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Influence of Some Antagonistic Bacteria against Early Blight (*Alternaria solani* (Ell. & Mart.) Jones & Grout.) of Tomato (*Lycopersicon esculentum* Mill.)

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ABSTRACT

Early blight caused by *Alternaria solani* (Ell. & Mart.) Jones & Grout., is amongst the most common foliar disease in tomato (*Lycopersicon esculentum* Mill.) farms of West Shewa sub-regions of Ethiopia that reduce yield and occasionally cause complete crop loss. To satisfy the contemporary market driven demand and supply for tomato products, the research need to focus on management options that are environmentally friendly. The present study was undertaken to evaluate the antagonistic effect of some rhizospheric bacteria (biocontrol agent) against *A. solani* and to study their influence on growth and development of tomato leaf of a farming cultivar, 'Romans VS'. Ten local antagonistic bacteria were screened *in vivo* for suppressing the pathogen. Five promising antagonists exhibiting higher zone of inhibition (ZOI) (38 mm and above) and percent disease control (ranging from 38.16 to 43.79%) were selected. These are *Pseudomonas fluorescens* TK-1, *P. fluorescens* TK-3, *Bacillus subtilis* TK-4, *P. fluorescens* TK-8 and *P. fluorescens* TK-10. The greenhouse experiment revealed *P. fluorescens* TK-3 as a best biocontrol agent which increased plant height by 35.20% and biomass by 52.28%. The efficacy test results of antagonistic bacterial isolates have clearly indicated that the indigenous strain, *P. fluorescens* TK-3 followed by *Bacillus subtilis* Tk-4 is an efficient biocontrol agent against *A. solani* with good *in vitro* and *in vivo* antagonistic activity.

Keywords: *Bacillus* spp., biocontrol, *Pseudomonas*, zone of inhibition
Abbreviations: CRD, Completely Randomized Design; **HCN**, hydrogen cyanide; **KB**, King's B medium; **NA**, nutrient agar; **PDA**, potato dextrose agar; **ZOI**, zone of inhibition

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) has become one of the most widely grown vegetables in the world and is regarded as a top priority vegetable. Tomato contributes to a healthy, well-balanced diet. It is rich in minerals, essential amino acids, sugars, dietary fibers and is considered to be fairly high in vitamins of high cash value and with potential for better-quality processing (Bebeli and Mazzucato 2008). Recently, there has been more emphasis on tomato production as a source of income and food security (Rice *et al*. 1987). Today the importance of tomato is increasing and is widely being accepted and used as a variety of dishes as raw cooked or processed products more than any other vegetable. Western Shoa of is one of the most important tomato-growing areas in Ethiopia. Current productivity under farmers' condition in Ethiopia is 90 q/ha whereas yield up to 400 q/ha recorded on research plots. Farmers get lower yield mainly due to diseases, pests and sub-optimal fertilization (Tesfaye 2008).

The tomato crop suffers from many diseases. Amongst them, *Alternaria solani*, causing early blight of potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*), is the most destructive (Reni and Roeland 2006) of field crops. The disease becomes wide spread and serious, causing large economic loss to the growers when the season begins with abundant moisture or frequent rains followed by warm and dry weather which are un favorable for the host and help in rapid disease development (Agrios 1988). The disease appears on leaves, stems, petioles, twigs and fruits under favorable conditions resulting in defoliation, drying off of twigs and premature fruit drop and thus causing loss from 50 to 86% in fruit yield (Mathur and Shekhawat 1986).

It causes diseases on foliage (blight), basal stems of seedlings (collar rot and damping off), stems of adult plants (stem lesions), fruits (fruit rot) of tomato and may also infect egg plant and pepper. This disease can be very destructive if left uncontrolled, often resulting incomplete defoliation of plants (Dater and Mayee 1985).

The conventional use of synthetic chemicals for combating destructive bacterial and fungal diseases has limitations due to development of resistance (Mustafa *et al*. 2004), high cost, harmful effects to the users, environment and consumers (Taechowisan and Lumyong 2003). Due to all-year round cultivation of Solanaceous crops in the highland tropics, disease and infectious propagules are continuously present, and biological control as cultural means such as crop rotation cannot be relied upon to control the disease (Lehman *et al*. 2005).

