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In vitro Antagonistic Potential of Fungal Isolates Against *Botrytis fabae* Sard

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ABSTRACT

Owing to its high protein content, faba bean (*Vicia fabae* L.) leaves' phylloplane harbors many microorganisms besides *Botrytis fabae* which could have antagonistic potential. The use of chemical fungicides against chocolate spot has been a common practice, but negative effects to the environment forces a search for alternative options. The objective of this study was 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, which yielded 72 fungal isolates. These isolates were evaluated for their biocontrol potential against *B. fabae in vitro*. ANOVA (Analysis of Variance) for Antibiosis, percent growth inhibition, growth rate and parasitism tests showed significant difference ($p<0.0001$) among fungal isolates. In antibiosis test, two isolates, *Trichoderma harzianum* and *Penicillium* spp. formed higher mean inhibition zone of 7.33 and 7.00 mm, respectively and the highest mean colony growth inhibition were recorded from *T. harzianum* (69%) and *T. oblongisporum* (58.1%) over the control. *Trichoderma* spp. showed higher mean growth rate (14-16.67 mm day^{-1}) than any of other fungal isolates and on the other hand higher mean diameter of lysed mycelium (12 mm) was recorded from *Aspergillus* spp. and *Penicillium* spp. This study revealed higher distribution of biocontrol agents and their antagonistic ability over *B. fabae*, in Ethiopia. Particularly *Trichoderma* spp. showed better potential in controlling *B. fabae* and can be further evaluated for its commercialization, either alone or as a component of integrated disease management.

Key words: Antagonistic fungi, biocontrol, growth rate, antibiosis, lysis, *Trichoderma*

INTRODUCTION

In Ethiopia, faba bean is produced widely and considered as one of the major food crops (Chopra *et al*., 1998). However, unlike world average (1.8 t ha^{-1}); the average yield of this crop in Ethiopia is very low, ranging 1.0 to 1.2 t ha^{-1} (Agegnehu *et al*., 2006). This is particularly due to the wider range of biological limitations such as inherently low yielding potential of the local cultivars and susceptibility to biotic and abiotic stresses (Mussa *et al*., 2008). Diseases such as, chocolate spot (*Botrytis fabae* Sard.), rust (*Uromyces Vicia fabae*) and black root rot (*Fusarium solani*) largely contribute to the low productivity of the crop; where chocolate spot is most destructive (Gorfu and Beniwal, 1987). According to Harrison (1988), several strategies were designed and can be employed for the management of chocolate spot. Crop hygiene, breeding for disease resistance and application of fungicides has been the most effective and widely used. It is also mentioned that biological control is another feasible option to the use of synthetic chemicals

(Obagwu and Korsten, 2003). In this context, *Trichoderma* spp. is the most important microorganisms used in biological control of many fungal phytopathogens (Saber *et al*., 2009). *Trichoderma* spp. controls phytopathogens through competing for nutrients and space, modifying micro-environmental, promoting plant defense mechanisms, antibiosis and mycoparasitism (Shakeri and Foster, 2007). Recent studies indicated the formation of localized and systemic resistance by *Trichoderma* spp. in a range of plants to a variety of phytopathogens (Reino *et al*., 2008).

Although, fungicidal management of plant diseases is an option, its problem arises when the development of fungicide resistance and/or adverse effects on the accompanying microflora occurs (Mahmoud *et al*., 2004). Now a days, bio-fungicides formulated with *T. harzianum*, named Trichodex, GlioGard and RootShield are used to control some soil-borne and phylloplane fungal pathogens (Barbosa *et al*., 2001). Little research conducted in Ethiopia for the biocontrol of *B. fabae* indicated high potential of local microbial agents (Sahle, 2008). Therefore, this study was proposed with the objective of exploring different areas for effective microbes for the management of chocolate spot *in vitro*.

MATERIALS AND METHODS

Collection of faba bean leaf samples: Two hundred thirty six 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 district were visited and from each farmer's 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*.

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 PDA (Potato Dextrose Agar), incubated at 20°C for 7 days and purified by repeated sub-culturing (Haggag *et al*., 2006). Likewise, 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 min each and macerated using mortar and pestle. The suspension was diluted at 10^{-2} times. Which is 1 mL of the original suspension was diluted by 9 mL of distilled water and again from the obtained 10 mL of suspension, 1 mL was diluted by 9 mL of distilled water). The final suspension was poured on PDA media and incubated at 25°C for 7 days. All visible fungal colonies were isolated, purified, coded and stored at 4°C. The fungal isolates which were later found effective antagonist were identified.

