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In vitro evaluation of fungicide and biocontrol agents against *Alternaria helianthi* causing leaf blight of sunflower (*Helianthus annuus* L.)

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ABSTRACT

Leaf blight caused by *Alternaria helianthi* is one of the major diseases of sunflower worldwide. It is responsible for causing upto10-15 per cent yield losses in sunflower. In this study, antagonistic effects of *Pseudomonas* and *Trichoderma* isolated from rhizosphere of sunflower were evaluated against *Alternaria helianthi* as potential biocontrol agents *in vitro*. Fungal inhibition tests were performed using dual plate culture technique. Overall the culture of *Pseudomonas* showed the maximum inhibition of 77 per cent on the growth of *A. helianthi* followed by *Trichoderma*. Fungicide Dithane M-45 (Mancozeb) was used at three different concentrations of 0.01, 0.05 and 0.1 ppm in inhibiting the radial growth of *A. helianthi* by poison food technique. All the three concentrations of Mancozeb inhibited the radial growth. Among them, Carbendazim at 0.1ppm was found to be the most effective against the pathogen. The growth parameters (plant height, root length and shoot length) were significantly increased by treating the seeds with bio control agent *Pseudomonas* compared to the untreated control. Results indicate that PGPR improve growth parameters and can also help in the biocontrol of pathogen.

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INTRODUCTION

Sunflower *(Helianthus annuus* L.) is an important oilseed crop in India popularly known as "Surajmukhi". Sunflower is a major source of vegetable oil in the world. Sunflower seed contains about 48 to 53 per cent edible oil. Sunflower oil is a rich source (64 %) of linoleic acid which is good for heart patients. It is used as nutritious meal for birds and animals. It is also used in the preparation of cosmetics and pharmaceuticals. Sunflower seeds are one of the most nutritious and healthy foods. It belongs to the family Asteraceae and genus *Helianthus*. Sunflower is native to the North America and being successfully cultivated under diversified geographical and agro-ecological zones.

This crop has gained importance due to its short duration of maturity, containing of excellent quality of oil, photo-insensitivity, wide adaptability into different kinds of cropping pattern. Oil cake is rich in high quality protein (40 – 44 %) and used as cattle and poultry feed. This crop is considered valuable from economic as well as ornamental point of view.

Sunflower dominates the oil seed sector in Karnataka. It is registered as the highest sunflower producing state in the country followed by Andhra Pradesh in the second position with 21 per cent. Karnataka with a production of 3.04 lakh tonnes from an area of 7.94 lakh hectares followed by Andhra Pradesh, Maharashtra, Bihar, Orissa and Tamil Nadu are major sunflower producing states of India. Sunflower production follows a systemic weather risk as about 80 per cent of the area is under rain-fed production. In terms of productivity, Bihar leads with 1402 kg/ha followed by Tamil Nadu with 1328.7 kg, although both the states have less than 25000 hectares under the crop which is mostly irrigated. The average productivity at all India level was 900 kg/ha depending on the climatic conditions and irrigation, which are critical factors for high yields (Khan, 2007).

Diseases are serious threat for the sunflower crop throughout world. It has been estimated that diseases can cause an average annual loss of 12 per cent in yield from nearly 12 million hectares of the world (Zimmer and Hoes, 1978 and Kolte, 1985). The incidence and severity of diseases are linked with the climatic factors and cultural practices. Among diseases, *Alternaria*, rust, verticillium leaf wilt, downy mildew, sclerotium wilt and charcoal rot are of worldwide occurrence. Prevalence or distribution of the disease is linked with the climatic factors, cropping pattern and cultural practices. In general, diseases cause 10-15 per cent net loss but under favourable conditions for the outbreak and development of the pathogen, they may claim failure of the crop (Sackston, 1981 and Xiaojian *et al*., 1988).

Among these diseases leaf blight caused by *Alternaria helianthi* is one of the major diseases. *A. helianthi* can cause upto 10-15 per cent yield losses (Khan, 2007). Sunflower is most susceptible to *A. helianthi* during anthesis and seed filling stage of growth. As there is no resistant variety/hybrid available against this disease, it has become inevitable to go for the management of the *Alternaria* through cultural practices, fungicides and biocontrol agents.