Research on biological control of plant pathogens has received much attention in recent years as a means of increasing crop production by avoiding a number of problems related to chemical control and developing practices compatible with sustainable agriculture (Bowen and Rovira 1999; Evidente *et al*. 2011). *Pseudomonas fluorescens* is worldwide in distribution and is present in all North American and globally. In nature, it is a harmless bacterial species that is found protecting the roots of plants from plant diseases (Molloy 2002). Among different bacteria, *Bacillus* spp., *Pseudomonas* and *Streptomyces* were widely used as biocontrol agents (Freir *et al*. 1991). Rhizospheric microorganism inhibits *A. solani*, disease causing microorganism extracted from tomato phylosphere in dual culture assay

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and also prevented the disease spread in field experiments (Ahmed and Saleh 1990). Various mechanisms are involved in disease suppression by *Pseudomonads* viz. production of siderophores, hydrogen cyanide (HCN) and antibiotics, competition, induction of synthetic resistance, etc. (Ryan 2004). For effective bio-control of the early blight disease, there is a need to isolate efficient microbes, preferably from the same environment to which they can be used, and to know their mechanism of disease suppression. Such isolates are ecologically fit than the strains introduced from other locations.

The main objective of this study was to evaluate the inhibition effect of some rhizospheric bacteria against *A. solani* and to study the effect of some rhizospheric antagonistic bacteria on growth and development of tomato.

MATERIALS AND METHODS

Description of the study area and topography

Screening experiment was conducted at Ambo Plant Protection Research Center (APPRC) Western Shoa, Ormomia Zone, Ethiopia and the samples of early blight and soils were collected from tomato farm of 0.5 ha located in Nagafile, at Tokke Kutaye Woreda during 2009-2010 off season. Tokee Kutaye has total geographical area of 78,887 sq. km and is located at 8°57' North latitude and 38°07' East longitude at an average elevation of 1800-2300 m above the sea level. While 23% of the area is high land, 60% is middle altitude, and the remaining 17% categorized as low land.

Sample collection

Infected tomato leaves and rhizosphere soils were collected from Nagafile farmers filed randomly. At each study site an area of 500 m^2 was chosen for sampling. The samples collected in triplicates were brought to Ambo Plant Protection Research Center laboratory in sealed plastic bags and stored at 5°C until processed for use. The leaves were washed thoroughly to free attached soil particles.

Isolation of the pathogen from infected leaves

A. solani was isolated from diseased leaves of tomato using a standard plating technique on potato dextrose agar medium (PDA) medium composed of infusion from 200 g, 20 g dextrose and 15 g agar/l of water which were made selective for isolation of fungus (Das *et al*. 2003). Accordingly, the infected leaves were washed thoroughly in distilled water and then surface sterilized in 75% alcohol for 90 sec, followed by washing three times in sterile distilled water and then inoculated on PDA medium. The plates were incubated at 28°C for 7-10 days. After incubation the development of colonies on the medium was observed and the results were recorded.

Isolation of native bacteria from soils

Bacterial species were isolated from rhizosphere soils of tomato using standard serial dilution plating technique (Waksman 1922). The soils were serially diluted in sterile distilled water. Dilutions such as 10^{-6}, 10^{-7} and 10^{-8} were taken for bacterial isolation. All isolate of *P. fluoresecens* were screened for their toxicity toward the pathogen on King's B medium (KB) agar plates in dual culture assays. Medium composed of 20 g Protease peptone No. 3 (Difco), 1.5 g $K_2HPO_4 \cdot 7H_2O$, 15 ml glycerol and 15 g agar/l of water (Ganesan and Gnanamanickam 1987). Nutrient agar (NA) medium was composed of 1 g beef extract, 2 g yeast extract, 5 g NaCl and 15 g agar/l of water. 15 to 20 ml of molten King's B agar medium was prepared and poured into sterile Petri dishes under aseptic conditions. Serially diluted samples were inoculated into the plate by spread plate method. The plates were incubated at 37°C for 24 h. Then the plates were observed and the results were recorded.

Dual culture technique

All the bacterial isolates were tested for antagonism against *A. solani* on PDA by dual culture technique (Anith and Manomahandas 2001). The agar suspension was then dispensed into Petri dishes and allowed to solidify and spot inoculated with the test strain from 24-h old cultures were used (Sakthivel *et al.* 1998). A mycelial plug from an actively growing *A. solani* on PDA was taken with a cork borer (10 mm diameter) and kept at the center of the agar medium in a Petri dish (90 mm diameter). The bacterial isolates were streaked on either side of the pathogen after 48 h of fungal inoculation. Plates were incubated at 30°C and the zone of inhibition (ZOI) of the fungal growth recorded after 7 days. Growth inhibition was calculated as:

% inhibition = (1 – (Fungal growth / Control growth)) × 100

Table 1 Treatment description in the experiment.