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

Zone	District	Altitude (m.a.s.l.)	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

***In-vitro* tests**

Antibiosis: Forty faba bean leave resident fungal isolates were tested for antibiosis against *Botrytis fabae* by dual culture technique. PDA plates were inoculated with 5 mm diameter mycelial disc of *B. fabae* from the edges of actively growing colony on one side and with a similar sized disc of fungal isolates on the other side at 6 cm apart from each other in three replications arranged in CRD (Completely randomized Design). The plates were incubated at 25°C for 2 days. The inhibition zone at the junction of fungal isolates and *B. fabae* were measured (mm) every 24 h (h) after 2 days of inoculation for three successive days. Culture plate with *B. fabae* alone (without the fungal isolate) was used as control.

Growth inhibition: Forty fungal isolates were evaluated in this test for their growth inhibition potential in three replications arranged in CRD. Five mm diameter mycelia disc of both *B. fabae* and fungal isolates were placed adjacent to each other at 6 cm apart as in section 2.3.1 above. Colony diameter growth was measured in millimeter (mm) every 24 h for three consecutive days starting from 2 days after inoculation. Plate inoculated with *B. fabae* alone was used as control. The colony diameter growth data was converted to percent colony growth inhibition (PGI) using the following formula:

$$PGI=\frac{R_1-R_2}{R_1}\times 100$$

where, R_1 is the radial growth of *B. fabae* in the control, R_2 is the radial growth of *B. fabae* in the presence of the antagonistic fungal isolate and PGI is the percent growth inhibition (Edgington *et al*., 1971).

Growth characterization: Forty six fungal isolates from faba bean leaves phylloplane were grown in petri dishes containing PDA medium, at 25°C for 2 days and evaluated for their growth rate in three replications arranged in CRD. Five mm mycelial disc of each isolate was placed at the center of the petri dishes and incubated for 2 days at 25°C. Colony growth diameter (mm) was measured from each isolate periodically every 24 h from 2 days after inoculation for the next 4 days. Colony growth rate (mm day^{-1}) was calculated from the measured colony growth on daily bases.

Parasitism: Thirty one fungal isolates found to be promising in the growth rate test were evaluated in three replications arranged in CRD for their parasitic potential against *B. fabae*. Five mm diameter of *B. fabae* mycelial disc was placed on PDA media and incubated at 25°C for 5 days. After incubation period, similar size agar disc of fungal isolates was placed at the center of *B. fabae* colony and incubated at 25°C for 2 days. Lysis of *B. fabae* colony was measured (mm) at 24 h interval starting from 2 days after inoculation for three days. Culture plate with *B. fabae* alone was used as a control.

Data management and statistical analysis: Data on inhibition zone (mm), colony diameter growth (mm) and parasitism (mm) were analyzed using the procedure of analysis of variance to know the effect of fungal isolates on growth and development of the pathogen *B. fabae*. Least Significant Difference (LSD) technique was used to separate between treatment means. ANOVA (Analysis of Variance) was performed using of SAS software (SAS, 2002).

RESULT AND DISCUSION

Antibiosis: Antagonistic effects of phylloplane fungi indicate the importance of such fungi as a biocontrol agent. In this study *B. fabae* growth was variably affected by different fungal isolates in different mode. Significant difference ($p<0.05$) was found among fungal isolates in their inhibition zone formation potential. The tested fungal isolates formed widely varying inhibition zone adjacent to the pathogen after three days in challenge. Those fungal isolates affecting growth of *B. fabae were* produced distinctly visible inhibition zone. This inhibition zone indicates the production of inhibitory compounds to the pathogen by the fungal isolates. The width of inhibition zone largely depends on the degree of diffusibility and solubility of the inhibitory compounds in the agar media as well as in their concentrations. The degree to which the inhibition zone was remained free of pathogen mycelia indicates the potency and stability of their activity. Out of the 40 fungal isolates tested, 20 isolates GB39-5, GB25-4, GB3-2, GB32-7, GB38-2, GB6-5 and A20-2 belonging to the species of *Penicillium*, isolates A12-2, A7-3 and GB2-3 of *Aspergillus* spp., A12-1 (*T. oblongisporum*), GA3-3 (*T. ovalisporum*), GB15-2 (*T. spirale*), GB25-1 (*T. citrinoviride*), GB25-3 (*T. virens*), GA2-3, GB6-3 and GO2-3 belonging to *T. harzianum*, GO3-2 (*T. gamsi*) and S11 (*T. hamatum*) produced 4 mm or higher inhibition zone 5 days after inoculation, indicating their strong antagonistic potential. In general, high inhibition zone were recorded from isolates GA2-3 (*T. harzianum*) (7.33 mm), GO3-2 (*T. gamsi*) (6.67 mm), GB25-3 (*T. virens*) (6.67 mm) and GA3-3 (*T. ovalisporum*) (6.67 mm) 5 days after inoculation (Table 2).

