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Broad Bean a New Host of Leaf Spot Disease Caused by *Alternaria tenuissima* in North Egypt.

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ABSTRACT

At the winter growing season (December, 2014), a leaf spot of broad bean (*Vicia faba* L.) was observed in fields belong to Researches and Production Station of National Research centre at Nubaria region, Beheira Governorate, North Egypt. The pathogenic isolated fungus was identified as *Alternaria tenuissima*. Efficacy of the bioagents, *Trichoderma harzianum*, *Pseudomonas fluorescence* and *Saccharomyces cerevisiae* as well as essential oils, lemon grass and thyme oil were evaluated against the growth of pathogenic fungus *in vitro*. All tested factors could inhibit the mycelia growth of the pathogen *A. tenuissima* at high percentages. Under natural infection, the growing broad bean plants were sprayed individually twice with interval of 15 days started from the second true leaves age. The applied bioagents spray completely inhibited the disease incidence. Meanwhile, essential oils reduced disease incidence to 10.0% and 8.3% at lemon grass and thyme oils comparing with 30.0% infection recorded at control treatment. It could be suggested that foliar spray with bioagents or essential oils considered as protective application against leaf spot disease of broad bean. It is thought that this study is considered the first report of leaf spot disease incidence of broad bean caused by *Alternaria tenuissima* in North Egypt.

Keywords: Bioagent; essential oils; Faba bean; foliar spray; foliar disease, leaf spot.

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INTRODUCTION

Broad bean *(Vicia faba* L.) is cultivated for use as a green or dried, fresh vegetable or for green manure in many parts of the world. It is also widely grown as a minor garden crop. Broad bean is used as human food in developing countries and as an animal feed in industrialized countries. In Egypt, broad bean is grown during September to March throughout the both river Nile sides in Delta and Upper Egypt regions as well as new reclaimed sandy lands. The production of broad bean is, however, constrained by several diseases including fungal diseases. Among them, chocolate spot (*Botrytis fabae* Sard.), rust (*Uromyces vicia-fabae*), black root rot (*Fusarium solani*) and foot rot (*Fusarium avenaceum*) which contributes to the low productivity of the crop [1].

A new leaf spot disease of broad bean was observed in fields of Researches and Production Station of National Research centre at Nubaria region, Beheira Governorate, Egypt. Infected plants with numerous concentric brown spots on older leaves were quite common in all locations of surveyed field. *Alternaria tenuissima* is a common pathogen on a number of plants described in several geographic regions of the world. Species of the genus Alternaria are widespread pathogens of wheat and other cereals. They are known to be a cause of wheat leaf blight, black point disease and as a source of food contamination by toxins [2]. *Alternaria tenuissima* was found to be able to infect various parts of plants belonging to different families. Many researchers have found this fungus to be a common pathogen on a number of plants in different parts of the world. It can, for example, induce late blight of pistachio in the USA [3], and was established as a major cause of apple dry core rot in South Africa [4]. *Alternaria tenuissima* can infect a high percentage of cereal grains [5-6]. The present work was aimed to isolate and identify the causal fungus of leaf spot diseases of broad bean as well as study its pathogenicity. Moreover, the efficacy of some bioagents and essential oils against the pathogen growth and disease incidence was also evaluated under laboratory and field conditions.

MATERIAL AND METHODS

Plant cultivar

Broad bean plants *Vicia faba* L. cv. Giza 3were grown in Researches and Production Station of National Research centre at Nubaria region, Beheira Governorate, Egypt.

Fungal isolation and identification

Diseased samples were obtained from fields belong to Researches and Production Station of National Research centre at Nubaria region, Beheira Governorate, North Egypt. Fungal isolates were collected mainly from sample leaves. Pieces of infected leaf, including a lesion tissue, were surface-sterilized with 70% ethanol for 20-30 sec. and then washed thoroughly with sterilized distilled water. Then leaf pieces were transferred to PDA medium plates and incubated at temperature 25^{o}C for 5-7 days. Hyphal tips from appeared fungal colonies were transferred to a new PDA medium plate, and a single-spore isolation was made from the resultant colony. Single spore isolates were maintained on slants medium of PDA. The fungal culture was maintained on PDA slants for further studies. The isolated fungus was identified based on morphological and microscopic characteristics.

