ISSN: 2277-5536 (Print); 2277-5641 (Online)

# BIOCHEMICAL MANAGEMENT OF FUSARIUM WILT

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**ABSTRACT**
In the present study, four plant extracts, two bio-control agents and four fungicides were evaluated in vitro. The antifungal activity of four plant extracts namely Annona, Datura, Ocimum, Neem are used in two solvents (acetone and methanol) with three concentration (5 ml, 10ml and15ml) were evaluated by using food poison technique. Among the poisonous plants Annona acetone extract is most effective and among medicinal plants Ocimum acetone extract and Neem acetone extract as well as Neem methanol extract were most effective in inhibiting mycelia growth of *Fusarium oxysporum F.sp. cathami.* The antagonistic activity of bio-control agents namely *Trichoderma harzianum and Trichoderma viride* were evaluated against wilt by dual culture technique. The T. viride found to be most effective against *Fusarium oxysporum. f. sp. carthami.* The evaluation of four fungicides viz-Bilitox, Captan, Thirum, Captan + Thirum Combi fungicides were taken among these Captan + Thirum combi fungicides was most superior in all three Conc$^{n}$ (0.5, 0.1 and 0.15 %) than others followed by thirum in inhibiting the mycelia growth of *fusarium oxysporum f.sp. carthami* by food poison technique.

**KEYWORDS:** Bio control agents, fungicides, Plant extracts, Safflower.

## INTRODUCTION

*Carthamus tinctorius* L. Commonly known as Safflower or Kardi an important oilseed Crop belonging to family Asteraceae of dicotyledonous plants and growing Worldwide. It is important edible oilseed Crop in India. It contains 40% edible oil which has high percentage of poly saturated fatty acids and lactic acid. The Potential Yield of this crop is affected by a number of diseases as well as seed borne Pathogens. Wilt of safflower caused by *fusarium oxysporum* f.sp. *Carthami* (Klisiewicz and Houston (FOC) is the most important disease. This disease manifesting in the form of unilateral infection on branches and golden yellow discoloration of leaves, followed by wilting, vascular browning appearing on one side of root and Stem. The pathogen is both soil and seed borne. The fungus being facultative parasite generally fungicidal spray was recommended, but recently there is an increased trend to manage crop diseases by innovative approaches. Biological control is one of the viable prepositions of such a pathogen (Naik and Sen 1991). Hence the present investigation was undertaken by using management of different plant extracts bio-control agents and fungicides in vitro for their efficacy against fusarium oxysporum f.sp. *carthami*.

## MATERIAL AND METHODS

*Fusarium oxysporum f.sp.carthami* was isolated from safflower crop variety Annagiri-1 growing in the vicinity of Parbhani field. This variety is susceptible to fusarium wilt disease causing a loss in crop yield. To evaluate the efficacy of plant extract the leaves of 4 plants were collected from Botanical Garden of Dnyanopasak College, Parbhani. Fresh leaves (100 gm) of Annona, Datura, Ocimum and Neem were collected and washed with distilled water and these leaves then chopped into small piece and kept in separate beaker containing 100 ml of Acetone, Methanol and water for 2 days. Then they were homogenized and kept at 27-30$^{o}$C for 48 hrs. The extracts were filtered through filter paper and the resulting mass extract is used as stock solution and it is kept open for evaporation of excess solvent. Three concentrations of leaf extract were prepared from stock solution 5ml, 10ml and 15ml were incorporated into 100ml of sterilized czpeks media (Cz) by using food poisoning technique. The Petri plate was prepared with 15ml of medium and a inoculum of *fusarium oxysporum* was inoculated by using 5mm of disc (filter paper no. 1). After inoculation the petri plates were incubated at 25 ± 2$^{o}$C for 4 days. The growth in diameter was recorded to detect antifungal activity. Same procedure is repeated separately for methanol solvent to detect the antifungal activity.