Antibiosis showing clear zone formation on agar plate was used to determine the antibiotic activity of the antifungal substances produced by fungal isolates against *B. fabae*. Where antibiosis has been involved, mostly hyphae of *B. fabae* were not observed to overlap with the colony of fungal isolates. In all cases, the isolates of *B. fabae* terminated growing before direct contact were made with fungal isolates. This might be in response to diffusible inhibitors that were released by the isolates (Lee and Wu, 1984). Growth of *B. fabae* was inhibited; this is due to the production of metabolites, possibly antibiotics produced by fungal isolates (Sahle, 2008). Secondary metabolites, possibly antibiotics and lytic enzymes released by antagonists are the main sources of antagonism in the biocontrol process. Antibiotics such as iturin and trichodermin and several enzymes, which hydrolyze fungal structures such as, chitinase, cellulose and galactase have been shown to be involved in antagonism process (Handelsman and Stabb, 1996 and De Melo and Faull, 2000). The diameter of inhibition zone varied with time as the amount of production of diffusible compounds in the fungal isolates increase and its action inhibits the growth of *B. fabae*. After 4 days of inoculation, the production of antibiotics (diffusible compounds) by the fungal isolates increased which was exhibited by the formation of high inhibition zone by the fungal isolates. After 5 days of inoculation, no further expansion of the formed inhibition zone was noted due to the reduction in the production of the diffusible compounds (antibiotics).

Growth inhibition: Out of the tested isolates one isolate was failed to compete and inhibit the growth of *B. fabae*. ANOVA for growth inhibition showed significant difference ($p<0.05$) among fungal isolates in their potential to inhibit the colony growth of *B. fabae*. The growth inhibition varied with time as the growth of *B. fabae* increases. In most of the isolates, the percentage of *B. fabae* growth inhibition was reduced from 3 days to 5 days after inoculation as the growth of *B. fabae* was increasing; while some of the isolates, particularly those with lower growth inhibition potential showed increasing trend of percent growth inhibition from 3 days to 5 days after inoculation (Table 3). However, after 5 days of inoculation, the growth of *B. fabae* was limited and

Table 2: *In vitro* effect of fungal isolates on *Botrytis fabae* as indicated by formation of inhibition zone at the junction in dual culture test