Preparation of spore suspension

A spore suspension was prepared by flooding plates of 7 day-old cultures with sterilized distilled water and dislodging spores with a glass rod. Spore concentration was adjusted using a hemacytometer.

Pathogenicity test

Detached healthy leaves of broad bean plants were used for pathogenicity test *in vitro* under laboratory conditions. Sterilized detached leaves were put onto moisten filter paper in Perti-dishes (18 mm diam.) A spore suspension of the isolated fungus, obtained by flooding with sterile water and rubbing 7-10-day-old cultures grown on PDA medium, was used as inoculum. Drop inoculations were carried out with a pipette on the upper leaf surface. Inoculated leaves were incubated at 25°C under dark for 5-7 days then examined for disease symptoms appearance.

Laboratory studies

The inhibitor activities of essential oils, lemon grass and thyme at concentrations of 0.5, 1.0 and 2.0 % (v:v), as well as antagonistic bioagents, *Trichoderma harzianum*, *Pseudomonas fluorescence* and *Saccharomyces cerevisiae* on the linear growth of the pathogen *Alternaria tenuissima* were evaluated. The tested essential oils were purchased from Cairo Company for oils and aromatic extractions CID, Egypt. Meanwhile, the bioagents were supplemented by culture collection unit, Plant Pathology Dept., NRC, Egypt.

For essential oils test, certain volumes of each of lemon grass and thyme were poured into individual flasks containing sterilized PDA before solidifying and rotated gently to ensure even distribution of proposed concentration, and then poured into 90-mm Petri dishes. The control treatment was untreated medium with essential oils. Amended plates were inoculated with a 5-mm disk of the tested pathogen fungus at the centre of a plate and incubated at 25±1^{o}C for 7 days, then the colony diameter growth compared to the control was calculated in percentages.

As for antagonistic bioagents test, the bioagent efficacy was also evaluated *in vitro* performed on PDA medium using the dual culture technique [7]. A 5-mm disk of each tested bioagent was placed onto the PDA plate 10 mm from the edge of the Petri dish. Another 5-mm disk of the pathogen growth was placed on the opposite side of the dish at the same distance. The control treatment was inoculated with mycelial disk of either a pathogen or antagonist alone.

Both experimental and control dishes were assigned to a completely randomized design, with five replicates per treatment. All inoculated Petri dishes were incubated at 25±1^{o}C and the diameter of fungal growth away from and toward the inhibitor agent was measured when the tested fungal growth in the control treatment had reached the edge of the PDA plate.

These tests were repeated three times and the inhibitor effect was calculated as the reduction percent in colony diameter growth compared to the control.

Field experiment

Field experiment was carried out at Researches and Production Station of National Research centre at Nubaria region, Beheira Governorate, North Egypt during 2015-2016 growing season to evaluate the efficacy of some foliar spray treatments for controlling leaf spot disease incidence of broad bean. This disease is recorded as the first occurrence at previous season 2014-2015 by the authors as naturally infected with *Alternaria tenuissima* pathogen of broad bean at the same field.

Lemon grass and thyme essential oils were sprayed at concentration of 2% (v:v), while the bioagents, *T. harzianum*, *P. fluorescence* and *S. cerevisiae* sprayed in spore or cell suspension at concentration of 10^{8}/cfu. All foliar spray treatment were applied twice, the first at the emerged broad bean plants (at 2 true leaves age) and the second after 15 days interval.

The experimental field consisted of plots (7x6 m) each comprised of 12 rows and 30holes/row which were conducted in completely randomized block design with five plots as replicates for each particular treatment as well as untreated check treatment. Broad bean seeds Giza, 3 cv. were sown in all treatments at the rate of 3seeds/hole. All plots received the traditional agricultural practices. Average percent of leaf spot disease infection was recorded after 15 days after each spray applied and the average accumulated disease incidence was calculated at the flowering stage of plant growth.

Statistical analysis

All *in vitro* and *in vivo* experiments were set up in a complete randomized design. One way analysis of variance (ANOVA) was used to analyze the obtain results concerning the following: 1. Differences between the tested essential oil and bioagent inhibitor effect and linear growth of pathogenic fungus *in vitro*, 2. Differences between tested spray treatments of essential oils and bioagents on the incidence of leaf spot infection under field conditions. General Linear Model option of the Analysis System SAS [8] was used to perform the analysis of variance. Duncan's Multiple Range Test at $p \leq 0.05$ level was used for means separation [9].