Treatment number	Treatment
T_1	Pathogen + TK-1
T_2	Pathogen + TK-3
T_3	Pathogen + TK-4
T_4	Pathogen + TK-8
T_5	Pathogen + TK-10
T_6	Pathogen alone
T_7	Un-inoculated control
T_8	Chemical control (Mancozeb 0.2%)
T9	Pathogen + *Pseudomonas fluorescens* (TK-3) + *Bacillus subtilis* (TK-4)
T10	*Pseudomonas fluorescens* (TK-3) + *Bacillus subtilis* (TK-4)

Screening *in vivo*

All the bacterial isolates were screened *in vivo* for control of early blight disease on tomato plants. Three weeks old tomato seedlings were transplanted to plastic pots. The spore suspension of the pathogen containing 10^{-6} spores/ml was sprayed on to one-month-old tomato seedlings. Bacterial isolates grown in nutrient broth (10^{-6} × cells/ml) were sprayed onto tomato seedlings one day after the challenge inoculation. Percent Disease Index (PDI) and Percent Disease Control (PDC) were calculated using the formula given by (Wellker 1988). The percentage of disease control was scored.

$$PDC = \frac{DC\text{-}DT}{DC} \times 100$$

$$\%DC = \frac{C\text{-}T}{C} \times 100$$

where PDC – percentage disease in control; DC – disease in control; DT – disease in treated plants.

$$PDI = \frac{\sum}{X_n \times X_{xL}} \times 100$$

where PDI – percentage disease index; ∑– sum of all ratings; X_n – number of plants; X_{xL} – maximum rating grade.

Based on *in vitro* inhibition of the pathogen (zone of inhibition = 45.07-43.35) and percent disease control (43.97-42.89%) by the bacterial isolates, 5 promising antagonists were selected. In the inoculated treatment, the percentage disease control was 0%, i.e., all plants were infected and there was less produce. They were identified up to the generic level by assessing morphological and biochemical characters. The five promising antagonists were evaluated in greenhouse for their biocontrol and plant growth promotion efficiency. Detail of treatments is listed in **Table 1**.

Greenhouse experiment

The greenhouse experiment was conducted at the Ambo Plant Protection Research Center (PPRC), based on *in vitro* inhibition of the pathogen and percent disease control by the bacterial isolates. The five promising antagonistic bacterial isolates (*P. fluorescens* TK-1, *P. fluorescens* TK-3, *Bacillus subtilis* TK-4, *P. fluorescens*

TK-8 and *P. fluorescens* TK-10) were used to control early blight disease in tomato cv. 'Romans VS'. The experiment was laid out in a completely randomized design (CRD) with 10 treatments and 4 replications including the un-inoculated control.

Pot culture experiment

The experiment was carried out in 20 × 15 cm diameter plastic pot. Mixed soil of cattle manure, sandy and clay loam (1: 1: 2) respectively was autoclaved at 121°C for 2 h and 3 Kg of sterilized soil at pH 6.8, was filled into plastic pots. Two tomato plants were transplanted to each pot and were regularly watered with deionized water. The infection percentage, plant height and biomass of shoot and roots were recorded after 60 days.

Data analysis

Plants were harvested 60 days after transplanting. The plant height was measured and the total dry biomass (shoot + root) was determined after drying the plant samples in oven at 60°C for 48 h. The disease infection percent and percent disease control were calculated.

Statistical analysis

The results obtained were subjected to analysis of variance by complete randomized design and the treatment means were separated by Duncan's multiple range test ($P < 0.05$) (Little and Hills 1978). Statistical analyses were conducted with SAS v. 8.

