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**Original Research Article**

# Bioefficacy of Bioagents against Pathogenic Mycoflora of Sunflower Seeds

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**A B S T R A C T**

Sunflower (*Helianthus annuus* L.) crop is affected by number of pathogenic fungi, which are mostly seed borne, causing significant qualitative and quantitative losses. Though, majority of seed borne fungi are being managed with fungicidal seed treatments, but due to their harmful effects, their use needs to be reduced. Therefore, present *in vitro* study was conducted to asses bioefficacy of nine bioagents against two major pathogenic seed borne fungi *viz.*, *Alternaria alternata* and *Fusarium oxysprorum*, by applying Dual Culture Technique. Two separate experiments were planned and conducted with Completely Randomized Design (CRD) and all the treatments replicated thrice. The results revealed that all of the nine test bioagents significantly inhibited mycelial growth of *A. alternata* and *F. oxysprorum*, over untreated control. However, in case of *A. alternata* significantly highest mycelial growth inhibition was achieved with *A. niger* and *T.virens* (79.80% and 79.40%, respectively) followed by *T. viride* (78.10%), *T. koningii* (77.50%), *T. harzianum* and *T. lignorum* (each 77.20%), *T. hamatum* (74.60%) all of which were on par with each other. *P. fluorescens* and *B. subtilis* were found least effective. Whereas, in *F. oxysprorum*, significantly highest mycelial growth inhibition was resulted with *T. koningii* (76.20%), followed by *T. lignorum* (75.50%), *A. niger* (75.30%) and *T. harzianum* (73.10%), all of which were on par with each other. Rest of the bioagents found effective in their order of merit were *T. hamatum* (60.70%), followed by *T. viride* (59.66%), *T.virens* (57.60%), *P. fluorescens* (48.50%) and *B. subtilis* (34.70%).

**Keywords**

Pathogenic Mycoflora of Sunflower Seeds, Bioagents

## Introduction

Sunflower (*Helianthus annuus* L.) is one of the major oilseed crops grown for edible oil, throughout world. It's seeds are highly nutritious containing about 20% proteins and 40 to 50% vegetable oil of high calorific value along with 60 to 73% linoleic acid, minerals (calcium and iron) and vitamins like A, B, E and K (Gosal *et al.*, 1988). In India, during 2014-15 sunflower was cultivated on an area of 5.516 lakh ha, production of 4.15 lakh tones and productivity of 752 kg/ha, which is popularly grown in the state of Maharashtra, Andhra Pradesh and Karnataka. (Anonymous, 2015). Sevaral phytopathogenic and saprophytic fungal species have been reported on sunflower seeds. The most important seedborne pathogens represents the genera of *Alternaria* (*A. helianthi, A. alternata*), *Plasmopara halstedii, Fusarium (F. oxysprorum, F. solani, F. chlamydosporum),*

*Macrophomina phaseolina, Aspergillus (A. niger, A.flavus), Rhizopusstolonifer, Verticillium dahlia, Sclerotinia sclerotium, Rhizoctonia solani, Sclerotium rolfsii* etc. These phytopathogenic fungi associated with sunflower seeds cause qualitative losses by reducing oil quality due to increased free fatty acids as well as quantitative losses by seed deterioration and seedling mortality, leading to accountable seed yield losses (Mathur and Manandhar, 2003; Sangawan *et al.*, 2005; Khan, 2007; and Afzal *et al.*, 2010). Therefore, present study on *in vitro* bioefficacy of bioagents against pathogenic mycoflora of sunflower seeds was planned and conducted at the Department of Plant Pathology, College of Agriculture, Latur, during 2016-17.

## Materials and Methods

### Isolation, identification and pathogenicity of seed borne fungi

Previous season stored seeds of sunflower Hyb. LSFH-171 and Var. Morden were collected from Oilseeds Research Station, Latur. These seeds were plated aseptically onto autoclaved and cooled Potato Dextrose Agar medium, in separate sterile glass petri plates and incubated at room temperature. After a week of incubation, various fungal colonies developed on PDA plates were observed under stereomicroscope, distinguished on the basis of colony colour and growth habit, further re-isolated on fresh PDA plates and incubated at room temperature. Based on morpho-cultural characteristics and microscopic observations, the most predominant fungi identified were *Alternaria alternata* and *Fusarium oxysporum.* The pathogenicity of both fungi was proved by seed inoculation and standard blotter paper techniques, by using the surface sterilized (2% Sodium hypochlorite solution) seeds of sunflower Hyb. LSFH-171 and Var. Morden.