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RESULTS AND DISCUSSION

The leaf spot disease was observed in the field from the end of November until the middle of December during 2014-2015 growing season. This field belongs to Researches and Production Station of National Research centre at Nubaria region, Beheira Governorate, North Egypt. The recorded percentage of infected plants ranged from 20 to 30. Disease symptoms on broad bean plants illustrated in Figure (1). Lesions appeared at first on older leaves then spread to the new ones as well as plant stem. Initially, lesions were distinct, brown, water soaked, and circular or slightly irregular. Then the lesion enlarged and became concentric, and lesions on mature leaves had extensive necrosis. Under field conditions, the observed severe disease on leaves at a flowering growth stage, suggesting that repeated infection cycles may be took place for the necessity of disease to reach an economic level. Thus, the disease occurred irrespective of the plant's growth stage.

The isolated fungus from the lesions on broad bean leaves growing on PDA medium plates. Colonies formed on PDA developed aerial hyphae of greyish white colonies, and later on turned to olive-green to black. The fungus produced conidia in unbranched chains. Based on morphological and microscopic characteristics, the fungus identified as *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire as described by [10-11-12].

Under *in vitro* pathogenicity test, symptoms appeared after drop inoculation of detached leaves. Repeated isolation of *A. tenuissima* from a naturally infected leaf spot, production of the same disease symptom after inoculation of plants completion of Koch's postulates and complete agreement with the natural disease in surveyed field provided sufficient evidence that the leaf spot disease of broad bean is caused by *A. tenuissima*. In this concern, leaf spot of broad been caused by *Alternaria tenuissima* was earlier recorded in Japan [13-14], in Korea [15]. Also, the fungus *A. tenuissima* has been isolated throughout the world from many diseased crops such as chillies [16], soybean [17], cowpea [18], pigeon pea [19], cotton [20], wheat [21], Jojoba [22] and apricot [23].