RESULTS AND DISCUSSION

Screening and *in vitro* study of inhibition of *A. solani* by native bacterial isolates

As many as 10 bacteria were isolated from rhizosphere soil samples of healthy tomato plants from a Nagafile farmer's field at Toke Kutaye Woreda. From the place where the soil sample was taken, King's B mediums were used to isolate bacteria as it is known to support growth of all types of bacteria. All 10 bacterial isolates were tested for *in vitro* inhibition of the pathogen showing a ZOI ranging from 0 to 45.0 mm. The results are in agreement with the work of Hafeez *et al*. (2001) and Venugopal *et al*. (2003) who reported the inhibition of *A. solani* by microorganisms in the range of 45 to 50 mm diameter. As many as 5 isolates were found to be strong antagonists with a ZOI or limited the growth of the fungus of ≥ 40.0 mm diameters. The highest ZOI of 45.0 was produced by TK-3, followed by TK-4, TK-8, TK-10 and TK-1 isolates. The least ZOI of 0 and 0.6 mm diameter were produced by TK-9 and TK-7 strains, respectively; the remaining strains were moderate inhibitors of *A. solani* (**Table 2**). The antagonistic effect appeared two days after bacteria and fungi were co-incubated and advanced in the days after inoculation. The viability test was recorded 7 days after inoculation. Bacterial isolates were identified up to the species level by assessing morphological and biochemical characteristics.

B. subtilis were tested for its ability to reduce disease incidence in pot culture. Among the isolates, TK-3 isolate alone with the pathogen gave the maximum disease control followed by TK-4 alone. Amongst the isolates tested, TK-3 remained efficient throughout the period of experimentation. Amaresh (2000) reported similar results where foliar spray of *P. fluorescens* showed 48.9% control of *Alternaria* blight and chemical spraying (Mancozeb 0.2%)® remained superior with a percent disease control of 61.30%. Similarly, Yegesh *et al*. (2008) reported that native isolates Pf-551 and Pf-572 individually and in combination with Pf-173 and Pf-547 were found effective with respect to plant growth promotion and bio-control against *Fusarium solani* in peas.

Screening of bacterial isolates by *in vivo* for the control of *A. solani*

All the 10 bacterial isolates were tested *in vivo* for their ability to control the early blight disease in tomato seedling under pot culture conditions. The results indicated that the percent disease control by the inoculated bacterial isolate varied from 0 to 43.79% (**Table 3**). Out of 10 bacterial isolates, 5 isolates showed very high disease control ranging above 38% after 60 days of sowing, and the remaining five isolates showed very low disease control (**Table 3**).

In conformity with the findings of Broadbent *et al*. (1971), Burr *et al*. (1978) and Jagadeesh 2000), the present study revealed little correlation between the *in vitro* inhibition of the pathogen and *in vivo* control of the disease. Based on *in vitro* and *in vivo* performances of the isolates, 5 promising isolates were selected. A large population of antagonists seems to be made from *F. fluorescens pseudomonads* and non-*F. fluorescens pseudomonads* put together. Non-fluorescent rhizobacteria antagonistic to a number of phytopathogens have been reported by others (Rangeswaran and Prasad 2000; Venugopal *et al*. 2003).

Fluorescens pseudomonas is a ubiquitous bacterium in agricultural soils and have many traits that make them well suited as PGPRs. The simultaneous screening of *fluorescens* rhizobacteria for growth promotion under gnotobiotic conditions and *in vitro* production of auxins is a useful approach for selecting effective PGPRs.

Plant growth promotion and bio-control activities of indigenous antagonistic bacteria against *A. solani* in tomato

Five promising isolates were identified up to generic and species level. Out of five isolates, four belonged to *F. pseudomonads* (TK-1, TK-3, TK-8, TK-10) and one *B. subtilis* (TK-4) (**Table 4**).

The group *F. pseudomonads* entails different species, which possess many beneficial activities such as production of antibiotics, chitinolytic enzymes, siderophore, HCN, plant growth hormones, mineral solubilization, etc. (Davision 1988; Defago and Hass 1990).

Various mechanisms are involved in disease suppression by *Pseudomonads* viz. production of siderophores,

Table 2 *In vitro* inhibition of *Alternaria solani* by the indigenous isolates of bacteria (dual culture method).

Serial No.	Isolate No.	ZOI (mm)
1	TK-3	45.07 a
2	TK-4	44.17 b
3	TK-10	44.12 b
4	TK-8	43.95 b
5	TK-1	43.35 c
6	TK-2	22.12 d
7	TK-5	21.92 d
8	TK-6	8.25 e
9	TK-7	6.10 f
10	TK-9	0.00

Means in the same column followed by different letters indicate highly significant differences according to DMRT ($P < 0.05$)

Table 3 Disease control of the native isolates bacteria against *Alternaria solani* (*in vivo*).

