. sp)	0
Wogera	2650-2943	9	-	30-1(A. n)	0
Debark	2740-3053	7	-	49(A. n)	0
Farta	1975-3000	11	51-bT, 52B-2T	56(A. n)	11.2
Lay Gaint	2794-3184	10	63T,	62(P. sp), 68(A. fl)	5.6
Meket	2779-3319	9	-	-	0
Gubalafto Woldia	1900-3033	8	87	81(P. sp)	5.6
Ambasel Tehuledrae	1908-2196	8	108-1T, 108-3T, 108-4T,	108-2(P. sp), 104(F. sp)	16.7
Kutaber	2144-3250	13	114-3T, 117-2, 118T, 120-2T, 122-1T	119-2(P. sp), 117B(P. sp), 119-B(P. sp), 122-2(A. n), 112(A. n), 126(A. fl), 130-2(F. sp), 130-1(F. Sp), 120-2y1(Ph. sp)	33.3
Desei Zuria	2055-3138	7	140-2	134-2(P. sp), 140-3(A. n)	5.6

[1]. -, No isolate obtained

[2]. A. fl = *Aspergillus flavus* ; A. n = *Aspergillus niger* ; F. sp = *Fusarium* sp. ; P. sp = *Penicillium* sp. ; Ph. sp = *Phailophora* sp.

TABLE 2. Colony and growth characteristics of different *Trichoderma* species isolates from northwestern Ethiopia

District	Isolate code	Altitude (m.a.s.l)	Colony diameter (mm)			Colony		Media reverse colour
			48 hr	72 hr	96 hr	Type	Colour	
Yilmana Densa	6-1T	2702	15.33	23.00	39.45	A	W/g	W
Hulet Ejue Ensae	14bT	2424	17.78	28.33	44.60	A	W/g	G
Hulet Ejue Ensae	18-2T	2544	21.11	31.67	47.30	A	G	G
Hulet Ejue Ensae	18-3T	2544	20.00	30.00	46.20	A	G	Y
Farta	51-bT	2727	25.56	38.33	58.40	S	G/y	W
Farta	52-2T	2702	23.78	35.67	51.67	A	G/y	W
Lay Gaynt	63T	2145	18.89	28.33	44.33	A	W/g	W
Gubalafto Woldia	87T	2810	22.22	33.33	49.45	A	W/g	W
Ambasel Tehlederae	108-1T	2003	16.00	24.00	50.00	A	G	G
Ambasel Tehlederae	108-3T	2003	23.33	35.00	51.23	S	G	W
Ambasel Tehlederae	108-4T	2003	30.00	45.00	61.55	S	G/y	Y
Kutaber	114-3T	2264	25.56	38.33	58.30	A	G	G
Kutaber	117-2T	2407	23.33	35.00	51.33	A	G	G/y
Kutaber	117-3T	2407	18.89	28.33	44.00	S	G	G
Kutaber	118T	2216	25.56	38.33	58.00	S	W/g	W
Kutaber	120-2T	2505	25.56	38.33	58.33	A	G	G
Kutaber	122-1T	2567	16.67	25.00	41.45	A	G	W
Dessei Zuria	140-2T	2784	25.56	38.33	58.25	A	G	W

A = aerial; S = suppressed; W/g = white to green; G/y = Green to yellow; G = green; W = white; Y = yellow; G/y = gray to yellow

varying degrees. All *Penicillium* isolates produced 4-5 mm inhibition zones. *Aspergillus niger* isolates produced 5-6 mm inhibition zones in comparison to 4-5 mm by *A. flavus* and *Fusarium* species. Single isolate of *Phailophora* sp. produced 6 mm inhibition zone against *B. fabae* (Table 5).

Lytic potential in fungal isolates. All the *Trichoderma* isolates when placed on mycelium of *B. fabae* caused lysis to varying extent. Isolates 6-1T, 52-2T, 87T, 108-1T, 108-3T and 120-2T caused 8-10 mm of lysis around them. *Trichoderma* isolates 6-1, 18-2, 18-3, 51-b, 52-2, 63, 87, 108-1, 108-3, 108-4, 114-3, 117-2, 117-3, 118, 120-2,122-1 and 140-2 overgrew upon the pathogen mycelium (Table 4).