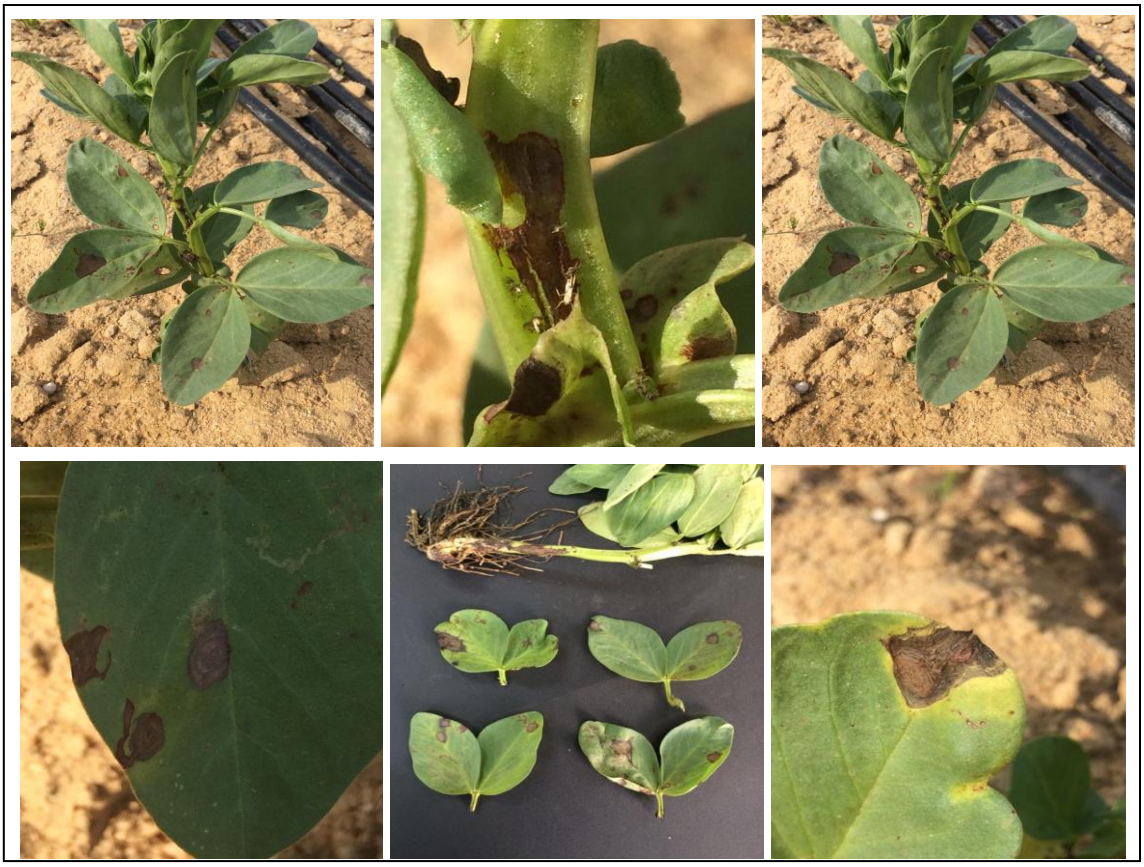

Figure 1: Symptoms of leaf spot of broad bean plants (*Vicia fabae* L.). The typical Circular lesions appeared on plant leaves and stem.

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Laboratory studies

Under *in vitro* conditions, either lemon grass or thyme oils could inhibit the growth of *A. tenuissima* (Table 1). This growth inhibition was increased by increasing the essential oils concentration in growth medium to reach its maximum at concentration of 2%. At this concentration the fungal growth was decreased by 70.0 and 65.5% at lemon grass or thyme oils treatments, respectively (Fig. 2). Essential oils have been reported as promising alternative compounds which have an inhibitory activity on the growth of pathogens. It was reported that essential oils could be used in plant disease control as the main or as adjuvant antimicrobial compounds [25]. It is well established that some plants contain compounds able to inhibit the microbial growth [26]. Also, [27] reported that Thyme, Lemon grass, Peppermint, Clove and Mint oils had higher inhibitor effect on mycelial growth of *Fusarium oxysporum*. Fungal mycelial growth decreased significantly as the concentrations of essential oils were increased, to reach the fungal growth's minimum at the highest concentration used. Moreover, lemongrass oil expressed antifungal activity against *Colletotrichum coccodes, B. cinerea, Cladosporium herbarium, Rhizopus stolonifer* and *A. niger in vitro* [28]. Also, thyme oil proved to be extremely effective as a fumigant as well as a contact fungicide against a range of the economically significant fungi *Alternaria* spp., *Aspergillus* spp., *Botrytis cinerea, Erysiphe graminis* [29].

Table 1: Inhibitor effect of some essential oils and bioagents on the linear growth of *Alternaria tenuissima in vitro*

Tested essential oils and bioagents	Concentration %	Linear growth (mm)
Lemon grass oil	0.5	57 c
	1.0	45 d
	2.0	27 f
Thyme oil	0.5	62 b
	1.0	52 c
	2.0	31 e
Trichoderma harzianum	-----	22 f
Pseudomonas fluorescence	-----	27 f
Saccharomyces cerevisiae	-----	22 f
Untreated (Control)	-----	90 a

Mean values within columns followed by the same letter are not significantly different (P ≤ 0.05).

On the other hand, presented data in Table (1) and Figure (2) showed highest antagonistic effect of tested bioagents against the growth of *A. tenuissima.* The pathogenic fungal growth decreased by 70.0, 76.5 and 76.5% by the antagonists, *P. fluorescence, T. harzianum* and *S. cerevisiae,* respectively. In this regards, these microorganisms had been reported to have antagonistic effect against several pathogenic fungi. *Trichoderma harzianum* is reported as biocontrol agent that attacks a range of pathogenic fungi and can be used in the biological control of several plant diseases [30-31-32-33]. Also, *Pseudomonas cepacia* or *Pseudomonas fluorescence* applied to pea seeds act as biological control agent against Pythium damping-off and Aphanomyces root rot and was able to reduce diseases incidence [34-35]. Also, [36] reported that *S. cerevisiae* was reported to have a reduction potential against radial growth of pathogenic fungi *Macrophomina phaseolina* and *Fusarium solani*, the cause of root rot diseases in tomatoes and eggplants.