Alternaria leaf spot is caused by fungus and presents as circular spots with brownish or grayish middles and yellow edges. Symptoms of *Alternaria* leaf spot include dark circular spots on the leaves. Sometimes the spots run together, causing the leaves to wither and die. Some spots may have yellow halos (Fig. A). Dark streaks, flecks or diamond-shaped spots may appear on the stems or petioles. Sometimes these lesions can cause the stem to break. Dark spots can also appear on the back of the head. *Alternaria* infections are worse during warm, wet weather.

Fig. A : Symptoms of *Alternaria* leaf spot on sunflower

There are a number of fungicides that have activity against *Alternaria* fungi like chlorothalonil, captan, fludioxonil, imazalil, iprodione, maneb, mancozeb, thiram and selected copper fungicides have varying degrees of efficacy against *Alternaria* species. Not only fungicides, some of the bio control agents are also effective against *Alternaria*. Some species of *Trichoderma* and *Pseudomonas* are among the major micro-organisms that have shown great potential for biological control of several plant pathogens. Biological control of crop disease is receiving increased attention as an environmentally sound alternative to chemical pesticides. *Pseudomonas fluorescens* play a major role in control of *Alternaria.*

Pseudomonas fluorescens encompasses a group of common, non-pathogenic saprophytes that colonize soil, water and plant surface environments. It is a common gram negative, rod-shaped bacterium.Certain members of the *P. fluorescens* have been shown to be potential agents for the bio control which suppress plant diseases by protecting the seeds and roots from fungal infection. They are known to enhance plant growth promotion and

reduce severity of many fungal diseases (Hoffland *et al.*, 1995 and Wei *et al.*, 1996).

P. fluorescens may also be an important factor in disease control. It belong to plant growth promoting rhizobacteria (PGPR), the important group of bacteria that play a major role in the plant growth promotion, induced systemic resistance, biological control of pathogens etc. Many strains of *Pseudomonas fluorescens* are known to enhance plant growth promotion and reduce severity of various diseases. The efficacy of bacterial antagonists in controlling fungal diseases was often better as alone and sometimes in combination with fungicides.

Considering the above facts, the present study has therefore, been under taken with the following objectives:

– Screening of fungicide that inhibit the pathogen under *in vitro*

– Isolation and screening of antagonists inhibiting the pathogen under *in vitro*

– To study the plant growth promoting activity using bio control agents.

MATERIAL AND METHODS

Isolation of the pathogen:

Pathogen causing sun flower leaf spot was isolated from the collected samples by tissue segment method on potato dextrose agar (PDA) and they were purified by single spore isolation method and maintained on PDA medium (Dhingra and Sinclair, 1985).

Screening of fungicides *in vitro* against *Alternaria helianthi*:

The inhibitory effect of fungicide on the growth of fungi was evaluated by poisoned food technique (Dhingra and Sinclair, 1985). Fungicides were used @ 0.01 per cent, 0.05 per cent and 0.1 per cent concentrations in autoclaved PDA medium. Twenty ml of such medium was poured in each sterilized Petri plate and solidified. After solidification, 5 mm disc of seven days old cultures of *A. helianthi* were cut by using sterile cork borer and placed in the centre of Petri plates containing different concentrations of fungicides and incubated at 25 ±1ºC. Treatments were replicated thrice along with suitable control in which fungicide was omitted in the medium. The radial growth of *A. helianthi* was measured after 7 days.

Isolation of bio control agents:

The bio control agents were isolated from rhizosphere soil by serial dilution using king's B medium for *Pseudomonas* and PDA for *Trichoderma* (Dennis and Webster, 1971).

Screening of antagonists against *Alternaria helianthi* :

Antagonist activity of *Pseudomonas* and *Trichoderma* against *A. helianthi* was tested using dual culture technique (Kumar and Honda, 2007). Mycelial discs measuring five mm diameter from seven days old cultures of antagonist and the test pathogen were placed at equidistant on sterile Petri plates containing PDA medium. The Petri plates were then incubated at 28 ± 1ºC. Three replications of each treatment were maintained and observed for a period of eight days. Suitable controls were kept without antagonist. Growth of the pathogenic fungi was measured at 24 hours intervals upto eight days of inoculation of antagonist. Percentage inhibition of mycelial growth of test pathogen was calculated using the formula (Vincent, 1947).