Isolate No.	% Disease control
TK-3	43.79 a
TK-4	43.08 b
TK-10	42.94 b
TK-8	42.92 b
TK-1	42.89 b
TK-2	23.28 c
TK-5	23.21 c
TK-6	22.81 c
TK-7	21.73 d
TK-9	0.00

Means in the same column followed by different letters indicate highly significant differences according to DMRT ($P < 0.05$)

Table 4 Influence of antagonistic bacteria on percent of disease control and disease index of *A. solani* in cv. 'Romans VS'.

Treatments	% Disease index	% disease control	
		45 DSA	75 DAS
Pathogen + *Pseudomonas fluorescens* (TK-1)	22.58 ± 1.64 c	40.16 ± 1.50 c	45.34 ± 0.79 c
Pathogen + *Pseudomonas fluorescens* (TK-3)	21.15 ± 0.82 d	43.79 ± 1.63 b	47.79 ± 0.82 b
Pathogen + *Bacillus subtilis* (TK-4)	21.23 ± 0.78 c	41.27 ± 2.34 c	47.12 ± 0.86 b
Pathogen + *Pseudomonas fluorescens* (TK-8)	22.60 ± 0.02 c	38.16 ± 1.86 e	40.40 ± 1.89 e
Pathogen + *Pseudomonas fluorescens* (TK-10)	22.60 ± 0.72 c	39.64 ± 2.04 d	42.74 ± 0.91 d
Chemical spraying (Mancozeb 0.2%)	19.45 ± 0.76 e	61.30 ± 1.03 a	63.40 ± 1.15 a
Pathogen + *Pseudomonas* spp.+ *Bacillus subtilis*	20.46 ± 1.07 e	39.84 ± 1.62 d	41.65 ± 0.86 d
Pseudomonas spp.+ *Bacillus subtilis*	-	-	-
Pathogen alone	58.60 ± 0.82 a	-	-
Un-inoculated control	32.58 ± 0.59 b	-	-

Means in the same column followed by different letters indicate highly significant differences according to DMRT ($P < 0.05$)

Table 5 Effect of antagonistic bacteria on plant height and biomass of tomato cv. 'Romans VS'.

Treatment	Plant height (cm)	Dry weight (g)		
		Shoot	Root	Total
Pathogen + *Pseudomonas fluorescens* (TK-1)	32.45 ± 0.99 e	33.30 ± 1.6 ef	14.25 ± 0.87 c	48.30 ± 0.17
Pathogen + *Pseudomonas fluorescens* (TK-3)	35.20 ± 0.88 bc	36.43 ± 0.62 d	15.43 ± 0.62 bc	52.28 ± 0.66
Pathogen + *Bacillus subtilis* (TK-4)	33.43 ± 0.82 de	34.38 ± 0.95 e	14.25 ± 0.75 c	49.25 ± 0.79
Pathogen + *Pseudomonas fluorescens* (TK-8)	32.45 ± 1.62 e	32.48 ± 0.60 f	14.43 ± 1.53 c	47.40 ± 0.80
Pathogen + *Pseudomonas fluorescens* (TK-10)	32.48 ± 1.06 e	32.65 ± 0.90 f	14.55 ± 0.79 c	46.50 ± 0.80
Chemical spraying (Mancozeb 0.2%)	38.50 ± 0.79 a	42.65 ± 0.70 a	18.50 ± 0.73 a	60.43 ± 1.72
Pathogen + *Pseudomonas fluorescens* + *Bacillus subtilis*	34.28 ± 0.65 dc	38.43 ± 0.70 c	16.38 ± 0.79 b	55.40 ± 0.70
Pseudomonas spp.+ *Bacillus subtilis*	36.38 ± 0.77 b	40.55 ± 1.88 b	16.53 ± 1.39 b	57.45 ± 0.99
Pathogen alone	27.38 ± 1.09 f	28.43 ± 1.11 h	11.53 ± 0.74 d	39.58 ± 2.08
Un inoculated control	27.18 ± 0.79 f	30.53 ± 0.58 g	12.54 ± 1.00 d	42.35 ± 0.74

Means in the same column followed by different letters indicate highly significant differences according to DMRT ($P < 0.05$)

hydrogen cyanide (HCN) and antibiotics, competition, induction of synthetic resistance, etc. (Ryan 2004).