### *In vitro* evaluation of bioagents

A total of nine biocontrol agents (Table. 1) were evaluated *in vitro* against *A.alternata* and *F. oxysporum* separately, by applying Dual Culture Technique (Dennis and Webster, 1971).

Two separate experiments were planned and conducted with Completely Randomized Design (CRD) and all the treatments replicated thrice. Observations on linear mycelial growth of the test fungi and test bio-agents were recorded separately at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test fungi. Per cent mycelial growth inhibition of the test fungi with the test bioagents, over untreated control was calculated by applying following formula (Arora and Upadhyay, 1978).

$$\text{Per cent growth inhibition} = \frac{\text{Colony growth in Control plate} - \text{Colony growth in Intersecting plate}}{\text{Colony growth in Control Plate}} \times 100$$

The data obtained was statistically analyzed (Panse and Sukhatme, 1978) and the results were interpreted thereof.

## Results and Discussion

The results obtained on per cent of mycelial growth inhibition of two test fungi viz., *A. alternata* and *F. oxysporum*, with the test bioagents are depicted in PLATE-I and presented in Table 1 and Fig. 1.

### *A. alternata* inhibition

The results (Plate.1, Table.1 and Fig.1) revealed that all of the nine test bioagents significantly inhibited mycelial growth of *A. alternata*, over untreated control, which was ranged from 38.40 – 79.80 per cent.

**Plate-I**


**A. *Alternaria alternata***


**B. *Fusarium oxysporum***

$T_1$ :*Trichoderma viride*

$T_2$ :*T. harzianum*

$T_3$ :*T. hamatum*

$T_4$ :*T. koningii*

$T_5$ :*T. lingorum*

$T_6$ :*T. virens*

$T_7$ :*Aspergillus niger*

$T_8$:*Pseudomonas fluroscens*

$T_9$ : *Bacillus subtilis*

$T_{10}$ :Control (Untreated)

***In vitro* evaluation of various bioagents against *alternate* (A) and *F. oxysporum*(B)**

**Fig.1** *In vitro* efficacy of various bioagents against *A. alternate* and *F. oxysporum* associated with sunflower seeds

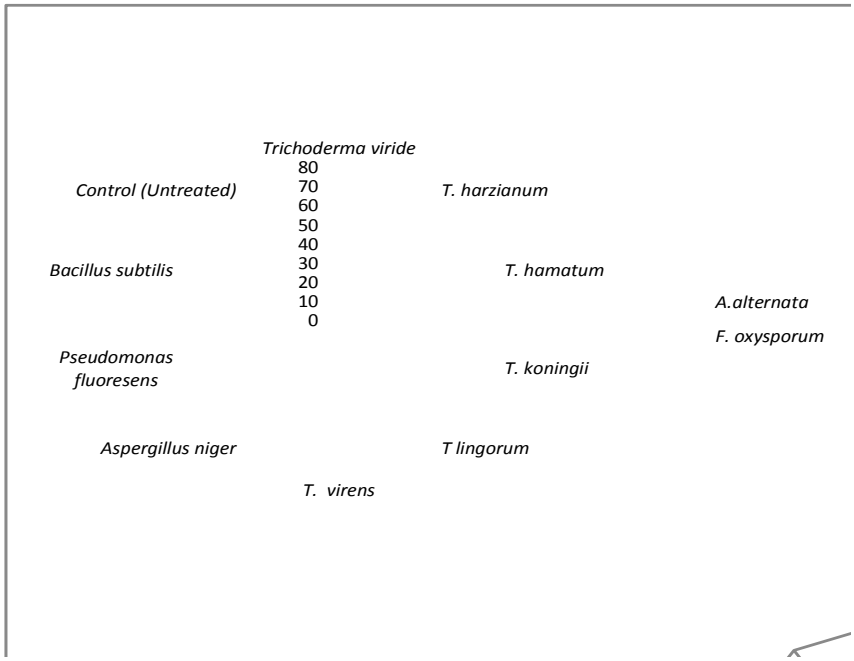


**Table.1** *In vitro* efficacy of various bioagents against *A. alternata* and *F. oxysporum* associated with sunflower seeds

