Plant Archives Vol. 15 No. 1, 2015 pp. 411-416 *ISSN 0972-5210*

IN VITRO AND *IN VIVO* EVALUATION OF ANTAGONISTIC MICROORGANISMS ON THE INCIDENCE OF LEAF BLIGHT OF SUNFLOWER CAUSED BY *ALTERNARIA HELIANTHI*

M. E. Shilpa, H. M. Vikas[1], P. Sowmya[2], M. Srikantaiah and A. V. D. Dorajeerao[3]

Department of Agricultural Microbiology, University of Agricultural Sciences, G.K.V.K., Bangalore-65 (Karnataka), India.
[1]College of Horticulture, University of Horticultural Sciences Campus, G.K.V.K., Bangalore - 65 (Karnataka), India.
[2]Department of Sericulture, University of Agricultural Sciences, G.K.V.K., Bangalore-65 (Karnataka), India.
[3]Department of Horticulture, University of Agricultural Sciences, Dharwad (Karnataka), India.

Abstract

Biological control of leaf blight of sunflower caused by *Alternaria helianthi* was investigated by four antagonistic microorganisms like *Trichoderma viride*, *Pseudomonas fluorescens*, *Streptomyces griseus* and *Bacillus subtilis* under *in vitro* conditions by dual culture technique. Inhibition zone in mm was recorded and the per cent inhibition was calculated. Glasshouse and field studies were conducted to test the ability of antagonistic microorganisms on incidence of leaf blight of sunflower. The least per cent disease incidence was recorded in the treatment combination having soil application of *A. chroococum* + *B. megaterium* + *T. viride* with 75% NP + 100% K and foliar spray of *T. viride* and maximum disease incidence was recorded in the uninoculated control plant under glasshouse study and field experiments showed that least disease incidence was recorded in the chemical treated plots. Among the bioagents, plants supplemented with 100% NPK + soil application of microbial consortium (*A. chroococcum*, *B. megaterium* and *T. viride*) + foliar spray of *T. viride* (T_3) (14.27%) showed least disease severity.

Key words : *Alternaria helianthi,* antagonistic microorganisms, biological control, sunflower.

Introduction

Sunflower (*Helianthus annuus* L.) is a major edible oilseed crop next to soybean and groundnut at the global level. Sunflower oil is rich in PUFA (poly unsaturated fatty acids) and contains high concentration of linoleic acid. The crop is affected by several biotic and abiotic stresses. Low productivity in sunflower seems to be due to poor seed setting and high per cent of chaffy seeds in the capitulum. Among the several biotic factors, susceptibility to diseases is one of the major constraints for successful sunflower production. The major diseases of sunflower in India are leaf blight (*Alternaria helianthii*), wilt (*Sclerotium rolfsii*) and root rot caused by *Rhizoctonia solani* and *Macrophomina phaseolina*. Gulya and Masirevie (1991) listed 80 pathogens occurring on sunflower. Among this leaf blight caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara has been considered as potentially destructive disease in many parts of the sunflower growing countries.

Biofertilizers play a pivotal role in sustainable agriculture to improve soil health and crop productivity through efficient nutrient supply. They control soil-borne diseases and induce resistance in crops against pests and diseases. A successful microbial bio-control agent is the one that compete with pathogen in the infected tissues. Antagonistic micro-organisms like *Trichoderma viride*, *Streptomyces griseus*, *Pseudomonas fluorescens* and *Bacillus subtilis* etc., act against number of plant pathogens due to their ability to produce secondary metabolites such as siderophores, antibiotics, HCN production, volatile compounds, enzymes and phytohormones. They can elicit host crop growth in addition to disease control (Baker *et al*., 1984); hence they are called as microbial bio-control agents and as yield enhancers.

With the growing awareness of harmful effects of pesticides, integration of disease tolerant cultivar, crop rotation or sanitation practices and use of bio-agents and

plant extracts to reduce fungicidal spray is gaining importance. In this study, an attempt was made to study the effect of biofertilizer and microbial biocontrol agents to overcome leaf blight disease of sunflower.

Materials and Methods

Isolation of fungi

The sunflower leaves infected by *Alternaria helianthi* showing typical dark brown to black, circular to irregular spots were collected from the field in *kharif* 2012 and brought to the laboratory for isolation of the causal agent. *Alternaria* infected leaves exhibiting typical *Alternaria* leaf spot symptoms were selected and pathogen was isolated by following standard tissue isolation method. The infected leaf bits measuring about 2 mm were transferred to petri plates containing Potato Dextrose Agar (PDA) (3 pieces/dish). These plates were incubated at 27 ± 1°C. After 8 days of incubation the growth of the fungus in association with the leaf spot was observed.