		Mean inhibition zone (mm)[1]		
Isolate code	Fungal species	3 days	4 days	5 days
GO3-2	*Trichoderma gamsi*	5.33^{a}	6.33^{ab}	6.67^{abc}
GB25-3	*Trichoderma virens*	5.33^{a}	5.67^{bcd}	6.67^{abc}
GB32-7	*Penicillium* spp.	5.00^{ab}	5.33^{cde}	6.00^{cd}
S11	*Trichoderma hamatum*	5.00^{ab}	5.67^{bcd}	6.00^{cd}
GA3-3	*Trichoderma ovalisporum*	5.00^{ab}	6.00^{bc}	6.67^{abc}
GB25-4	*Penicillium* spp.	4.67^{abc}	5.33^{cde}	5.67^{d}
GB2-3	*Aspergillus* spp.	4.67^{abc}	6.00^{bc}	6.67^{abc}
GO2-3	*Trichoderma harzianum*	4.67^{abc}	5.67^{bcd}	5.67^{d}
A7-3	*Aspergillus* spp.	4.33^{bcd}	5.67^{cd}	6.67^{abc}
GB15-2	*Trichoderma spirale*	4.33^{bcd}	5.00^{de}	6.00^{cd}
GA2-3	*Trichoderma harzianum*	4.00^{bcd}	7.00^{a}	7.33^{a}
GB6-3	*Trichoderma harzianum*	4.00^{cde}	4.67^{ef}	6.00^{cd}
GB25-1	*Trichoderma citrinoviride*	4.00^{cde}	5.67^{bcd}	6.00^{cd}
A12-2	*Aspergillus* spp.	4.00^{cde}	6.00^{bc}	6.33^{bcd}
A12-1	*Trichoderma oblongisporum*	3.67^{ed}	4.67^{ef}	5.67^{d}
GB3-2	*Penicillium* spp.	3.67^{ed}	4.00^{fg}	4.33^{e}
GB38-2	*Penicillium* spp.	3.67^{ed}	5.33^{cde}	6.00^{cd}
GB6-5	*Penicillium* spp.	3.33^{e}	6.33^{ab}	7.00^{ab}
GB39-5	*Penicillium* spp.	3.33^{e}	3.50^{gh}	4.00^{e}
A20-2	*Penicillium* spp.	2.33^{f}	4.00^{fg}	4.33^{e}
S2-3	*Penicillium* spp.	2.33^{f}	2.67^{hi}	2.67^{gh}
D5-1	*Fusarium moliniformae*	1.67^{fg}	2.00^{ij}	2.67^{gh}
B34-2	*Fusarium* spp.	1.67^{fg}	2.00^{ij}	3.67^{ef}
D7-3	*Fusarium* spp.	1.33^{g}	1.67^{jk}	2.00^{h}
D14-1	*Fusarium moliniformae*	1.33^{g}	1.67^{jk}	2.00^{h}
B20-2	*Fusarium* spp.	1.33^{g}	1.67^{jk}	2.00^{h}
B28-2	*Fusarium* spp.	1.33^{g}	1.67^{jk}	2.00^{h}
D15-1	*Fusarium* spp.	1.33^{g}	1.33^{jk}	2.00^{h}
4B-T	*Fusarium* spp.	1.33^{g}	2.00^{ij}	2.00^{h}
D22-2	Unknown	1.33^{g}	2.00^{ij}	2.33^{gh}
B24-2	*Fusarium moliniformae*	1.33^{g}	2.00^{ij}	2.33^{gh}
D27-4	*Fusarium moliniformae*	1.00^{g}	1.33^{jk}	2.00^{h}
B28-1	Unknown	1.00^{g}	2.00^{ij}	3.00^{fg}
B37-3	*Fusarium moliniformae*	1.00^{g}	1.00^{k}	2.33^{gh}
D15-2	*Fusarium* spp.	1.00^{g}	1.33^{jk}	2.33^{gh}
B34-1	*Geotrichum* spp.	1.00^{g}	1.67^{jk}	2.00^{h}
B20-1	*Geotrichum* spp.	1.00^{g}	1.00^{k}	1.00^{i}
B13-1	*Penicillium* spp.	1.00^{g}	1.33^{jk}	2.00^{h}
D20-3	*Fusarium moliniformae*	1.00^{g}	2.00^{ij}	2.67^{gh}
D22-1	Unidentified	1.00^{g}	1.33^{jk}	2.33^{gh}
Control		0.00^{h}	0.00^{l}	0.00^{j}
LSD (5%)		0.91	0.91	0.91
CV (%)		21.05	16.26	13.99

Means designated with the same letter in the same column are not significantly different at $p<0.05$, [1] Mean inhibition zone (millimeter) formed by the fungal isolates from three replications

Table 3: *In vitro* growth inhibition (%) effect of fungal isolates on *Botrytis fabae*