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

where,

I = Per cent radial mycelial growth inhibition

C =Radial growth of pathogen in check Petri-plate.

T =Radial growth of pathogen in dual culture.

Effect of antagonist in plant growth promotion:

The plant growth-promoting activity of the bio control agents was assessed based on the seedling vigour index by the standard roll towel method (ISTA, 1993). Ten seeds were placed on presoaked germination paper. The seeds were held in position with another presoaked germination paper strip and gently pressed. The polythene sheet along with the seeds was then rolled up and incubated in a growth chamber for 10 days. Three replications were carried out for each treatment. The root length and shoot length of individual seedlings were measured and the per cent germination of the seeds was calculated. The seedling vigour index was calculated using the formula (Abdul Baki and Anderson, 1973).

Vigour index = (Mean root length+ Mean shoot length) × % Germination

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Mycelial growth of *Alternaria helianthi* on PDA media:

A. helianthi exhibits brown colour mycelial growth on culture media with concentric rings. The mycelia completely covered the culture media at 7-8 days after inoculation. This mycelium was white in colour at its early stage later it turns brown in colour (Fig. 1).

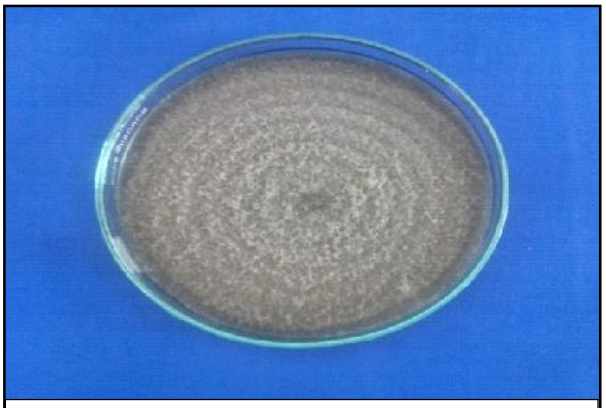

Fig. 1 : Culture of *Alternaria helianthi*

Screening of fungicide *in vitro* against the mycelial growth of *A. helianthi*:

Evaluation of 3 different concentrations of the fungicide Dithane M-45 (Mancozeb) was done by employing the Poisoned food technique. The results of the experiment showed that all the three concentrations (0.01ppm, 0.05 ppm and 0.1 ppm) of Dithane M-45 (Mancozeb) inhibited the radial growth of *A. helianthi.* Among these 0.1 ppm found to be more effective against the fungus *A. helianthi.* The results of the present study suggested that Dithane M-45 (Mancozeb) is the most effective fungicide against *A. helianthi*, which is in agreement with Kamble *et al.* (2000) and Deora *et al.* (2004). Deora and his coworkers found out that out of eight fungicides tested, Dithane M-45 @ 0.25 per cent was found to be the most effective in controlling *A. solani* in tomato.

Meena *et al*. (2004) reported that fungicide mancozeb caused 100 per cent reduction in mycelial growth of *Alternaria brassicae* over control*in vitro*. Among the various fungicides tested, Mancozeb was the best among all the treatments, resulting in the lowest disease severity on leaves of mustard.