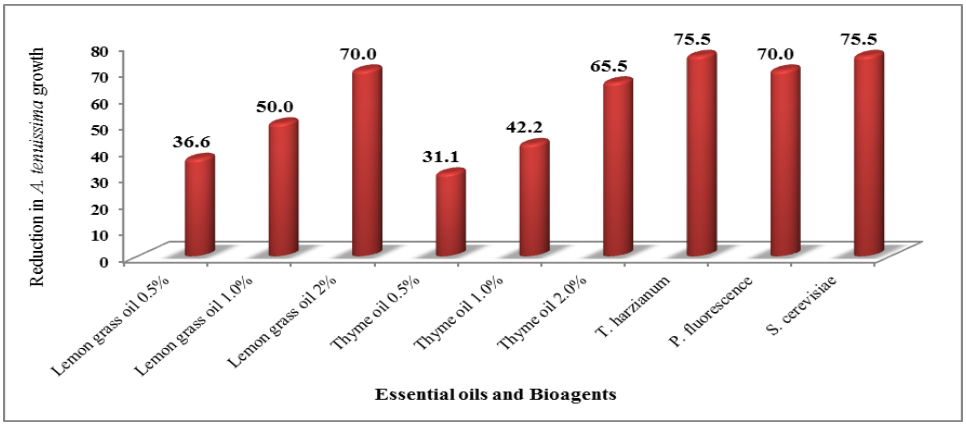

Figure 2: Reduction in *Alternaria tenuissima* growth in response to some essential oils and bioagents *in vitro*

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Field experiment

According to the present study, a newly emerging devastating disease "broad bean leaf spot" was proved to be dominant both in terms of occurrence and intensity season after another (2013/2014 – 2014/2015) at the same study area. The successive occurrence of this disease could be further complicated by addition of new disease in the region and the country as a whole. Furthermore, the occurrence of disease at early growth stage of the plant suggesting that repeated infection cycles to reach an economic level. Therefore, the control of this disease is considered important. The control of diseases in broad bean mainly relies on chemical treatments. However, a number of authors indicate that the effect of leaf pathogens can be reduced by biological control with *Trichoderma* [37-38].

In the present study, the efficacy of some foliar spray treatments for controlling leaf spot disease incidence of broad bean was evaluated under field conditions. Presented data in Table (2) and Figure (3) revealed announced reduction recorded as 100% at bioagents treatments. Meanwhile, spray with essential oils reduced disease incidence by 66.6 and 72.3% at treatments of lemon grass and thyme oils, respectively.

These results were in agreement with several previous reports. Early studies on microbial ecology of the phyllosphere showed that there was considerable potential for use of microbial antagonists for control of leaf spot diseases of many crops. In a study of [39] spraying vegetables, Cucumber, Cantaloupe, tomato and Pepper with the bio-agents, *T. harzianum, T. viride, B. subtilis, P. fluorescens* and *S. cerevisiae* was effectively able to reduce the foliar diseases comparing with untreated control. Also, [40] stated that *Trichoderma harzianum* regarded as a model to demonstrate bio-control under commercial field conditions. He added that this bio-control agent controls some cucumber foliar diseases, *i.e.* gray mold (caused by *Botrytis cinerea*), downy mildew (caused by *Pseudoperonospora cubensis*), foliar blight (caused by *Sclerotinia sclerotiorum*) and powdery mildew (caused by *Sphaerotheca fusca*) under commercial greenhouse conditions. Moreover, [41] stated that foliar application of *Pseudomonas fluorescens* combined with a half recommended dose of azoxystrobin was effective control of downy and powdery mildews of cucumber. Also, [42] suggest that some bacterial isolates, *i.e. Paenibacillus macerans, Serratia plymuthica, Bacillus coagulans, Serratia marcescens-GC, Bacillus pumilis* and *Pantoea agglomerans* reduced the disease severity of early blight caused by *Alternaria solani* in tomato when applied as foliar spray.

Table 2: Efficacy of some bioagents and essential oil on leaf spot disease incidence of broad bean under field conditions

Spray treatment	Disease incidence
Lemon grass oil	10.0 b
Thyme oil	8.3 b
Trichoderma harzianum (10^8 spores/ml)	0.0 c
Pseudomonas fluorescence (10^8 cells/ml)	0.0 c
Saccharomyces cerevisiae (10^8 cells/ml)	0.0 c
Untreated (Control)	30.0 a

Mean values within columns followed by the same letter are not significantly different (P ≤ 0.05).