		DAI I		
Isolate	Fungal species	3	4	5[1]
GA2-3	*Trichoderma harzianum*	86.09^{a}	73.01^{a}	68.99^{a}
A12-1	*Trichoderma oblongisporum*	75.30^{b}	65.38^{b}	58.12^{b}
GB25-3	*Trichoderma virens*	73.73^{b}	60.09^{c}	47.75^{c}
GB15-2	*Trichoderma spirale*	72.23^{b}	53.68^{d}	45.47^{c}
GB25-4	*Penicillium* spp.	72.16^{b}	53.65^{d}	45.89^{c}
GA3-3	*Trichoderma ovalisporum*	67.57^{c}	53.07^{d}	49.50^{c}
S11	*Trichoderma hamatum*	67.56^{c}	50.14^{d}	44.51^{c}
GB25-1	*Trichoderma citrinoviride*	57.46^{d}	40.18^{e}	31.05^{defg}
GO2-3	*Trichoderma harzianum*	52.10^{e}	35.48^{f}	29.25^{defghi}
GO3-2	*Trichoderma gamsi*	44.43^{f}	26.06^{g}	17.56^{klmno}
S16-2	*Trichoderma polysporum*	41.30^{fg}	24.31^{ghij}	18.01^{jklmno}
A7-3	*Aspergillus* spp.	38.19^{gh}	24.31^{ghij}	21.61^{ijklmn}
GB32-9	*Penicillium* spp.	35.12^{hi}	23.15^{ghijkl}	17.28^{lmno}
GB32-7	*Penicillium* spp.	35.10^{hi}	24.10^{ghi}	17.14^{lmno}
GB38-12	*Aspergillus* spp.	32.81^{ij}	21.97^{hijklm}	16.15^{mnopq}
GB6-5	*Penicillium* spp.	32.76^{ij}	24.92^{ghi}	14.82^{nopqr}
GB6-3	*Trichoderma harzianum*	31.21^{ijk}	24.10^{ghi}	18.46^{jklmno}
GB39-2	*Aspergillus* spp.	30.46^{jkl}	21.38^{ijklm}	17.98^{jklmno}
GB7	*Penicillium* spp.	29.67^{jkl}	20.21^{klmno}	12.21^{opqrs}
GB3-2	*Penicillium* spp.	28.13^{kl}	16.13^{pqrstu}	12.15^{opqrs}
GB26-6	*Penicillium* spp.	27.41^{klm}	19.02^{mnopqr}	16.68^{mnop}
GB20-2	*Penicillium* spp.	27.35^{klm}	13.77^{tu}	10.83^{opqrs}
GB38-2	*Penicillium* spp.	26.62^{lm}	14.95^{stu}	13.02^{opqrs}
GB39-5	*Penicillium* spp.	23.49^{mn}	15.54^{qrstu}	8.96^{pqrs}
S16-1	*Penicillium* spp.	22.00^{no}	14.94^{stu}	8.04^{rs}
S2-3	*Penicillium* spp.	18.87^{op}	13.19^{u}	8.51^{qrs}
A20-2	*Penicillium* spp.	18.86^{op}	14.35^{stu}	13.44^{opqrs}
A13-2	*Aspergillus* spp.	15.76^{pq}	12.58^{u}	11.22^{opqrs}
B13-1	*Penicillium* spp.	11.83^{qr}	25.47^{fgh}	32.37^{def}
B24-2	*Fusarium moliniformae*	9.58^{rs}	15.53^{qrstu}	29.19^{defghi}
D27-4	*Fusarium moliniformae*	9.56^{rs}	23.73^{ghijk}	24.74^{fghijkl}
GB32-14	*Aspergillus* spp.	7.28st	6.72^{v}	5.81st
B20-2	*Fusarium* spp.	6.54^{stu}	20.80^{jklmno}	26.54^{efghi}
D15-1	*Fusarium* spp.	5.79^{stu}	12.58^{u}	30.15^{defgh}
D15-2	*Fusarium* spp.	5.76^{stu}	17.86^{nopqrs}	25.19^{fghijk}
B20-1	*Geotrichum* spp.	5.72^{stu}	15.50^{rstu}	30.15^{defgh}
B28-2	*Fusarium* spp.	5.01^{tu}	7.30^{pqrst}	30.63^{defg}
D7-3	*Fusarium* spp.	4.97^{tu}	19.08^{mnopq}	35.98^{d}
B34-1	*Geotrichum* spp.	4.97^{tu}	19.04^{mnopqr}	25.64^{efghij}
D20-3	*Fusarium moliniformae*	4.26^{tuv}	19.05^{mnopqr}	26.51^{efghi}
Control		0.00^{v}	0.00^{w}	0.00^{t}
LSD		4.53	3.58	7.77
CV (%)		10.48	9.01	19.05