In vitro evaluation of bioagents

The antagonistic microorganisms like *Trichoderma viride*, *Pseudomonas fluorescens*, *Streptomyces griseus* and *Bacillus subtilis* were evaluated for their antagonistic effect in *in vitro* conditions against soil borne pathogen *Alternaria helianthi* by dual culture technique. The cultures of antagonistic microorganisms used in the present study were obtained from the Department of Agricultural Microbiology, University of Agricultural Sciences, Bengaluru (Karnataka), India.

Dual culture technique

In dual culture technique, 20 ml of sterilized and cooled potato dextrose agar was poured into sterilized petri plates. Fungal antagonists were evaluated by inoculating the pathogen at one side of the petri plate and the antagonist was inoculated exactly on opposite side of the same plate by leaving 3-4 cm gap. For this, actively growing cultures were used. In case of bacterial antagonist evaluation, two mycelial discs of pathogen were inoculated and bacterial antagonist was streaked at the centre of the plate. Each treatment was replicated three times. After required period of incubation *i.e.*, after mycelial growth in control plate reached 90 mm diameter, the radial growth of pathogen was measured. Per cent inhibition over control was worked out according to equation given by Vincent (1947).

$$I = \frac{(C-T)}{C} \times 100$$

I = Per cent inhibition of mycelium.

C = Growth of mycelium in control.

T = Growth of mycelium in treatment.

Preparation of bio-control agents for spraying

Fungal bio-control agents were first grown on PDA plates and then 5 mm mycelial disc of bio-control agent was transferred to sterile potato dextrose broth aseptically and incubated at 28 ± 1°C on a rotary shaker at 150 rpm for 5 days, then shaking was stopped and allowed to form mat and after 7 days the bio-control agent culture was homogenized thoroughly to break the mycelial bits and the culture is filtered aseptically using Whatman no. 44 filter paper and the filtrate containing cell suspension was used for spraying at the rate of 0.5% concentration.

Bacterial bio-control agents were first grown on the respective medium and then transferred to sterile respective broth aseptically and incubated at 28 ± 1°C on a rotary shaker at 150 rpm for 5 days, then shaking was stopped and allowed till good turbidity was formed, the biocontrol agent culture was homogenized thoroughly and filtered aseptically using Whatman no. 44 filter paper and the filtrate containing cell suspension was used for spraying at the rate of 0.5% concentration.

A pot culture experiment was conducted under glass house condition to screen an efficient microbial biocontrol agents for control of leaf blight of Sunflower. The experiment was laid out in Complete Randomized Design (CRD), which had 16 treatments and three replications. Sunflower plants were raised in 5 kg capacity polythene bags containing uniform soil mixture in a glass house by following the recommended package of practices. The recommended dose of fertilizers for sunflower is 62.5:75:62.5 Kg NPK/ha. 75% of nitrogen was supplied at the rate of 0.25 g per pot of soil in the form of urea, 75% of phosphorus and 100% of potassium at the rate of 0.87g and 0.09 g per pot, respectively were supplied in the form of single super phosphate and muriate of potash. The pathogen inoculum suspension from ten days old culture was prepared and sprayed on to the plants. The plants were covered with polyethylene cover and were incubated for 48 hours to ensure successful penetration of the pathogen into the tissue. The polythene covers were removed after five days and seedlings were kept under greenhouse conditions. Observations were made regularly for the appearance and development of disease symptoms. Disease severity was recorded at 60 DAS by using 0-9 scale (Mayee and Datar, 1986).

Rating value	Description
0	No symptoms on the leaf
1	Small circular, scattered, brown spots covering 1 per cent of the leaf area.
3	Spots enlarging, dark brown in colour covering 1 to 10 per cent of the leaf area and infection on lower most leaves of the plant.
5	Spots enlarging, dark brown in colour covering 11 to 25 per cent of leaf area and infection half of the plant.
7	Spots dark brown coalescing, occupying 26 to 50 per cent of leaf area and covering 1/3 of the plant.
9	Spots uniformly dark brown, coalescing, covering 50 per cent or more leaf area, severe infection.