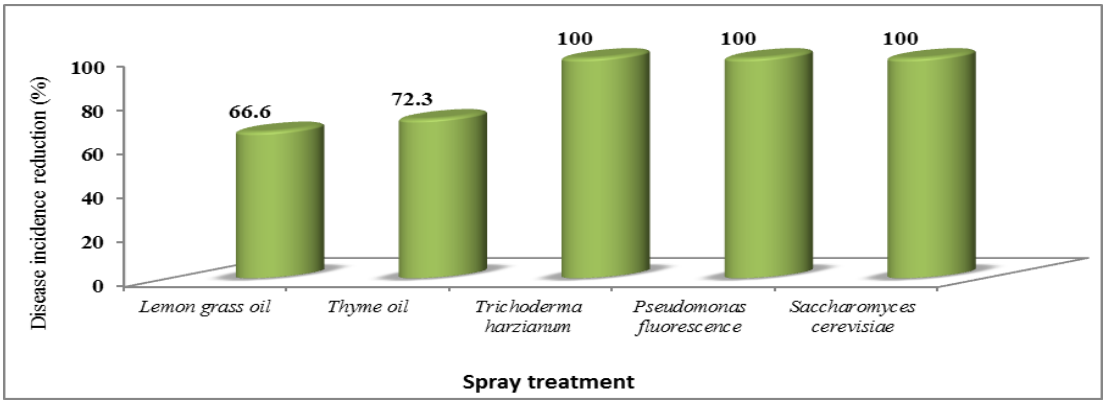

Figure 3: Reduction (%) in leaf spot disease incidence of broad bean in response to application of some bioagents and essential oil under field conditions

The mode of action of biocontrol mechanisms was explained by many investigators. *Trichoderma* spp. have received major attention as biocontrol agent against several plant pathogenic fungi and are excellent candidates for successful exploitation [31]. Proposed mechanisms of antagonism resulting in biocontrol are antibiosis [43-44], mycoparasitism [45], and competition [46]. Furthermore, [40] stated that The BCA (an isolate T39 of *Trichoderma harzianum)* suppressed enzymes of *B. cinerea,* such as pectinases, cutinase, glucanase and chitinase, through the action of protease secreted on plant surfaces. A combination of several modes of action is responsible for biocontrol. However, biocontrol is not achieved by means of antibiotics or by mycoparasitism, in spite of the fact that BCA has the potential to degrade cell-wall polymers, such as chitin. Recently, it has been known the potential of *Bacillus* sp. to synthesize a wide variety of metabolites with antifungal activity [47]. Most of these substances belong to lipopeptides, especially from surfactin, iturin and fengicin classes [48]. Also, antibiotics of the iturin group were found to act upon the sterol present in the cytoplasmic membrane of the fungi [49]. On the other hand, the mode of action of antagonistic yeasts might be attributed to competition for space and nutrients [50], production of cell-wall lytic enzymes [51], and induction of host resistance [52]. Moreover, the bio-control activity of *S. cerevisiae* against pathogenic fungi might have possibly resulted from mycoparasitism [53], secretion of lytic enzymes such as *β*-1,3 glucanase [54] and production of antibiotics [55].

In the present study, foliar spray with essential oils proved to have high potential effect for reducing disease incidence. Essential oils as natural alternatives that are user friendly and demonstrate low toxicity to humans are desirable to be tested in the present work. Thyme oil applied showed effective reduction in foliar diseases incidence more than 50%. In this regards, several investigators reported the antifungal effect of essential oils. Thyme and Egyptian geranium oils are considered antimycotic natural compounds may be useful for inhibition of mold fungi on wood in service or during storage of building materials [56]. Moreover, [57] had the first report on the use of Thymol for controlling a plant disease under field conditions, which indicated that this compound provided effective control of bacterial wilt on susceptible tomato cultivars. Also, Thymol has been reported to have fungicidal activities and fumigation with thymol has been used for control of postharvest fungal diseases [58-59]. Modes of action of the antibacterial property of thymol appeared to include disruption of bacterial cell membrane integrity by altering protein reactions [60-61].

The preliminarily recorded results in the present work showed that foliar spray with bioagents or essential oils can have a considerable activity against leaf spot of broad bean under field conditions. Their non chemical properties suggest potentials for commercial formulation and application which could suggested as a broad spectrum use against foliar pathogens under field conditions. The present findings demonstrate that the future use of antagonistic microorganisms and essential oils on a commercial scale for controlling such diseases. Considering their attribute and broad-spectrum activities, successful development of such compounds as antifungal would not only provide a potent tool for control of plant foliar diseases, but also could promise success in multipurpose biorational alternatives to conventional fungicides for the management of plant diseases.