Figures in the same column with the same letter are not statistically different at p<0.05, [1]Mean colony growth inhibition (%) of fungal isolates against *B. fabae* (mean of three replications). DAI-days after inoculation

the reduction in growth inhibition as a function of the pathogen growth was reduced by the fast growth of antagonistic fungal isolates tested. Out of the tested isolates, most of them possessed the ability to inhibit the growth of *B. fabae* with varying degrees. Showing PGI of 68.99 and 58.12% over the control, GA2-3 (*T. harzianum*) and A12-1 (*T. oblongisporum*), respectively, are promising isolates for the management of *B. fabae*. Whereas, GB32-14 (*Aspergillus* spp.) is the lowest in inhibiting *B. fabae* colony growth with PGI value of 5.81% over the control 5 days after inoculation (Table 3). *Trichoderma* spp. gave 90% growth inhibition (Evueh and Ogbebor, 2008), while Abou-Zeid *et al.* (2008) found 100% growth inhibition and Amin *et al.* (2010) reported 88% growth inhibition. In most cases colony growth inhibition of the pathogen indicates that the isolates produced antibiotics that diffuse in the agar medium and affect the growth of the pathogen. Production of antibiotics (antibiosis) has been recognized as one of the modes of action of microbial antagonists by which they suppress the activity of target pathogens on different crops (El-Ghaouth *et al.*, 2004). For instance, bacterial antagonists like *Bacillus subtilis* and *Pseudomonas cepacia* are known to kill pathogens by producing the antibiotic iturin (Gueldner *et al.*, 1988; Pusey, 1989).

Growth characteristics of fungal isolates from phylloplane: Significant differences ($p<0.05$) were resulted among fungal isolates for their growth rate. Most of the fungal isolates grew considerably faster than *B. fabae*. Among the tested antagonistic fungal isolates GO2-3 (*T. harzianum*), GA3-3 (*T. ovalisporum*) and GB15-2 (*T. spirale*) showed faster growth rate of 16.67, 15.67 and 15.56 mm day^{-1}, respectively (Table 4). Growth rates of antagonists affect their interactions with other fungi (Widden and Hsu, 1987). Maximum and optimum temperatures for growth differ among species of *Trichoderma* (Goldfarb, 1986). The degree of antagonism exhibited by various fungi towards Plant pathogens also could affect the rate of replacement. Goldfarb *et al.* (1989) reported the varying nature of the growth rate of *Trichoderma* with species and temperature. In their study, the growth rate of *Trichoderma* spp. varies from 12.7 to 23.4 mm day^{-1} depending on the species at 20°C of temprature. In another study, Saber *et al.* (2009) found daily growth rate of different fungal antagonist in the range of 15 to 35 mm day^{-1} which is reasonably higher than the pathogen and is similar with the result of current study.

Parasitism: ANOVA showed significant difference ($p<0.05$) among fungal isolates in their potential to lyse *B. fabae* mycelium. Most of the tested fungal isolates lysed the pathogen mycelia as compared to the untreated control (Table 5). Higher diameter of lysed mycelium (12 mm) was recorded from isolates GB38-12 (*Aspergillus* spp.), S16-1(*Penicillium* spp.) and A13-3 (*Penicillium* spp.) which is followed by S16-2 (*T. polysporum*), A7-3 (*Aspergillus* spp.) and GB32-14 (*Aspergillus* spp.) with lysed pathogen mycelium diameter of 11.67, 11.33 and 11.33 mm, respectively 5 days after inoculation (Table 5). Where as in case of two fungal isolates, 52-BT (*T. longibrachiatum*) and S25-9 (*T. viride*) overgrowth was observed. Similar results were reported by Sahle (2008), in their study conducted at Haramaya University they have found different antagonistic fungal isolates particularly of *Trichoderma* spp. showing lytic activity in varying degrees against *B. fabae*. Among isolates they tested, different species of *Trichoderma*, *Penicillium*, *Aspergillus* and *Phylophora* isolates that gave lysed diameter of *B. fabae* mycelium ranging from 6.7-12.5 mm and in some *Trichoderma* species overgrowth was reported. *Trichoderma* spp. exerts direct biocontrol by detecting, growing towards and parasitizing a range of fungi; this matches with

Table 4: Growth rate (mm day^{-1}) of some fungi isolated from faba bean leaf phylloplane on PDA medium