### Antagonistic activity of bio agent

Antagonistic activity of bio-agents viz –*Trichoderma harzianum*, T. *viride* were collected from Agarkar Institute, Pune. For evaluation of bio control agents against *fusarium oxysporum* F.sp. *carthami* on PDA by using dual culture technique. (Dennis and Webstar, 1986). Pour 20 ml of melted cooled PDA in each sterilized Petri plates. The 5mm disc of antagonistic fungal culture and *fusaruim oxysporum* were inoculated at the opposite side on each Petri plate containing PDA media. Each treatment was replicated three times and incubated at 27 + 2$^{o}$C. Observations were made after 9 days of incubation.

### Evalution of fungicides

Testing of efficacy of some fungicides against *Fusarium oxysporum f.sp.* carthami by food poison technique (Nene and Thaplial 1993 Sindhan *et al*., 1999). Four fungicides namely Bilitox, Captan, Thirum, Captan + Thirum combi fungicides with three Conc$^{n}$ (0.05, 0.10m 0.15%) were taken. These Concentrations were incorporated into 50ml of

PDA. The calculated amount of fungicides was added to Luke warm PDA in flask before pouring. The media without fungicide were used as control. The three replication of each treatment was taken. These plates were incubated with 5mm disc of actively growing mycelium of the organism. These plates were incubated at room temperature 25 + 2°C for 4 days. After 4 days the colony growth in diameter and percent growth inhibition was recorded.

## RESULTS AND DISCUSSION

### Efficacy of leaf extract

Inhibitory effect of leaf extract in acetone solvent of Annona was increased with increasing concentration. Although Annona acetone extract was most effective at 0.15% conc$^{n}$. Datura acetone extract shows less inhibitory effect as compared to Annona acetone extract followed by methanol among poisonous plant. Among medicinal plants ocimum acetone extract and Azardirahcta acetone extract at 15 ml Conc$^{n}$ shows complete inhibition against *Fusarium oxysporum*. f. sp. *carthami* (Table 1).

**Table 1. Efficacy of plant extract against *Fusarium oxysporum F.sp. carthami***

| Sr.No. | Plant extract | Conc$^{n}$ | Growth in diameter (mm) | |
|---|---|---|---|---|
| | | | Acetone | Methanol |
| 1) | Poisonous plants. *Annona Squamosa (sitaphal)* | 5ml | 28.00 | 30.00 |
| | | 10ml | 25.00 | 28.50 |
| | | 15ml | **0.00** | 23.00 |
| | | Control | 30.00 | 35.10 |
| 2) | Datura innoxia (Datura) | 5ml | 38.00 | 40.00 |
| | | 10ml | 31.67 | 35.00 |
| | | 15ml | 28.00 | 30.00 |
| | | Control | 40.00 | 42.50 |
| 1) | Medicinal Plants *Azardirachta indica (Neem)* | 5ml | 15.00 | 18.00 |
| | | 10ml | 3.00 | 5.00 |
| | | 15ml | **0.00** | **0.00** |
| | | | 25.00 | 30.00 |
| 2) | Ocimum Santum (Tulas) | 5ml | 32.50 | 34.00 |
| | | 10ml | 9.00 | 12.00 |
| | | 15ml | **0.00** | 10.00 |
| | | Control | 35.00 | 35.00 |

* Mean was calculated of three replicates.

The efficacy of leaf extract to control *Fusarium oxysporum*. Ahmed and prasad (1995) found that the extract of *Azardirachta indica*, *Lantana camera*, *M. exotica*, *Ocimum santum* and *Datura innoxia* Inhibited against *Fusarium*. Pokhar et al (2003) found the extract of medicinal plant like *Azardirachta indica, Ocimum santum* and Poisonous plant *Datura strominum* were most effective to inhibit the growth of *Fusarium*. Raghuwanshi *et al*. (2003) also shows antifungal activity against *fusarium oxysporum.* Narayanlal et al (2005) reported the plant extract of *Azardirachta indica, Allium sativum, Ocimum santum, Madhuca Indica, Zingiber officinal, Datura strominum* all these plant extract shows antifungal activity against *fusarium oxysporum.* Abdul Latif (2006) studied efficacy of various concentration of four plant extract and these plant tested against the fungi isolated from mustard seed. Like *Alternaria, Fusarium, Aspergillus, Rhizopus, Curvularia and Penicillium*. It was found that Neem Leaf extract was moderately effective in removal of mycoflora of Mustard Seed.