Treatments details

T_1 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *T. viride*

T_2 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *T. viride* + FS of *S. griseus*

T_3 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *T. viride* + FS of *T. viride*

T_4 : 75% NP + 100% K+ SA of *A. chroococum* + *PSB* + *T. viride* + FS of *B. subtilis*

T_5 : 75% NP + 100% K+ SA of *A. chroococum* + *PSB* + *T. viride* + FS of *P. fluorescens*

T_6 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *B. subtilis*

T_7 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *B. subtilis* + FS of *S. griseus*

T_8 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *B. subtilis* + FS of *T. viride*

T_9 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *B. subtilis* + FS of *B. subtilis*

T_{10} : 75% NP +100% K + SA of *A. chroococum* + *PSB* + *B. subtilis* + FS of *P. fluorescens*

T_{11} : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *P. fluorescens*

T_{12} : 75% NP +100% K + SA of *A. chroococum* + *PSB* + *P. fluorescens* + FS of *S. griseus*

T_{13} : 75% NP +100% K + SA of *A. chroococum* + *PSB* + *P. fluorescens* + FS of *T. viride*

T_{14} : 75% NP +100% K + SA of *A. chroococum* + *PSB* + *P. fluorescens* + FS of *B. subtilis*

T_{15} : 75%NP + 100% K +SA of *A. chroococum* + *PSB* + *P. fluorescens* + FS of *P.fluorescens*

T_{16} : Control- without any chemical fertilizers and biofertilizers

Note

1. Biofertilizers treatment : Applied to soil a day after sowing.

2. SA : Soil Application

3. FS : Foliar Spray @ 0.5% given at 30 and 45 days after sowing.

The field experiment was conducted at Zonal Agricultural Research Station (ZARS), University of Agricultural Sciences, G.K.V.K., Bengaluru (12°58 North, 77°35 East, altitude of 899 m) on sunflower (*Helianthus annuus* L.), hybrid KBSH 44 at different levels of nitrogen and phosphorous fertilizers (100% recommended dose and 75%) during *Kharif* season 2012 to evaluate the antagonistic microorganisms on the incidence of leaf blight of sunflower caused by *Alternaria helianthi*. The soil under experimentation was red sandy loam in texture with 165.5 kg ha^{-1} available N, 26.6 kg ha^{-1} P and 65.7 kg ha^{-1} K. The experiment was laid out in a Randomized Complete Block Design, which had 12 treatments with three replications.

The pure cultures of *Azotobacter chroococcum*, *Bacillus megaterium* and *Trichoderma viride* were obtained from the culture collection of Biofertilizers Scheme, Department of Agricultural Microbiology, University of Agricultural Sciences, G.K.V.K., Bengaluru and were mass multiplied in the laboratory. The final carrier based product was applied in soil 7 days after sowing at the recommended rate (*Azotobacter chroococcum* - 10 kg ha^{-1}, *Bacillus megaterium* - 10 kg ha^{-1} *Trichoderma harzianum*- 10 kg ha^{-1}). The amount of inoculum applied to each plant was 10 g .

Treatment details of the field experiment

T_1 : 100% NPK

T_2 : 75% NP +100% K

T_3 : T_1 + S. A. of *A. chroococum* + *PSB* + *T. viride* + F.S of *T. viride*

T_4 : T_2 + S. A. of *A. chroococum* + *PSB* + *T. viride* + F.S of *T. viride*

T_5 : T_1 + S.A. of *A. chroococum* + *PSB* + *T. viride* + F.S. of *S. griseus*

T_6 : T_2 + S.A. of *A. chroococum* + *PSB* + *T.viride* + F.S of *S. griseus*

T_7 : T_2 + S.A. of *A. chroococum* + *PSB* + *B. subtilis* + F.S of *T. viride*

T_8 : T_2 + S. A. of *A. chroococum* + *PSB* + *B. subtilis* + F.S of *T. viride*

Table 1 : Dual culture technique : *In vitro* evaluation of microbial bioagents on *Alternaria helianthi*.

Treatments	Radial growth of pathogen	Per cent inhibition over control (%)
A. helianthi	4.50 cm	——
Trichoderma viride + *A. helianthi*	0.75 cm	83.30
Streptomyces griseus + *A. helianthi*	1.40 cm	68.80
Bacillus subtilis + *A. helianthi*	1.87 cm	58.33
Pseudomonas fluorescens + *A. helianthi*	2.18 cm	51.55

Alternaria leaf blight caused by *Alternaria helianthii* and the effect of bio protectants on sunflower was determined by scoring as disease severity. *Alternaria* leaf blight appears first on lower leaves and later spreads to the whole plant. The disease severity was assessed on three leaves of randomly selected plants at 50% seed filling stage using 0-9 scale (Mayee and Datar, 1986). The field experimental data was analysed statistically by Fischer's method of analysis of variance as given by Panse and Sukhatme (1967).