Species of *Penicillium* placed on *B. fabae* also caused lysis of its mycelium, which ranged from 6.7 – 11.2 mm. Isolates 119-2, 62, 14, 29, 134-2, 81, 10-p-2 and 119-B caused higher lysis ranging from 9-11.2 mm. Species of *Aspergillus* produced lysis ranging from 6-12.5 mm and isolates 49, 140-3, 122-2, 56, 112, 30-1, 18-1 and 2 of *A. niger* and 68 of *A. flavus* showed higher lytic potential. Except for isolate 130-2, *Fusarium* species produced lesser lysis of mycelium of pathogen. *Phailophora* sp. proved effective in lysing the pathogen by 9.5 mm (Table 5).

Effect of Trichoderma species on chocolate spot *in vivo*. Effect of 3 isolates 6-1T, 18-3T and 87T belonging to *T. ovalisporum* and 3 isolates 52-BT, 108-1T and 108-4T of *T. longibrachiatum* on development of chocolate spot on four genotypes of faba bean was studied *in vivo* using detached leaf technique (Table 6). All isolates were found to reduce chocolate spot severity, when inoculated with the pathogen. However, their effect varied with isolate and genotype. Isolates 108-1T, 108-4T and 52-BT, of *T. longibrachiatum* were the most effective in reducing the mean disease severity on all the four genotypes and provided 43-47% mean disease control. Out of three isolates of *T. ovalisporum,* 6-1T proved better than other isolates of this species and reduced the disease by 40%, but was less effective than those of *T. longibrachiatum*. Isolates 18-3T and 87T of *T. ovalisporum* were very effective on susceptible genotypes EH91011-6-2, EH0013-18 and local check, but failed to reduce severity on

TABLE 3. Colony and cultural characteristics of different fungal isolate

District	Fungi	Isolate code	Altitude (m. a. s. L.)	Colony growth diameter (mm)		Colony	Character at 96 hr
				72 hr	96 hr	Colour	Media reverse colour
Kutaber	*Penicillium* sp.	119-2	2430	15.33	31. 21	B/g	Y
Lay Gaynt	*Penicillium* sp.	62	2100	12.00	28.17	B/g	B/g
Hulet Eju Ensae	*Penicillium* sp.	14	2424	14.00	30.19	B/g	G
AmbaselTehulederae	*Penicillium* sp.	108-2	2003	11.33	27.16	B/g	W
Gonder Zuria	*Penicillium* sp.	29	2075	14.67	30.20	B/g	W
Desei Zuria	*Penicillium* sp.	134-2	2679	12.67	28.17	Blue	Y
Gubalafto Woldia	*Penicillium* sp.	81	3020	11.00	27.15	Blue	B
Kutaber	*Penicillium* sp.	117B	2407	15.33	31.21	B/g	W
Yilmana Densa	*Penicillium* sp.	10p-2	2360	14.00	30.19	B/g	W
Kutaber	*Penicillium* sp.	119-B	2430	15.00	31.21	B/g	W
Debark	*Aspergillus niger*	49	3122	25.67	41.36	Br	W
Dessei Zuria	*Aspergillus niger*	140-3	2784	23.00	39.32	Br	W
Kutaber	*Aspergillus niger*	122-2	2567	24.00	40.33	Br	W
Farta	*Aspergillus niger*	56	2851	23.00	39.32	Br	W
Kutaber	*Aspergillus niger*	112	2131	25.00	41.35	Br	W
Wogera	*Aspergillus niger*	30-1	2241	16.08	32.22	Br	W
Yilmana Densa	*Aspergillus niger*	2	2443	24.00	40.33	Br	W
Hulet EjuEnsae	*Aspergillus niger*	18-1	2544	32.67	48.45	Br	W
Lay Gaynt	*Aspergillus flavus*	68	2731	21.00	37.29	Y	W
Kutaber	*Aspergillus flavus*	126	2336	17.67	33.25	Y	W
Gonder Zuria	*Aspergillus flavus*	25	2268	21.00	37.29	Y	W
Gonder Zuria	*Fusarium* sp.	24	2647	23.00	39.32	W/r	W/r
Ambasel Tehulderae	*Fusarium* sp.	104	2679	22.00	38.31	W/r	W/r
Kutaber	*Fusarium* sp.	130-2	2838	24.33	4034	W/r	W
Kutaber	*Fusarium* sp.	130-1	2838	24.00	40.33	P	P
Kutaber	*Phailophora* sp.	120-2y1	2505	25.56	41.36	O	W