CONCLUSION

The present study, however, appears to be the first description of disease symptoms, morphology, and pathogenicity of *A. tenuissima*, as a pathogen of broad bean causing a destructive leaf spot disease observed in fields located in North Egypt. Moreover, this disease occurred during two successive growing seasons at the same area under natural conditions as well as under *in vitro* under artificial infestation the causal fungus proved to be *A. tenuissima*

This finding disagreed with a recent study of [24] who recorded that fourteen fungi related to 9 genera were isolated from diseased leaves of broad bean collected from different cultivated regions in Qena governorate in Upper Egypt. They found that eight fungal species (represent 57.15% of total fungi tested) were positive and successfully able to infect broad bean leaves appearing leaf spot symptoms. Meanwhile, six species (42.85%) including *Alternaria* tenuissima had negative pathogenicity result and unable to infect the leaves of broad bean plants and failing to produce any leaf spot symptoms.

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REFERENCES

[1] Nigussie T, Seid A, Derje G, Tesfaye B, Chemeda F, Adane A, Abiy T, Fekede A, Kiros M. Abraham Tadesse (Eds.). Increasing Crop Production Through Improved Plant Protection 2008; 1: 85-124.
[2] Rotem J. The genus Alternaria. Biology, epidemiology and pathogenicity. St. Paul, Minnesota: APS Press, 1994; pp. 326.
[3] Pryor BM and Michailides TJ. Phytopathology 2002; 92: 406-416.
[4] Serdani M, Kang JCh, Andersen B, Crous PW. Mycol Res 2002; 106: 561–569.
[5] Andersen B, Thrane U, Svendsen A, Rasmussen IA. Can J Bot 1996; 74: 854–858.
[6] Gannibal PhB. Micologiya i Fitopatologiya 2004; 38: 19–28.
[7] Ferreira JHS, Mathhee FN, Thomas AC. Phytopathology 1991; 81: 283-287.
[8] SAS Statistical Analysis System. User's Guide: Statistics (PC-Dos 6.04). SAS Institute Inc., Cary, NC, USA, 1996.
[9] Winer BJ. Statistical Principles in Experimental Design. 2nd ed. MiGraw-Hil Kogakusha, LTD 1971; pp. 596.
[10] Simmons, E.G. Mycotaxon 1990; 37: 79-119.
[11] Simmons, E.G. Host-specific toxin producers. Mycotaxon 1999; 70: 325-369.
[12] Navi SS, Bandyopadhyay R, Hall AJ, Bramel-Cox PJ. Information Bulletin No. 59 (In En. Summaries in En, Fr). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics 1999; p. 118.
[13] Honda Y, Rahman MZ, Islam SZ, Muroguchi N. Plant Dis 2001; 85: 95.
[14] Rahman MZ, Honda Y, Islam SZ, Muroguchi N, Arase S. J Gen Pl Pathol 2002; 68 (1): 31-37.
[15] Kwon Jin-Hyeuk, Park and Chang-seuk. Res Pl Dis 2002; 8(2): 117-119.
[16] Kamal M, Tahirudden S. West Pakistan J Agric Res 1970; 8: 38-49.
[17] Mishra B and Parakash 0. Indian J Mycol Pl Pathol 1975; 5: 95 (Abstr.).
[18] Shukla P, Lal B, Singh RP, Singh PN. Indian J Mycol Pl Pathol 1977; 7: 159-160.
[19] Kannaiyan J and Nene YL. Trop Grain Legume Bull 1977; 7: 34.
[20] Davis ND, Diener UL, Morgan-Jones G. Appl Environ Microb 1977; 34: 155-157.
[21] Patil LK and Wani PV. J *Maharashtra* Agric *Univ* (India) 1980; 5(3): 253-254.
[22] Gupta A and Ghosh RN. Indian Phytopathol 1981; 34: 397-399.
[23] Oprea M, Balan V, Dragoescu E, Ivascu A. Acta Hort 1986; 192: 231-238.
[24] Saleem A, El-Said AHM, Maghraby TA, Hussein MA. J Plant Pathol Microb 2012; 3:141
[25] Kaur J and Arora D. Int J Antimicrob Agents 1999; 12: 257–262.
[26] Naqui SHA, Khan MSY, Vohora SB. Fitoterapia 1994; 62: 221–228.
[27] Ragab MMM, Ashour AMA, Abdel-Kader MM, El-Mohamady R, Abdel-Aziz A. Int J Agric Forest 2012; 2(2): 70-77.
[28] Tzortzakis NG and Economakis CD. Innov. *Food Sci.* Emerg. Technol 2007; 8 (2): 253-258.
[29] Alefyah A and Avice MH. Plant Pathology-Global Perspectives of an Applied Science. E:\potato\The BSPP-Archives-BSPP Presidential Meeting 1997.htm 1997.
[30] Papavizas GC. Phytopathology 1982; 72: 121-125.
[31] Chet I. Innovative Approaches to Plant Disease Control (Chet I ed.), John Wiley & Sons: New York, 1987; pp: 137-160.
[32] Whipps JM and Lumsden RD. Commercial use of fungi as plant disease biological control agents: status and prospects. CABI Publishing, Wallingford, United Kingdom. 2001; pp: 9-22.
[33] McLean KL, Dodd SL, Sleight BE, Hill RA, Stewart A. New Zealand Pl Prot 2004; 57: 72-76.
[34] Parke JL, Rand RE, Joy AE, King EB. Plant Dis 1991; 75: 987-992.
[35] King EB and Parke JL. Plant Dis 1993; 77: 1185-1188.
[36] Attyia SH and Youssry AA. Egypt J Biol 2001; 3: 79-87.
[37] Elad Y. Biocontrol Sci Technol 2000; 10: 499–507.