Results and Discussion

In vitro evaluation of bio agents by dual culture technique

A dual culture test is extensively used as one of *in vitro* tests for preliminary screening of biological control agents, the antagonistic microorganisms *viz*., *Trichoderma viride*, *Streptomyces griseus*, *Bacillus subtilis* and *Pseudomonas fluorescens* were evaluated for their antagonistic effect against *Alternaria helianthi* through dual culture technique. Maximum inhibition of mycelial growth of *Alternaria helianthii* was noticed in *Trichoderma viride* (83.30%) followed by *Streptomyces griseus* (68.80%), *Bacillus subtilis* (58.33%) and least inhibition of mycelia growth was observed in *Pseudomonas fluorescens* (51.55%). Antibiotic

Table 2 : Effect of microbial inoculants on disease severity (%) in sunflower hybrid KBSH 44–pot culture study.

Treatments	Disease Severity (%) at 60 DAS
T_1 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *T. viride*	20.33
T_2 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *T. viride* + FS of *S. griseus*	16.67
T_3 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *T. viride* + FS of *T. viride*	9.33
T_4 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *T. viride* + FS of *B. subtilis*	18.33
T_5 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *T. viride* + FS of *P. fluorescens*	19.67
T_6 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *B. subtilis*	25.00
T_7 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *B. subtilis* + FS of *S. griseus*	17.67
T_8 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + B. subtilis + FS of *T. viride*	11.33
T_9 : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + B. subtilis + FS of *B. subtilis*	18.33
T_{10} : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + B. subtilis+ FS of *P. fluorescens*	20.00
T_{11} : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *P. fluorescens*	38.33
T_{12} : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *P. fluorescens* + FS of *S. griseus*	27.00
T_{13} : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *P. fluorescens* + FS of *T. viride*	24.33
T_{14} : 75% NP + 100% K + SA of *A. chroococum* + *PSB* + *P. fluorescens* + FS of *B. subtilis*	31.67
T_{15} : 75%NP + 100% K + SA of *A. chroococum* + *PSB* + *P. fluorescens* + FS of *P.fluorescens*	34.33
T_{16} : Control- without any chemical fertilizers and biofertilizers	45.00
F – test	*
S.Em±	2.67
C.D. at 5%	8.67

T_9 : T_1 + S.A. of *A. chroococum* + *PSB* + *B. subtilis* + F.S of *S. griseus*

T_{10} : T_2 + S.A. of *A.chroococum* + *PSB* + *B. subtilis* +F.S of *S. griseus*

T_{11} : T_1 + Mancozeb spray at 0.3%

T_{12} : T_1 : + Propiconozole spray at 0.1%

This study was carried out to know the effect of microbial inoculants on soil borne diseases of sunflower under field condition. The crop is highly susceptible to

Table 3 : Effect of microbial inoculants on disease severity at 50% seed filling stage in sunflower Hybrid KBSH 44 - field study.

Treatments	Disease severity (%) at 60 DAS
T_1: 100 % NPK	25.33
T_2: 75 % NP +100 % K	26.53
T_3: T_1 + S.A of *A. chroococum* + *PSB* + *T. viride* + F.S of *T. viride*	14.27
T_4: T_2 + S.A of *A. chroococum* + *PSB* + *T. viride* + F.S of *T. viride*	18.27
T_5: T_1 + S.A of *A. chroococum* + *PSB* + *T.viride* + F.S of *S. griseus*	16.73
T_6: T_2 + S.A of *A. chroococum* + *PSB* + *T.viride* + F.S of *S. griseus*	19.73
T_7: T_1 + S.A of *A. chroococum* + *PSB* + *B. subtilis* + F.S of *T. viride*	17.13
T_8: T_2 + S.A of *A.chroococum* + *PSB* + *B. subtilis* + F.S of *T.viride*	19.53
T_9: T_1 + S.A of *A. chroococum* + *PSB* + *B. subtilis* + F.S of *S. griseus*	20.40
T_{10}:T_2+ S.A of *A.chroococum* + *PSB* + *B. subtilis* +F.S of *S. griseus*	21.47
T_{11}: T_1 + Mancozeb spray at 0.3 %	12.27
T_{12}: T_1 + Propiconozole spray at 0.1 %	11.00
F – test	*
S.Em.±	0.61
C.D. at 5%	1.79