B/g = blue to green; G = green; B = blue; Br = brown; Y = yellow; W = white; W/r = white to red; P = pink; O = orange

moderately resistant genotype CS20DK. Highly significant disease control (75%) was provided by *T. ovalisporum* (isolates 108-1T and 108-4T) on genotype EH0013-18. Highest disease pressure developed on local check and all the 6 isolates could reduce disease severity. In general, all the isolates effectively reduced the disease on the two susceptible genotypes, but were less effective on moderately resistant genotypes EH91011-6-2 and CS20DK.

DISCUSSION

Trichoderma species predominantly occurred on faba bean leaves in Ethiopia. Species of *Trichoderma* were encountered from 10.5% leaf samples from 12 districts, indicating their natural adaptability to faba bean leaves. However, a variation in isolates of this genus was also found to be widespread. Within the total 110 isolates of *Trichoderma* species obtained from leaves, 18 distinct isolates showing clear differences in colonies and morphology were established. Besides, *Trichoderma*, 26 distinct isolates of *Penicillium, Aspergillus*, *Fusarium* and *Phailophora* were also found to be prevalent on faba bean leaves. A total of 11 isolates belonging to *A. niger* and *A. flavus*, 10 isolates of *Penicillium,* 4 isolates of *Fusarium* and one isolate of *Phialophora* were obtained from faba bean leaves. There was no co-relation of species of fungi with altitude as all of them

TABLE 4. Effect of *Trichoderma* species on growth *Botrytis faba*

Isolate code	Inhibition zone(mm)	Lyses (mm)	Efficacy (%)	*Trichoderma* species colony growth at 72 hr (in mm)	Mean growth of *B. fabae* at 72 hr (in mm)
6-1T	5	10.25	50.2	23.00 b	22.8ab
14bT	4	7.00	61.8	28.33 ab	17.47b
18-2T	2	7.00	69.2	31.67 ab	14.13b
18-3T	4	7.00	65.5	30.00 ab	15.8b
51-bT	3	7.00	83.7	38.33 ab	7.47c
52-2T	5	10.25	77.9	35.67 ab	10.13bc
63T	2	7.00	61.9	28.33 ab	17.47b
87T	4	10.25	72.8	33.33 ab	12.47bc
108-1T	4	10.25	47.6	24.00 b	21.8ab
108-3T	5	8.00	76.4	35.00 ab	10.8bc
108-4T	4	7.00	98.3	45.00 a	0.8d
114-3T	3	7.00	83.7	38.33 ab	24.47a
117-2T	4	7.00	76.4	35.00 ab	10.8bc
117-3T	2	7.00	61.9	28.33 ab	17.47c
118T	5	6.25	83.7	38.33 ab	7.47c
120-2T	4	10.25	83.7	38.33 ab	7.47c
122-1T	4	7.00	54.6	25.00 b	20.8ab
140-2T	4	7.00	83.7	38.33 ab	7.47c
Mean	3.78	7.14	72.1	33.02	12.78
Control			0		45.80
LSD (5%)	NS	NS		18.59	6.59

NS = non significant. Over growth has bean occurred during 96 hr growth

occurred at all altitudes from where samples were collected. This clearly indicated their wide adaptability to different environments. Goldfarb *et al.* (1989) reported the varying nature of the growth rate of *Trichoderma* with species and temprature. In their study, Goldfarb and his co-workers found the growth rate of *Trichoderma* spp. to vary from 12.7-23.4 mm day^{-1} depending on the species at 20 ^{0}C of temprature. In another experiment, Saber *et al.,* (2009), found daily growth rate of different fungal antagonist in the range of 15-35 mm/day. In their experiment conducted in Egypt, the author reported that all of the fungal antagonists tested showed reasonably higher growth rate than the pathogen *B. fabae*.