[38] Elad Y, Freeman S. The Mycota, XI. Agricultural Applications, Springer, Heidelberg, Germany Kempken F (ed.) 2002; pp. 93-109.
[39] Abdel-Kader MM, El-Mougy NS, Aly MDI, Lashin SM, Abdel-Kareem F. Adv Life Sci 2012; 2: 98-103.
[40] Elad Y. Crop Prot 2000; 19: 709-714.
[41] Anand T, Chandrasekaran A, Kuttalam S, Raguchander T, Samiyappan R. J Agric Sci Tech 2009; 11: 211-226.
[42] Yazici S, Yanar Y, Karaman I. Afr J Biotechnol 2011; 10: 1573- 1577.
[43] Dennis C and Webster J. J. Brit Mycol Soci 1971; 57: 25-39.
[44] Dennis C and Webster J. Brit Mycol Soci 1971; 57: 41-48.
[45] Ayers WA and Adams PB. Biological control in crop production, Papavizas GC (ed), Allanheld, Osmun,Totowa, NJ. 1981; pp. 91-103.
[46] Sivan A and Chet I. Phytopathology 1989; 79: 198-203.
[47] Ahimou F, Jacques P, Deleu M. Enzyme Micro Tech, 2000; 27: 749-754.
[48] Moyne AL, Shelby R, Cleveland TE, Tuzun S. J Appl Microbiol 2001; 90: 622-629.
[49] Worthington PA. Nat Prod Rep 1988; 5: 47-50.
[50] Janisiewicz WJ, Tworkoski TJ, Sharer C. Phytopathology 2000; 90: 1196-1200.
[51] El-Ghaouth A, Wilson CL, Wisniewski M. Phytopathology 1998; 88: 282-291.
[52] Droby S, Wisniewski M, El-Ghaouth A, Wilson C. Postharvest Biol Technol 2003; 27: 127- 135.
[53] Hajlaoui MR and Belanger RR. Biocontrol Sci Technol 1993; 11: 427-434.
[54] Punja ZK. Can J Pl Pathol 1997; 19: 35-323.
[55] Benyagoub M, Rhlid RB, Belanger RR. J Chem Ecol 1996; 22: 405-413.
[56] Yang VW and Clausen CA. Int Biodeterior Biodegradation 2007; 59: 302-306.
[57] Momol MT, Olson SM, Pradhanang PM., Jones JB. Plant Dis 2005; 89: 497-500.
[58] Paster N, Menasherov M, Ravid U, Juven B. J Food Prot 1995; 58: 81-85.
[59] Liu WT, Chu CL, Zhou T. Hort Sci 2002; 37: 151-156.
[60] Juven BJ, Kanner J, Schved F, Weisslowicz H. J Appl Bacteriol 1994; 76: 626-631.
[61] Walsh SE, Maillard JY, Russell AD, Catrenich CE, Charbonneau DL, Bartolo RG. J Appl Microbiol 2003; 94: 240-247.