Isolate	Fungal species	Mean growth rate (mm day^{-1})
GO2-3	*Trichoderma harzianum*	16.67^{a}
GA3-3	*Trichoderma ovalisporum*	15.67^{ab}
GB15-2	*Trichoderma spirale*	15.56^{abc}
GB25-3	*Trichoderma virens*	15.22^{abcd}
S16-2	*Trichoderma polysporum*	15.00^{abcd}
GB6-3	*Trichoderma harzianum*	14.89^{abcd}
GB25-4	*Penicillium* spp.	14.78^{abcd}
GB25-1	*Trichoderma citrinoviride*	14.44^{abcd}
S11	*Trichoderma hamatum*	14.44^{abcd}
A12-1	*Trichoderma oblongisporum*	14.33^{abcde}
GB6-5	*Penicillium* spp.	14.00^{abcde}
GB20-2	*Penicillium* spp.	13.78^{abcdef}
S16-1	*Penicillium* spp.	13.78^{abcdef}
GB7	*Penicillium* spp.	13.44^{abcdefg}
GB39-5	*Penicillium* spp.	13.22^{abcdefg}
A20-2	*Penicillium* spp.	12.89^{bcdefg}
GB34.2	*Penicillium* spp.	12.22^{bcdefgh}
A7-3	*Aspergillus* spp.	12.11^{cdefgh}
GB32-7	*Penicillium* spp.	12.1^{cdefgh}
GO3-2	*Trichoderma gamsi*	11.89^{defghi}
S2-3	*Penicillium* spp.	10.89^{efghij}
GB3-2	*Penicillium* spp.	10.33^{fghijk}
GB38-2	*Penicillium* spp.	10.11^{ghijkl}
GB32-14	*Aspergillus* spp.	9.00^{hijklm}
GA31-3	*Penicillium* spp.	8.56^{ijklmn}
B28-2	*Fusarium* spp.	7.44^{jklmno}
D14-1	*Fusarium moliniformae*	7.11^{klmno}
D27-4	*Fusarium moliniformae*	7.11^{klmno}
GB26-6	*Penicillium* spp.	7.11^{klmno}
A13-2	*Aspergillus* spp.	7.11^{klmno}
B20-1	*Geotrichum* spp.	7.00^{klmno}
GB39-2	*Aspergillus* spp.	7.00^{klmno}
GB38-12	*Aspergillus* spp.	6.89^{klmno}
B24-2	*Fusarium moliniformae*	6.78^{lmno}
D20-3	*Fusarium moliniformae*	6.44^{mno}
B13-1	*Penicillium* spp.	6.44^{mno}
D15-1	*Fusarium* spp.	5.78^{mno}
B34-1	*Geotrichum* spp.	5.67^{mno}
B20-2	*Fusarium* spp.	5.44^{no}
B37-3	*Fusarium moliniformae*	5.33^{no}
4B-T	*Fusarium moliniformae*	5.11^{no}
D7-3	*Fusarium* spp.	4.89^{o}
B34-2	*Fusarium* spp.	4.33^{o}
D15-2	*Fusarium* spp.	4.11^{o}
D5-1	*Fusarium moliniformae*	4.11^{o}
GB32-9	*Penicillium* spp.	4.00^{o}
LSD		3.48
CV (%)		22.16

Means in the same column with the same letter are not statistically significantly different at $p<0.05$, [1]Mean daily growth rate (millimeter day^{-1}) of fungal isolates from the three replications, PDA-potato dextrose agar