The dual culturing of pathogen with 18 isolates of *Trichoderma* and 26 of other fungi revealed clearly potential of control in some of the isolates. Thirteen isolates of *Trichoderma* produced 4 mm or higher inhibition zone on agar medium. These might be producing antibiotics or extracellular enzymes, which inhibited growth of the pathogen. Similar strong antagonistic behaviour of some isolates was observed in lysing the pathogen mycelium in agar plates. Some isolates proved effective in antibiosis as well as in lysis, while some others were better in antibiosis and some better in lysing. This reflects the differences in the spectrum and degree of their antibiotic and enzyme production. The genus *Trichoderma* comprises a great number of fungal strains that act as biological control agents, the antagonistic properties of which are based on the activation of multiple mechanisms. Elad and Stewart (2004) have also reported that *Trichoderma*, *Gliocladium* and *Ulocladium* have greatest potential for *Botrytis* diseases and commercial success has been achieved in glasshouse and post-harvest environments for disease control. In the activity of biological control, micro-organisms action is not limited to direct influence on the target diseases, in addition to

TABLE 5. *In vitro* effect of fungal isolates on the growth of *Botrytis fabae*

Fungi	Isolate code	Inhibition zone (mm)	Lyses (mm)	Efficacy (%)	72 hr growth diameter (mm) of fungal	Botrytis mean radial growth (mm) at 72 hr
Penicillium sp.	119-2	4b	11.00 b	30.5	15.33 b	33.47cd
Penicillium sp.	62	5ab	10.75 ab	33.8	12.00 b	26.2dc
Penicillium sp.	14	5ab	9.25 ab	31.8	14.00 b	30.57cd
Penicillium sp.	108-2	4b	6.75 b	34.5	11.33 b	24.74d
Penicillium sp.	29	5ab	11.00 ab	31.1	14.67 b	32.03cd
Penicillium sp.	134-2	4b	10.75 b	33.1	12.67 b	27.66dc
Penicillium sp.	81	5ab	11.25 ab	34.8	11.00 b	24.02d
Penicillium sp.	117B	4b	6.75 b	30.5	15.33 b	33.47cd
Penicillium sp.	10p-2	5ab	10.50 ab	31.8	14.00 b	30.57cd
Penicillium sp.	119-B	4b	11.25 b	30.8	15.00 b	32.75cd
Aspergillus niger	49	4b	11.00 b	20.1	25.67 ab	56.05a
Aspergillus niger	140-3	4b	12.00 b	22.8	23.00 ab	50.22bc
Aspergillus niger	122-2	5ab	10.50 ab	21.8	24.00 ab	52.41bc
Aspergillus niger	56	5ab	11.00 ab	22.8	23.00 ab	50.22bc
Aspergillus niger	112	6a	10.00 a	20.8	25.00 ab	54.59b
Aspergillus niger	30-1	6a	11.00 a	29.7	16.08 b	35.11cd
Aspergillus niger	2	5ab	11.25 ab	21.8	24.00 ab	52.41bc
Aspergillus niger	18-1	6a	12.50 a	13.1	32.67 a	71.33a
Aspergillus flavus	68	4b	6.00 b	24.8	21.00 ab	45.85cb
Aspergillus flavus	126	4b	7.00 b	28.13	17.67 ab	38.58c
Aspergillus flavus	25	5ab	9.25 ab	24.8	21.00 ab	45.85cb
Fusarium sp.	24	5ab	5.00 ab	22.8	23.00 ab	50.22bc
Fusarium sp.	104	5ab	4.50 ab	23.8	22.00 ab	48.03bc
Fusarium sp.	130-2	4b	11.25 b	21.47	24.33 ab	53.67bc
Fusarium sp	130-1	4b	6.75 b	21.8	24.00 ab	52.41bc
Phailophora sp.	120-2y1	6a	9.50 ab	20.24	25.56 ab	55.81b
Control						45.80
LSD (5%)		2.39	1.939		16.35	16.35

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 mechanisms and antibiosis against, the pathogen or direct mechanisms such as mycoparasitism. *T. harzianum* and *T. viridi* have been reported as biocontrol agents for chocolate spot of grape, apple and strawberry caused by *B. cinerea* (Sutton *et al.,* 1997; Hjeljord *et al.,* 2001).