All the 5 promising isolates were tested for their ability to reduce the disease incidence in pot culture. At the 45th day after sowing (DAS) (= 15 days after inoculation), maximum disease control was recorded in the chemical check (61.30 ± 1.03). Among the isolates, TK-3 gave the maximum disease control (43.79%), followed by TK-4 (41.27%). At 75 DAS also, chemical spraying resulted in higher disease control of 63.40%. This was followed by TK-3 (47.80%). Among the isolates tested, TK-3 remained efficient throughout the experimental period. It is clear that TK-3 is an efficient biocontrol agent with good in *vitro* and in *vivo* antagonistic activity.

Among the different treatments, *P. fluorescens* + *B. subtilis* followed by *P. fluorescens* (TK-3) showed maximum plant height, shoot and root dry biomass (**Table 5**). However, the lowest plant height, shoot and root biomass were recorded in plants inoculated with *A. solani* alone and also the treatment *P. fluorescens* with the pathogen showed highest plant height and root shoot biomass. In the last two decades endophytic bacteria especially *P. fluorescens* have received considerable attention as potential biocontrol agent of a number of soil-borne pathogens. Unfortunately, the seemingly inherent variable performance of most biocontrol strains between field locations and cropping seasons has hampered commercial development, and relatively few biological agents are registered for use in production agriculture Cook (1996). The plant growth-promoting rhizobacteria (PGPRs), of which fluorescent Pseudomonads are major players, could play an active role in plant growth promotion and disease suppression as an active component of organic farming Yegesh *et al*. (2008).

In the present study, the indigenous isolate TK-3 was found effective with respect to plant growth promotion and biocontrol against *A. solani* in tomato. However, field experimentation is required to conclusively prove the biocontrol potential and plant growth promotion with yield of the isolate for its commercial exploitation as a bio-control agent.

CONCLUSION AND RECOMMENDATIONS

For effective bio-control of early blight disease, there is a need to isolate efficient microbes, preferably from the same environment to which they can be used, and their mechanism of disease suppression is known. Such isolates are ecologically fit than the strains introduced from other locations. The efficacy test results of antagonistic bacterial isolates in this experiment have clearly indicated that the indigenous strain, *P. fluorescens* (TK-3) is an efficient biocontrol agent against *A. solani* with good *in vitro* and *in vivo* antagonistic activity. However, field experiments are required to conclusively prove the bio-control potential and plant growth promotion with yield of the isolate for its commercial exploitation as a bio-control agent. The findings of the current study indicate the potential of Ethiopian *P. fluorescens* strain as an efficient biological control agent against *A. solani* in tomato. Considering the condition of Ethiopian farmers, the results of this research could be used as eco-friendly technology with relatively low cost than inorganic chemicals.

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REFERENCES

Agrios GN (1988) *Plant Pathology*, Academic Press Ltd., London, 803 pp

Ahmed MA, Saleh EA (1990) Phyllosphere microflora of tomato leaves and their antagonistic activity against *Alternaria solani*. *Food Science and Microbiology* **4**, 106-122

Amaresh YS (2000) Epidemiology and management of *Alternaria* leaf blight on sunflower (*Helianthus annuus* L.). PhD thesis, University of Agricultural Sciences, Dharward, Karnataka, India

Anith KN, Manomhandas TP (2001) combined application of *Trichoderma harzianum* and *Alcaligenes* sp. strain AMB and for controlling nursery rot disease of black pepper. *Indian Phytopathology* **54**, 335-339

Bebeli PJ, Mazzucato A (2008) The Solanaceae – A Review of Recent Research on Genetic Resources and Advances in the Breeding of Tomato, Pepper and Eggplant. In: Passam H (Ed) The Fruiting Species of the Solanaceae. *The European Journal of Plant Science and Biotechnology* **2 (Special Issue 1)**, 3-30

Broadbent P, Bakker KF, Waterworth Y (1971) Bacteria and actinomycetes antagonistic to fungal root pathogen in Australian soil. *Australian Journal of Biological Science* **24**, 925-944

Burr TJ, Schroth MN, Suslow TV (1978) Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonads fluorescens* and *P. putida*. *Phytopathology* **68**, 1377-1383

Cook RJ (1996) Assuring the safe use of microbial biocontrol agents. A need for policy based on real rather than perceived risks. *Canadian Journal of Plant Pathology* 1**8**, 439-466

Das BC, Devi P, Dutta P (2003) Management of *Sclerotium rolifsii* in tomato by fungal antagonists, *Journal of Agricultural Science Society* **13**, 101-103