Table 5: *In vitro* lysis (mm) effect of fungal isolates on *Botrytis fabae*

		DAI I		
Isolate	Fungal species	3	4	5
GB38-12	*Aspergillus* spp.	8.33^{a}	10.00^{a}	12.00^{a}
4B-T	*Fusarium moliniformae*	8.00^{ab}	10.00^{a}	10.67^{d}
GB25-1	*Trichoderma citrinoviride*	7.67^{bc}	9.00^{b}	11.00^{cd}
GB32-9	*Penicillium* spp.	7.33^{cd}	9.00^{b}	11.00^{cd}
A7-3	*Aspergillus* spp.	7.33^{cd}	9.00^{b}	11.33^{bc}
S16-1	*Penicillium* spp.	7.00^{d}	10.00^{a}	12.00^{a}
A13-3	*Penicillium* spp.	7.00^{d}	10.00^{a}	12.00^{a}
GB25-4	*Penicillium* spp.	7.00^{d}	9.00^{b}	11.00^{cd}
GB26-6	*Penicillium* spp.	7.00^{d}	9.00^{b}	11.00^{cd}
GA32-7	*Penicillium* spp.	7.00^{d}	8.67^{b}	10.00^{e}
GB32-14	*Aspergillus* spp.	7.00^{d}	10.00^{a}	11.33^{bc}
GO2-3	*Trichoderma harzianum*	7.00^{d}	9.00^{b}	11.00^{cd}
GO3-2	*Trichoderma gamsi*	7.00^{d}	9.00^{b}	11.00^{cd}
S16-2	*Trichoderma polysporum*	7.00^{d}	9.00^{b}	11.67^{ab}
GB39-2	*Aspergillus* spp.	7.00^{d}	8.00^{c}	10.00^{e}
GB7	*Penicillium* spp.	6.33^{e}	8.67^{b}	11.00^{cd}
A12-1	*Trichoderma oblongisporum*	6.00^{e}	7.00^{d}	9.00^{gh}
A20-2	*Penicillium* spp.	6.00^{e}	7.00^{d}	8.33^{ij}
GB20-2	*Penicillium* spp.	6.00^{e}	8.00^{c}	9.00^{gh}
S2-3	*Penicillium* spp.	6.00^{e}	8.00^{c}	9.33^{fg}
GA3-3	*Trichoderma ovalisporum*	6.00^{e}	7.67^{c}	9.00^{gh}
GB25-3	*Trichoderma virens*	6.00^{e}	8.00^{c}	9.67^{ef}
GB39-4	*Penicillium* spp.	5.33^{f}	7.00^{d}	8.00^{jk}
D2-1	*Aspergillus* spp.	5.00^{fg}	6.00^{ef}	7.00^{mn}
GB6-3	*Trichoderma harzianum*	5.00^{fg}	6.00^{ef}	7.67^{kl}
GB38-2	*Penicillium* spp.	5.00^{fg}	7.00^{d}	8.67^{hi}
9-T	*Trichoderma harzianum*	5.00^{fg}	6.00^{ef}	6.67^{n}
GB8-2	*Fusarium Moliniformae*	5.00^{fg}	6.33^{e}	7.33^{lm}
6-T	*Trichoderma harzianum*	4.67^{g}	5.00^{g}	5.67^{o}
GB3-2	*Penicillium* spp.	4.00^{h}	5.00^{g}	7.00^{mn}
112-An	*Trichoderma koningii*	4.00^{h}	5.67^{f}	6.00^{o}
Control		0.00^{i}	0.00^{h}	0.00^{p}
LSD		0.44	0.47	0.58
CV (%)		4.45	3.74	3.82

Treatment means in the same column with the same letter are not statistically different at $p<0.05$, [1]Lysis diameter (millimeter) of fungal isolates (mean of the three replications). DAI-Days After Inoculation

the current study result (Benitez *et al*., 2004). Lyses of mycelia of the pathogen *B. fabae* by fungal isolates markedly affected growth of the pathogen giving the colonies a ruptured appearance. This is due to the production of several enzymes, which hydrolyze fungal structures such as, chitinase, cellulose and galactase have been shown to be involved in antagonism process (Handelsman and Stabb, 1996; De Melo and Faull, 2000). The challenged mycelia of the pathogen showed a disintegrated appearance when examined microscopically. Lytic activity against mycelium of *B. cinerea* was reported from yeast isolates (Castoria *et al*., 1997). The yeast *Candida famata*

reduced green mold decay (*Penicillium digitatum*) on orange, electron microscopic observations indicated rapid colonization and partial lyses of *P. digitatum* hyphae by the antagonists (Arras, 1996).

CONCLUSION

The finding from this and some other studies depicted that there is strong potential of phylloplane fungi to be biocontrol agents against foliar and soil born plant pathogens. *In vitro* tests of phylloplane fungi indicated their strong antagonistic potential towards wide array of plant pathogens, hopefully this will turn new page in the plant disease management strategy. Exploiting this area of research will give away to shift from dependence on synthetic pesticides to biological method and/or biocontrol incorporated integrated disease management method. Future research directions should consider on exploring wide agro-ecological area to search for the availability of biocontrol agents, it should be repeatedly tested against different plant pathogens and users' friendly formulation and commercialization should be considered. As already started on some biocontrol agents like *T. harzianum;* formulation of these biocontrol agents should be given due attention.

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