The study showed that there were promising antagonistic species of fungi prevalent on faba bean leaves, which can be exploited for the control of chocolate spot. Although, different genera of fungi were found to have antagonistic ability against the pathogen *in vitro*, from the point of view of wider antagonistic spectrum, *Trichoderma* species were considered more feasible for further exploration. Therefore, isolates 6-1T, 18-3T and 87T of *T. ovalisporum* and 52-BT, 108-1T and 108-4T of *T. longibrachiatum* were further tested *in vivo* by detached leaf technique. All of them were found to reduce the development of chocolate spot on two susceptible and two moderately resistant genotypes of faba bean, though degree of reduction varied and also depended on genotype of faba bean. Isolates provided a higher extent of control in susceptible genotypes. These antagonistic isolates were fast growing and reduced the colony growth of *B. fabae*, when grown in dual culture.

TABLE 6. Effect of *Trichoderma* species on development of chocolate spot on genotype of faba bean

Trichoderma species	Isolate no.	Faba bean genotype											
		EH91011-6-2			EH0013-18			CS20DK			Local check		
		48 hr	72 hr	96 hr	120 hr	48 hr	72 hr	96 hr	120 hr	48 hr	72 hr	96 hr	120 hr	48 hr	72 hr	96 hr	120 hr
T. ovalisporum	6-1T	1	1	1	1.5b	1.5cb	2bac	2bac	2.5bac	1.5	1.5ba	1.5	1.5	1c	1cb	2.5b	2.5b
T. ovalisporum	18-3T	1	1	1	1b	2b	2.5ba	2.5ba	3ba	2	2ba	2	2	1c	1c	2b	2b
T. ovalisporum	87T	1	1	1	1.5b	1c	1.5bc	1.5bc	2bc	2	2ba	2.5	2.5	1c	1c	2.5b	2.5b
T. longibranchiatum	52BT	1	2	2	2ba	1c	1c	1c	1c	1.5	1.5ba	1.5	1.5	1.5cb	1.5cb	3b	3b
T. longibranchiatum	108-1T	1	1	1	1.5b	1c	1c	1c	1c	1.5	1.5ba	1.5	1.5	2b	2b	3b	3b
T. longibranchiatum	1108-4T	1	1	1	1.5b	1c	1.5bc	1.5bc	1.5bc	1	1b	1.5	1.5	2b	2b	2.5b	2.5b
	Control	2	2	2	3a	3a	3a	3a	4a	2	2ba	2	2	3a	4a	5a	5a
	LSD (5%)	NS	NS	NS	1.5	1.00	1.19	1.19	1.73	NS	1.84	NS	NS	0.65	0.99	1.94	1.94

[1] Disease rating was based on 1-5 scale for detached leaf test where, 1 = 1-25%, 2 = 26-50%, 3 = 51-70%, 4 = 71-90% and 5 = 91-100% area affected (ICARDA, 1986) [2] NS = non significant

This study has revealed isolates of *T. ovalisporum* and *T. longibrachiatum* as effective antagonists of *B. fabae* for the first time. *Trichoderma ovalisporum* is an endophytic type of fungus and was first identified as a new and novel biocontrol agent from Amazon basin of South America for frosty pod rot (*Moniliopthora rori*) and wittches' broom (*Crinipellis* spp.) of cocoa (Holmes *et al*., 2004; Holmes *et al*., 2006).

Trichoderma strains are known to control pathogens either indirectly by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, or directly by mechanisms such as mycoparasitism (Benítez *et al*., 2004). *Trichoderma* species *s*uch as *T. harzianum, T. viridi* and *T. polysporum* are well known antagonists. Ten commercial products of these three species were developed for controlling diseases on different crops (Frevel *et al.,* 1998). *T. harzianum* and *T. viridi* were reported as biocontrol agent for chocolate spot of grape, apple and strawberry caused by *B. cinerea* (Sutton *et al*., 1997; Hjeljord *et al*., 2001).

Other fungi prevalent on faba bean leaves also exhibited antagonistic activities against *B. fabae in vitro*. Isolates 62, 29, 10-p2 of *Penicillium*; 140-3, 122-2, 56, 18-1 and 112 of *A. niger*, 25 of *A. flavus* and 24 of *Fusarium* and 120-2yl of *Phailophora* caused wide inhibition zone and lysis of mycelium. The antagonists evaluated in this study showed significant differences in reducing pathogen growth and their effects ranged from 24.47 to 0.8 mm (Table 5). Earlier also *Penicillium brevicompactum* and *Cladosporium cladosporioides* isolated from faba bean leaves were found to have significant antagonistic activity against *B. fabae in vitro* and *in vivo* (Jackson *et al*., 1997). Commercial products like Biofox C and Fusaclean having non-pathogenic strains of *Fusarium oxysporum* have been developed for controlling soil borne diseases (Frevel *et al.,* 1998). De Cal *et al.* (2008) reported biological control of powdery mildew on strawberry leaves by *Penicillium oxalicum* applications, it was achieved on different cultivars and lines in growth chambers