| Tr. No. | Treatments | Inhibition* (%) | |
|---|---|---|---|
| | | ***A. alternata*** | ***F. oxysporum*** |
| $T_1$ | *Trichoderma viride* | 78.10 (62.10) | 59.66 (50.57) |
| $T_2$ | *T. harzianum* | 77.20 (61.48) | 73.10 (58.76) |
| $T_3$ | *T. hamatum* | 74.60 (59.70) | 60.70 (51.18) |
| $T_4$ | *T. koningii* | 77.50 (61.68) | 76.20 (60.80) |
| $T_5$ | *T. lignorum* | 77.20 (61.48) | 75.50 (60.33) |
| $T_6$ | *T. (Gliocladium) virens* | 79.40 (63.01) | 57.60 (49.37) |
| $T_7$ | *Aspergillusniger* | 79.80 (63.29) | 75.30 (60.20) |
| $T_8$ | *Pseudomonas fluorescens* | 50.40 (45.23) | 48.50 (44.14) |
| $T_9$ | *Bacillus subtilis* | 38.40 (38.29) | 34.70 (36.09) |
| $T_{10}$ | Control (Untreated) | 00.00 (00.00) | 00.00 (00.00) |
| **SE±** | | **0.92** | **0.97** |
| **CD (P=0.01%)** | | **2.74** | **2.92** |

*: Mean of three replications. Figures in parentheses are Arcsine values

However, significantly highest mycelial growth inhibition was achieved with *A. niger* (79.80%), followed by *T.virens* (79.40%), *T. viride* (78.10%), *T.koningii* (77.50%), *T. harzianum* and *T. lignorum* (each 77.20%), *T. hamatum* (74.60%) all of which were on par with each other. *P. fluorescens* and *B. subtilis* caused comparatively moderate mycelial growth inhibition of 50.40 and 38.40 per cent, respectively.

### *F. oxysporum* inhibition

The results (Plate.1, Table.1 and Fig.1) revealed that all of the nine test bioagents significantly inhibited mycelial growth of *F.oxysporum*, over untreated control and it was ranged from 34.70 – 76.20 per cent.

However, significantly highest mycelial growth inhibition was resulted with *T. koningii* (76.20%), followed by *T. lignorum* (75.50%), *A. niger* (75.30%) and *T. harzianum* (73.10%), all of which were on par with each other. Rest of the bioagents found effective in their order of merit were *T. Hamatum* (60.70%), followed by *T. viride* (59.66%), *T.virens* (57.60%), *P. fluorescens* (48.50%) and *B. subtilis* (34.70%).

Various species of *Trichoderma, Aspergillus niger, P. fluorescens* and *B. subtilis* are most commonly and commercially exploited bioagents/ antagonists to combat several seed borne and / or soil borne plant pathogens. Fungicidal / fungistatic effects of these bioagents have been attributed to various mechanisms exerted such as antibiosis, lysis, mycoparasitiam, competition, production of volatile / non-volatile compounds etc. In present study also, various species of *Trichoderma, Aspergillus niger, P. fluorescens* and *B. subtilis* were found as efficient antagonists against two major seed borne pathogenic fungi *viz*., *A. alternate* and *F. oxysporum* of sunflower.

These results of the present study are in agreement with previous findings of several workers (Bardia and Rai, 2007; Jamwal *et al.,* 2011; Savitha *et al.,* 2011; Mahmood *et al.,* 2015 and Jat *et al.,* 2017). Bardia and Rai (2007) reported that various native strains of *Trichoderma harzianum*, *T. viride*, *T. virens*, A. niger and *P. fluorescens* isolated from cumin rhizosphere significantly inhibited mycelial growth of *F. oxysporum* f. sp. *cumini*, causing cumin wilt. Jamwal *et al.,* (2011) reported that significantly highest mycelial growth inhibition of *Fusarium oxysporum* f. sp. *lycopersici*, causing tomato wilt was caused by *Trichoderma harzianum* (77.30%), *T. viride* (75.00%) and *P. fluorescens* (66.15%). Savitha *et al.,* (2011) reported maximum mycelial growth inhibition of *A. sesame* with *T. harzanium* (87.00%), followed by *T. viride* (82.18%), *P. fluorescens* (76.23 - 80.00%) and *B. subtilis* (68.84 - 71.39%).