Inhibitory effect of antagonists on the growth of *A. helianthi:*

Both Pseudomonas and *Trichoderma* strains were

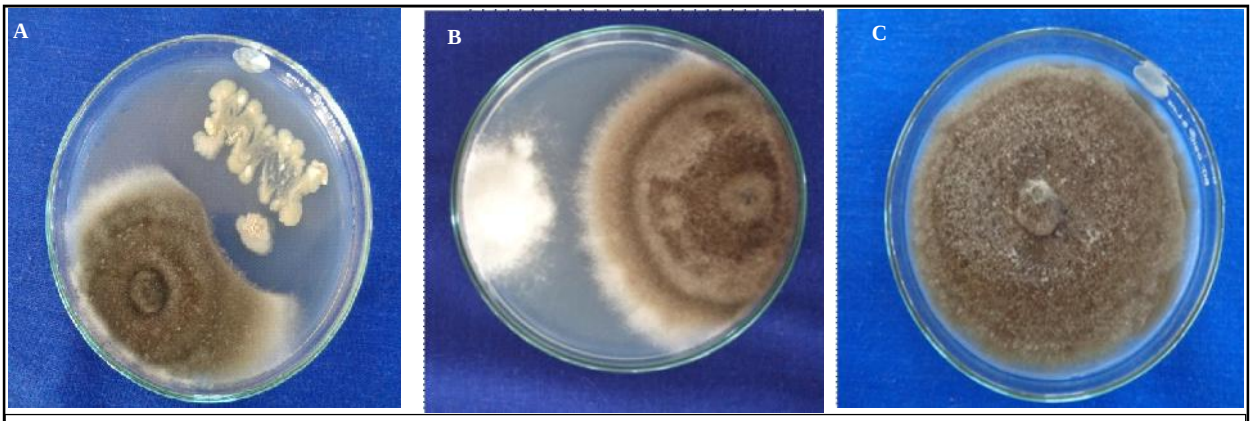

Fig. 2 : Efficacy of *Pseudomonas* and *Trichoderma* on the growth of *A. helianthi* (A- *Pseudomonas*, B- *Trichoderma*, C- Control)

Table 1 : Showing effect of antagonists on the radial growth of *Alternaria helianthi*

Sr. No.	Name of the antagonist	Colony diameter of pathogen (in mm)	% inhibition (in mm)
1.	*Pseudomonas*	16	77
2.	*Trichoderma*	20	71
3.	Control	70	00

evaluated by dual culture technique for their antagonistic effect against *A. helianthi* under *in vitro* conditions. Per cent Inhibition was calculated. In dual culture test, *Pseudomonas* was shown to have more growth inhibition (77%) of pathogen on the PDA medium than *Trichoderma* strains (71%) (Table 1 and Fig. 2). Similar results have previously been reported (Erdogan and Benlioglu, 2010; Zheng *et al*., 2011; Khot *et al*., 1996 and Eleftherio *et al*., 2004).The inhibition of mycelial growth may be due to the production of antibiotics/volatile and non-volatile metabolites produced by the bio control agents (Karimi *et al*., 2012).

Efficacy of antagonist in growth promotion :

Growth parameters such as shoot length and root length were significantly higher in seeds treated with *Pseudomonas* compared to untreated control (Table 2 and Fig. 3).

Previous work showed that many micro-organisms such as bacteria can had positive influence on plant growth and plant health as PGPR (Zakira *et al*., 2009; Erdogan and Benlioglu, 2010; Kloepper *et al*., 1980 and Dileep Kumar, 1999). The role of *Pseudomonas* spp. in promoting root and shoot growth of different crops has been demonstrated (Kamal *et al*., 2008). The use of rhizobacteria against leaf blight of sunflower caused by *A. helianthi* not only suppressed the disease incidence but also increased growth parameters of the plant. This gives them an advantage over the use of chemical fungicides against leaf blight in disease management. The work has to be intensified

Fig. 3 : Plant growth promoting activity of *Pseudomonas*

Table 2 : Plant growth promoting activity of *Pseudomonas*

Sr. No.	Shoot length (cm)		Root length (cm)	
	C	T	C	T
1.	6.5	5.5	9.0	10.5
2.	2.5	2.5	10.0	14.0
3.	7.0	8.5	6.5	13.0
4.	4.0	7.5	9.0	13.5
5.	2.0	6.7	8.5	6.5
6.	3.0	8.5	9.0	11.0
7.	3.0	6.0	8.0	7.5
8.	5.0	5.5	5.5	12.0
9.	2.0	3.0	3.5	11.0
10.	2.0	8.0	6.5	6.5
AVG	3.70	6.17	7.55	10.55

*C-control T-Treated

to study the mechanisms involved in disease control by mixtures of bio control agents.

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