and in open-field nurseries. Species of *Penicillium*, *Aspargillus* and *Fusarium* have been reported by Leibinger *et al.* (1997) as an antagonists against *Botrytis cinerea*. Mass production technology by solid state fermentation for conidi of *Penicillium frequentans*, a biocontrol agent of the fungal pathogen *Monilinia laxa* has been developed by using specially designed plastic bags (VALMIC ®) containing peat and vermiculite (De Cal *et al*., 2002).

ACKNOWLEDGMENT

This research was financed by IFAD and Haramaya University SIDA-SAREC support, Ethiopia. The authors thank Haymanot Bezuneh, Haramaya University, Yenework G/medhin Adet Agricultural Research Center, for their assistance in data collection and laboratory works.

REFERENCES

Bennett, A. W. and Lane, S. D. 1992. The potential role of *Trichoderma viride* in the in the integrated control of *Botrytis fabae*. *The Mycologist* 6:199-201.

Bretag, T. W. and Raynes, M. 2004. Importance of seed coloration in faba bean (*Vicia fabae*) grown in southern Australia. Proceedings of the Australian Conference, Australian Society of Agronomy.

Bouhassan, A., Sadiki, M., and Tivoli, B. 2004. Evaluation of a collection of faba bean (*Vicia fabae* L.) genotypes originating from the Maghreb for resistance to chocolate spot (*Botrytis fabae*) by assessment in the field and laboratory. *Euphytica* 135:55-62.

Calvet C., Pera J., and Barea, J. M. 1990. Interactions of Trichoderma spp. With two wilt pathogenic fungi . *Agricultural Ecology and Environment* 29:59-65.

Campbell, R.19989. Biological Control of Microbial Plant Pathogens. Cambridge University Press, Cambridge.

Dangachew Yirgou. 1967. Plant diseases of economic importance in Ethiopia. HSIU, College of Agriculture. *Experment Station Bulletin* 30. Debre zeit. pp. 30.

De Cal, A. , Larena, I., Guijarro, B and Melgarejo, P. 2002. Mass Production of Conidia of *Penicillium frequentans* , a Biocontrol Agent Against Brown Rot of Stone Fruits. *Biocontrol Science and Technology* 12 (6):715-725.

De Cal, A., Redondo, C. , Sztejnberg, A. and Melgarejo, P. 2008. Biocontrol of powdery mildew by *Penicillium oxalicum* in open-field nurseries of strawberries. *Biocontrol* 47(1):103-107.

De, R. K., Chaudhry, K. G. and Naimuddin, J. 1996. Comparative efficiency of biocontrol agents and fungicides for the controlling chickpea wilt caused by *Fusarium oxysporum. Indian Journal of Agricultural Science* 66:370-73.

Demoze, B. T. and Korusten, L. 2006. *Bacilus subtilis* attachment, colonization, and survival on avocado flowers and its mode of action on stem-end rot pathogens. *Biological Control* 376:68-74.

Dereje Gorfu. 1993. Studies on the Epidemology of Chocolate Spot *(Botrytis fabae* Sard*)* of faba beans *(Vicia faba* L.*)*. Masters Thesis, Alamaya University, Alamaya, Ethiopia.

Dereje Gorfu. 1999. Survival of *Botrytis fabae* Sard. Between seasons on crop debris in field soils at Holetta, Ethiopia. *Phytopathologya Mediterranean* 38:68-75.

Dhingra, O. D. and Sinclair, J. B. 1986. Basic Plant Pathology Methods. CRc Press, Inc. Boca Raton, Florida. pp. 355.

Dubos, B. 1984. Biocontrol of *Botrytis cinarea* on grapevines by antagonistic strain of *Trichoderma harzianum*. pp. 370-373. In: Current Perspectives in Microbial Ecology. American Society for Microbiology, Washington, D. C.