Dater VV, Mayee CD (1985) Chemical management of early blight of tomato. *Journal of the Maharashtra Agricultural University* **10 (3)**, 278-280

Evidente A, Andolfi A, Cimmino A (2011) Fungal phytotoxins for control of *Cirsium arvense* and *Sonchus arvensis*. In: Peng G, Wolf TM (Eds) *Bioherbicides*. *Pest Technology* **5 (Special Issue 1)**, 1-17

Freir JHS, Mathee FN, Thomas AC (1991) Biological control of Eutypalota on grapevine by an antagonistic strain of *Bacillus subtilis*. *Phytopathology* **81**, 283-287

Ganesan P, Gnanamanickam SS (1987) Biological control of S *sclerotium rolfsii sacc* in pea nut by inoculation with *P. fluorescens. Soil Biology and Biochemistry* **19**, 35-38

Hafeez A, Khan MA, Chohan RA (2001) *In vitro* evaluation of various antagonists and plant extracts against mycelia growth of *Altrnaria solani*. *Pakistan Journal of Phytopathology* **13 (2)**, 127-129

Jagadeesh KS (2000) Selection of rhizobacteria antagonistic to *Ralstonia solanacearum* E.F. Smith causing bacteria wilt in tomato and their bio control mechanism. PhD thesis, University of Agricultural science Dharwad, Karnataka

Lehman LJ, Randy JM, Caiyao Y, Denise MC, Orjala JC, Marone PG, Santamaria JIJ (2005) Metabolite from *Sterptomyces* strain NRRL accession No.B-30145 and mutants thereof for controlling plant disease US patent 6,852,317

Mathur K Shekhawat KS (1986) Chemical control of early blight in kharif sown tomato. *Indian Journal of Mycology and Plant Pathology* **16**, 235-238

Molloy DP (2002) Biological control of zebra mussels. In: *Proceedings of the 3rd California Conference on Biological Control*, University of California, Davis, pp 86-94

Mustafa SA, Tamer UA, Azer C (2004) Antibiotic of some actinomycetes isolated from farming soil of Turkey. *African Journal of Biotechnology* **3 (9)**, 441-446

Rangeswaran R, Prasad RD (2000) Isolation and evaluation of rhizosphere bacteria for biological control of chickpea wilt pathogen. *Journal of Biological Control* **14**, 9-15

Reni C E Roeland (2006) Tomato early blight (*Alternaria solani*) the pathogen, genetics, and breeding for resistance. *Journal of General Plant Pathology* **72 (13)**, 335-347

Rice RP, Rice LW, Tindall HD (1987) *Fruit and Vegetable Production in Africa.* Macmillan Publishers, UK, pp 371-399

Ryan K (Eds) (2004) *Sherris Medical Microbiology* (4th Edn), McGraw Hill, London

Sakthivel N, Sivamani E, Anuratha CS, Savithiry S, Gnanamickam SS, Mahadevar A (1998) Beneficial bacteria for plant disease management. In: *Proceedings of National Symposium on Phytobacteriology*, University of Madras, 14-15 March, 1998, Madras, India, pp 213-220

Taechowisan T, Lumyong S (2003) Activitey endophytic actinomycetes from roots of *Zingiber officinale* and *Alpina galangal* against phytopathogenic fungi. *Annals of Microbiology* **53 (3)**, 291-298

Tesfaye B (2008) Response of tomato cultivars differing in growth habit to nitrogen and phosphorus fertilizers and spacing on vertisol in Ethiopia. *Acta Agriculturae Slovenica* **91 (1)**, 103-119

Venugopal N, Earnapalli KS, Jagadeesh KS (2003) Screening of antagonistic bacteria for biological control of early blight caused by *Alternaria solani*. MSc thesis, University of Agricultural Sciences, Dharwad, India

Waksman SA (1922) A method of counting the number of fungi in the soil. *Journal of Bacteriol*ogy **7**, 339-341

Wellker DM (1988) Biological control of soil borne plant pathogens in the rhizosphere whit bacteria. *Annual Review of Phytophathology* **26**, 379-407

Yegesh K, Negi S, Garg K, Kumar J (2008) Plant growth promoting and biocontrol activities of cold tolerant *Pseudomonas fluorescens* isolates against root rot in pea. *Indian Phytopathology* **61 (4)**, 461-470