Mahmood *et al.,* (2015) reported that significantly highest mycelial growth inhibition of *Fusarium oxysporum* f. sp. *ciceri*, causing chickpea wilt was resulted with *P. fluorescens* (70.90%), *T. harzianum* (63.95%) and *B. subtilis* (57.68%). Jat *et al.,* (2017) reported *T. harzianum*, *T. viride*, *P. fluorescens* and *B. subtilis* as most efficient antagonist against *Fusarium oxysporum* f. sp. *coriandri*, with mycelial growth inhibition of 83.69, 81.17, 72.02 and 66.48 per cent, respectively.

Hence, from ongoing results and discussion, it is concluded that sunflower pathogenic two major seedborne fungi *viz*., *A. alternata* and *F. oxysporum* could be managed with the bioagents*viz*., *A. niger*, various species of *Trichoderma* and *P. fluorescens*.

## References


Afzal, R., Mughal, S. M., Munir, M., Sultana, K., Qureshi, R., Arshad, M. and Laghari, M. K. (2010). Mycoflora associated with seeds of different sunflower cultivars and its management. *Pak. J. Bot.* 42(1): 435-445.

Anonymous, (2015). AICRP (Sunflower) Directors report, Indian Institute of oilseeds research, Rajendranagar, Hyderabad. 30: 13-14.

Arora, D. K. and Upadhyay, R. K. (1978).Effect of fungal staling substances on colony interaction.*Pl. Soil*.49: 685-690.

Bardia, P. K. and Rai, P. K. (2007).*In vitro* and field evaluation of biocontrol agents and fungicides against wilt of cumin, caused by *Fusarium oxysporum* f. sp. *cumini*. *J. SpiceAron. Crops*16: 88-92.

Dennis, C. and Webster, J. (1971).Antagonistic properties of species groups of *Trichoderma*. *Trans. Br. Mycol. Soc*.57: 25-39.

Deokar, S. R., Thaware, D. S. and Wagh, S. S. (2014). Integrated disease managem ent of sunflower *Alternaria* blight (*Alternaria helianthi*). *Trends Biosci*. 7(19): 3013-3020.

Gosal, S. S., Vasiljevic, L. and Brar, D. S. (1988).Plant biotechnology and sunflower improvement. *Proceedings of 12th Int. Sunflower Conf. Novisad, Yugoslavia*.599 pp.

Jamwal, S., Jamwal, A. and Verma, V. S. (2011). Effect of biocontrol agents on wilt management and plant growth of tomato.*Indian Phytopath*.64: 381-382.

Jat, M. K., Ahir, R.R., Choudhary, S. and Kakraliya, G. L. (2017). Management of coriander wilt using biocontrol agents. *Internat. J. Chem. Studies*5: 523-525.

Khan, S. N., (2007). *Microphominaphaseolina* causal agent of charcoal rot of sunflower. *Mycopath*, 5(2): 111-118.

Mahmood, Y., Khan, M. A., Javed, N. and Arif, M. J. (2015).Comparative efficacy of fungicides and biological control agents for the management of chickpea wilt, caused by *Fusarium oxysporum* f. sp. *ciceri*. *J. Animal Pl. Sci*. 25: 1063-1071.

Mathur, S. B. and Manadhar, H. K. (2003) Fungi in seeds recorded at the Danish Government Institute of Seed Pathology for developing countries, Copenhagen, Denmark.

Panse, V. G. and Sukhatme, P. V. (1978) Statistical Methods for Agricultural Workers (3rded.). ICAR, New Delhi Pub: 157-165.

Sangawan, M., Mehata, M. and Saharanm, G. (2005) Diseases of oilseed crops. *Indus Publication. Co. India.*

Savitha, A. S., Naik, M. K. and Ajitkumar, K. (2011). Ecofriendly management of *A. sesame*, inciting blight of sesame. *J. Pl. Dis. Sci*. 6(2): 150-152.