Elda, Y. and Kapat, A. 1999. Role of *Trichoderma harzianum* protease in the biocontrol of *Botrytis cinerea. European Journal of Plant Pathology* 105:177-189

Elda, Y. and Kirshner, B. 1993. Survival in the phylloplane of introduced biocontrol agent (*Trichoderma harzianum*) and populations of the plant pathogen *Botrytis cinerea* as modified by abiotic conditions. *Phytoparasitica* 21:303-313.

Elad, Y. and Stewart, A. 2004. Microbial Control of Botrytis spp. pp. 223-241. In: Elad, Y., Williamson, B. , Tudzynski, P. and Delen, N (Eds). Botrytis: Biology, Pathology and Control. Springer Netherlands.

Frevel, D.R. ,William, J, Connick, Jr. and Lewis, J.A. 1998. Formulation of microorganisms to control plant diseases. pp. 187-202. In: Burges, H.D. (Ed.). Formulation of Microbial Biopesticides. Kluwer Academic publishers.

Goldfarb Barry, Earl E. N., Everett M. H., 1989. Trichoderma spp.: Growth rates and Antagonism to Phellinus weirii *in vitro*. *Mycologia* 81(3):375-381.

Guetsky, R., Elad, Y., Shtienberg, D.and Dinoor, A. 2002. Improved biocontrol of *Botrytis cinerea* on detached strawberry leaves by adding nutritional supplements to a mixture of *Pichia guilermondii* and *Bacillus mycoides*. *Biocontrol Science & Technology* 12 (5):625-630.

Guetsky, R., Shtienberg, D. Elad, Y., Fischer, E. and Dinoor, A. 2002. Improving biological control by combining biocontrol agents each with several mechanism of disease suppression. *Phytopathology* 92(9):976-985.

Gemechu, K., Musa, J., Tezera, W., and Millon, F., 2006. Faba bean and field pea mixed-cropping potential and limitations. Research report No.66, Ethiopian Institute of Agricultural Research, Ethiopia. pp. 38.

Gullino , M. L. and Garibaldi, A. 1988. Biological and integrated control of gray mold of grape vine results in Italy. *OEPP/ EPPO Bull*, 18:9-12.

Harrison, J.G. 1988. The biology of *Botrytis* spp. On Vicia beans and chocolate spot disease. A review. *Plant Pathology* 37:168 -201.

Hawthorne, W., 2004. Faba bean disease management strategy for southern region. http://www.sardi.sa.gov.au/pdfserve/ fieldcrops/publications/advicefactsheets/ brochure.pdf

Hjeljord, L. G., Stensvand, A. and Tronsmo, A. 2001. Antagonism of nutrient-activated conidia of *Trichoderma harzianum* (atroviride) P1 against *Botrytis cinerea*. *Phytopathology* 91(12):1172-1180.

Holmes, K. A., Karuss,Schroes, S.E., Thomas, H.C., Evans, H. C. and Samuels, G.J. 2004. Taxonomy and biocontrol potential of a new species *Trichoderma Ovalisporum*, from Amazon. *Mycological Progress* 3:199-210.

Holmes, K. A., Karuss, U., Samuels, G.J. 2006.*Trichoderma Ovalisporum*, a novel biocontrol agent offrost pod rot *(moniliophthora roreri)* of cacao *(theobroma cacao)*: From discovery to field. Proceedings of the first Internatinal Conference on plant microbe interactions: Endophytes and biocontrol agents. Saariselka, Lapland, Finland. pp. 54-65.

ICARDA,1986. Screening techniques for disease resistance in faba bean. International Center for Agricultural Research in the Dry Areas, Aleppo, Syria. pp. 59.

ICARDA, 2006. Technology Generations and Dissemination for Sustainable Production of Cereals and Cool Season Legumes. International Center for Agricultural Research in the Dry Areas, Aleppo, Syria. pp. 256.

Jackson, A.J., Walters, D.R. and Marshall, G. 1997. Antagonistic Interactions between the foliar Pathogen*Botrytis fabae* and isolates of *Penicillium brevicompactum* and *Cladosporium cladosporioides* on faba beans. *Biological Control* 8(2):97-106

Khalid, F. Al Mutalque, Carol, M. S. and Baniel , A. B. 1995. Procarbazone-sodium effect on rotational crops and its dissipation in soils http://docs.ksu.edu.sa/PDF/Articles35/ Article350947.