production, mycoparasitism, the production of cell wall degrading enzymes and competition for nutrients and space are considered as the actions involved in biocontrol of pathogen (Zeilinger and Omann, 2007; Vinale *et al*., 2008). *T. viride* as more efficient antagonistic fungi against *Alternaria burnsii* than *T. harzianum* with higher mycelial growth (Vihol, 2009). The antagonistic effect of various microorganisms have been reported by various scientists *viz*., *Pseudomonas fluorescens* (Amaresh, 2000); *Streptomyces* sp. (Srividya *et al*., 2012); *Bacillus subtili* (Vadivel and Ebenezar, 2006).

The least per cent disease incidence was recorded in the treatment combination of *A. chroococum* + *B. megaterium* + *T. viride* with 75% NP + 100% K and foliar spray of *T. viride* and maximum incidence was recorded in the uninoculated control plant. Similar results were obtained by Reshu and Khan (2012), who reported that *T. viride* inhibited the leaf blight of mustard due to action of enzyme produced by *Trichoderma* spp and production of volatile and non volatile chemical compound and toxins.

Conclusion

The investigation of this study has clearly showed that the use of microbial inoculants may offer a practical and alternative method for the management of *Alternaria* leaf blight in sunflower. Use of *Trichoderma* as a biocontrol agent is found most effective in controlling *Alternaria* leaf blight in sunflower and saves the application of pesticides. These microbial biocontrol agents are eco-friendly and have long lasting effect on plant compared to chemical pesticides in maintaining the disease.

References

Amaresh, Y. S. (2000). Epidemiology and management of *Alternaria* leaf blight and rust of sunflower. *Ph.D. Thesis*, University of Agricultural Sciences, Dharwad, pp. 321.

Barker, A. V. (1975). Organic versus inorganic nutrition of horticultural crops and quality. *Hort. Sci.*, **10** : 73-75.

Deshmukh, P. P. and G. J. Raut (1992). Antagonism by *Trichoderma* spp. on five plant pathogenic fungi. *New Agriculurist*., **3** : 127-130.

Gulya, J. J. and Masireview (1991). Common names for the plant diseases of sunflower (*Helianthus annuus* L.) and Jerusalem artichoke (*Helianthus tuberosa* L.). *Plant Disease*, **75** : 30.

Mayee, C. D. and V. V. Datar (1986). Phytopathometry, Technical Bulletin- I. Marathwada Agricultural University, Parbhani, pp. 46.

Panse, V. G. and P. V. Sukhatme (1967). Statistical methods for agricultural workers. ICAR, *Agri. Res*., New Delhi.

Reshu and Mahmud Khan (2012). Role of different microbial-origin bioactive antifungal compounds against *Alternaria* sp. causing leaf blight of mustard. *J. Plant Pathol*., **11(1)** : 1-9.

Srividya, S., Adarshana Thapa, Deepika V. Bhat, Kajingailu Gomei and Nilanjan Dey (2012). *Streptomyces* sp. as effective biocontrol agent against chilli soilborne fungal phytopathogens. *Euro. J. Exp. Bio*., **2(1)** : 163-173.

Vadivel, S. and E. G. Ebenezar (2006). Eco-Friendly Management of Leaf Blight of Tomato caused by *Alternaria solani*. *J. Mycol. Plant Pathol*., **36(1)**.

Vihol, J. B., K. D. Patel, R. K. Jaiman and N. R. Patel (2009). Efficacy of Plant Extracts, Biological agents and Fungicides Against *Alternaria* Blight of Cumin. *J. Mycol. Plant Pathol*., **39(3)** : 516-519.

Vinale, F., K. Sivasithamparam, E. L. Ghisalberti, R. Marra, M. J. Barbetti, H. Li, S. L. Woo and M. Lorito (2008). A novel role for *Trichoderma* secondary metabolites in the interactions with plants. *Physiol. Mol. Plant Pathol*., **72** : 80-86.

Vincent, J. M. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, **159** : pp. 850.

Zeilinger, S. and M. Omann (2007). *Trichoderma* biocontrol : signal transduction pathways involved in host sensing and mycoparasitism. *Gene Regul. Syst. Biol*., **1** : 227-234.