**ANTAGONISTIC ACTIVITY OF BIO AGENTS**: From (Table 2).

The growth of *Fusarium oxysporum* in presences of *T. harzianum* after 3 days of in after incubation was 36.7 mm whereas after 9 DAI the growth was recorded to 12.00 mm and Percent growth inhibition was 60.00%.

**Table2. Effect of *Trichoderma spp* on the growth *of Fusarium oxysporum***

| Sr. No. | Fungal antagonism | Days | Growth of *Fusarium oxysporium* (mm) | *Growth of* Trichoderma harzianum ***(mm)*** | Control | % growth inhibition |
|---|---|---|---|---|---|---|
| 1 | *T. harzianum* | 3 DAI | 6.33 | 1.66 | 10.0 | 36.7 |
| | | 6 DAI | 10.0 | 40.0 | 12.1 | 52.6 |
| | | 9 DAI | 12.0 | 12.0 | 12.0 | 12.0 |
| | | SE + | 0.46 | 1.86 | 1.10 | |
| | | 0.01 | 1.54 | 1.44 | 3.81 | |
| 2 | *Trichoderma Viride* | 3 DAI | 0.5 | 34 | 10.00 | 50 |
| | | 6 DAI | 7.66 | 42.0 | 21.00 | 63.5 |
| | | 9 DAI | 8.66 | 59.6 | 30.00 | 71.1 |
| | | SE + CD | 0.72 | 1.77 | 1.10 | |
| | | 0.01 | 2.48 | 6.13 | 3.81 | |

DAI – Days after incubation *Mean was calculated of three replicates

In presences of T. v*iride* after 3 DAI of the growth was 5mm of percent inhibition was 50%. Where as it is observed that 9 DAI was 8.66% and percent growth inhibition was 71.1%. It is clear that *T. viride* inhibit the maximum growth of *Fusarium oxysporum* as compared to *T.harzianum*. The similar result with Trichoderma spp. was reported earlier Gohil and Vala (1966). *T. viride* in vitro was most effective against soyabean wilt. Shalini et al (2005) reported *T. viride* and *T. harzianum* inhibits maximum antagonistic activity against *Fusarium oxysporum F.sp.* pisi. Prameela *et al.* (2005) reported maximum inhibition against was found in *Trichoderma viride*. C. Kishore *et al.* (2008) tested six bio agents among these *T. viride*, *T. hazianum* and *T. virens* inhibited the mycelial growth of *fusarium oxysporum* f.sp. dianthi. Muley *et al*. (2009) also reported *T. viride* inhibits the maximum growth of *Fusarium oxysporum.*

**Evaluation and fungicides**

With the increased importance of safflower oil in human health and economy in India, it was become necessary to sort out the problems uncounted in increasing production and productivity of safflower. From (Table-3) it is observed that at Normal (0.10), subnormal normal (0.05) and above normal (0.15) concentration significantly higher inhibition of growth was recorded at (0.15) Conc$^{n}$. In Captan + Thirium. The Similar results were recorded by Sharma (1984), Sastry (1997) and Somwanshi (2000).

**Table – 3 Effect of fungicide on the growth of F. oxysporum**

| Sr.No. | Name of fungicide | Conc$^{n}$ | Mean Colony diameter in (mm) | % of growth inhibition. |
|---|---|---|---|---|
| 1) | Bilitox | 0.05 (SN) | 38 | 92.68 |
| | | 0.10 (N) | 34 | 82.92 |
| | | 0.15 (AN) | 22 | 53.65 |
| | | Control | 42.33 | |
| 2) | Captan | 0.05 | 28 | 68.29 |
| | | 0.10 | 23 | 56.09 |
| | | 0.15 | 21 | 51.21 |
| | | Control | 30.00 | |
| 3) | Thirum | 0.05 | 25 | 60.97 |
| | | 0.10 | 22 | 53.65 |
| | | 0.15 | 16 | 39.02 |
| | | Control | 30.00 | |
| 4) | Captan + Thirum | 0.05 | 20 | |
| | | 0.10 | 14.33 | |
| | | 0.15 | 10.66 | |
| | | Control | 30.00 | |

SN = Sub normal N = Normal, AN=above normal

* Mean was calculated of three replicates.

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