Lawes, D.A., Bond, D. A., and Poulsen, M.H. 1983. Classification, origin, breeding methods and objectives. pp. 23-76. In: Hebblethwaite, P.D. (Ed.). The faba bean (*Vicia faba* L.), a basis for improvement. Butterworths, London, UK.

Leibinger, W., Barbara, B., Hahn, M. and Mendgen, K. 1997. Control of post harvest pathogens and colonization of the apple surface by antagonist microorganisms in the field. *Phytopathology* 87(11):1103-1110.

Ma, Y., Chang, Z., Zhao, J. and Zhou, M . 2008. Antifungal activity of *Penicillium triatisporum* Pst10 and its biocontrol effect

on Phytophthora root rot of chilly pepper. *Biological Control* 44(1):24-31.

Obagwu, J., Korusten,, L., 2003. Integrated control of citrus green and blue moulds using Bacillus subtilis in combination with sodium biocarbonate or hot water. *Postharvest Biolological Technology* 28:187-194.

Parry, D. W. 1990. Plant Pathology in Agriculture. Cambridge University Press, Cambridge.

Paul, C. St. Amand and Todd C. W. 1995. Green house, Detached-leaf and Field Testing method to determine Cucumber Resistance to Gummy Stem Blight. *Journal of American Society of Horticultural Science* 120(4):673-680.

Peng, G. and Sutton, J. C. 1990. Biological methods to control grey mould of strawberry. *Brighton Crop Protection Conference-Pests and Diseases* 30:233-240.

Reddy, M. V., Srinivasulu, B. and Devi, T. P. 2000. Biocontrol of pulse diseases. In: Upadhyay, R.R., Mukerji, K.G. and Chamola, B.P. (Eds.). Biocontrol potential and its exploitation in sustainable agriculture. Kluwer Academy Pllenum, New York. *Crop Diseases, Weeds, and Nematodes* 1:239-49.

Saber, W.I.A., K.M. Abd El-Hai and K.M. Ghoneem, 2009. Synergistic effect of *Trichoderma* and *Rhizobium* on Both biocontrol of chocolate spot disease and induction of nodulation physiological activities and productivity of *Vicia faba*. *Research Journal of Microbiology* 4:286-300.

Sahile, S., Fininsa, C., Sakhuja, P.K. and Seid, A., 2008a. Effect of mixed cropping and fungicides on chocolate spot (*Botrytis fabae*) of faba bean (*Vicia faba*) in Ethiopia. *Crop Protection* 27:275-282.

Sahile, S., Ahmed, S., Fininsa, C., Abang, M.M. and Sakhuja, P.K. 2008b. Survey of chocolate spot (*Botrytis fabae*) disease of faba bean (*Vicia faba* L.) and assessment of factors influencing disease epidemics in northen Ethiopia. *Crop Protection* 27:1457-1463.

Sherga,B. M, 1996. *Bacilus* isolates as potential biocontrol agents against chocolate spot on Faba beans. *Canadian Journal of Microbiology* 43:915-924.

Sumeet, R. and Mukerji, K. G. 2000. Exploitation of protoplast fusion technology in improving biocontrol potential. In: Upadhyay, R.R., Mukerji, K.G. and Chamola, B.P. (Eds.). Biocontrol potential and its exploitation insustainable agriculture.. Kluwer Academy

Pllenum, New York. *Crop Diseases, Weeds, and Nematodes* 1:39 - 48.

Sutton , J. C., Li, D., Peng, G. , Yu, H. and Zhang, P. 1997. *Gliocoladium raeum,* A versatile adversy of *Botrytis cinerea*. *Plant Disease* 81(4):317-328.

Torres, A. M., Roman, B., Avila, C. M., Satovic, Z., Rubiales, D., Sillero, J. C., Cubero, J.I. and Moreno, M. T. 2004. Faba bean breeding for resistance against biotic stresses: towards application of marker technology. *Euphytica* 147:67-80.

Upadhyay, R.K., Mukerji, K.G. and Chamola, B.P. 2000. Biocontrol potential and its exploitation in sustainable agriculture. Volume 1 Crop disease, weeds and nematodes. Kluwer Academic publishers. pp. 287.