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*Full Length Research Paper*

# *In vitro* and *In vivo* responses of different treating agents against wilt disease of safflower

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**In safflower Anucop, captan and Mancozeb M-45 were found extremely effective in reducing *Fusarium oxysporum* f.sp. *carthami* wilt. The seed treatments improved seed germination, seedling vigour and plant stand. Due to these treatments many of the seed-borne fungi failed to express in the normal way. Bioagents formulations viz., *Trichoderma harzianum, Bacillus subtilis* and *Pseudomonas fluorescens* reduced the wilt incidence both under greenhouse and field conditions, thereby enhancing the growth of the seedlings. These antagonists significantly reduced the population of *Fusarium oxysporum* f.sp. *carthami*, increased the seed germination and seedling vigour. Leaf extracts of *Becopa monniera* and *Adathoda vasica* were found effective in the control of safflower wilt. Comparatively *B. monniera* enhanced the seed germination and quality parameters of plants both under greenhouse and field conditions and also effectively suppressed the wilt up to flowering.**

**Key words:** Fungicides, bioagents, plant extracts, *Fusarium oxysporum* f.sp. *carthami*, disease management.

## INTRODUCTION

In safflower, seed-borne fungal diseases play a major role in the yield loss, among which is wilt caused by *Fusarium oxysporum* f.sp. *carthami* Klisiewicz and Houston, a serious disease in almost all major growing areas. Main symptoms of the disease are unilateral yellowing of the foliage followed by wilting, one sided infection on plant parts, golden yellow leaf discoloration, curving of the midrib towards yellow side and dark brown discoloration of vascular tissue of root and stem (Klisiewicz, 1963; Holdeman and McCartney, 1964). In severe infection, flowering is delayed and size of the head remains small and partially blossomed. A large number of ovaries fail to develop seeds or form blackish, small, distorted, chaffy seeds which are abortive (Chakrabarti, 1976). The severity of symptoms varies depending on the time of infection and the genotype with a grain yield loss ranging from 7.2 to 100% (Sastry and Ramachandran, 1993).

Lower dose of fungicides and several micro organisms have been successfully used to control the disease (Raghuchander et al., 1997; Vidyashekaran et al., 1997; Prasad and Rangeswaran, 1999; Krishnamurthy et al., 2001; Kumar and Dubey, 2001). In addition to this many workers have used plant extracts against pathogenic fungi (Tewari and Kurochevu, 1999; Varshnay, 2001). Though many attempts have been made to control the severity of the fungal diseases many lacunae have been left out with respect to dose, type of fungicides as well as bioagents. Since the effectiveness of the chemicals and potential of the organisms varies depending upon the agroclimatic conditions, in the present study few synthetic fungicides, biological agents and plant extracts were used for seed treatment to test their efficacy against safflower wilt disease causing fungus borne *in vitro* and *in vivo*.

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## MATERIALS AND METHODS

### Collection of seed samples and incidence of the wilt pathogen and their effect on seed quality

Seed samples of safflower cultivar Manjira were collected from Annigeri, Dharwad district of Karnataka, India during February 2002 and stored in polyethylene bags for further use. In order to assess the incidence of *F. oxysporum* f.sp. *carthami*, the seeds were subjected to standard blotter method. Seeds were plated on 3 layers of wet blotters in plastic plates and incubated following the procedures of ISTA (Anonymous, 1996). On 8th day of incubation, seeds were observed for the occurrence of *F. oxysporum* f.sp.*carthami* and its incidence was recorded and tabulated. To study effect of *F. oxysporum* f.sp. *carthami* on growing seedlings, 200 seeds were surface sterilized with 2% NaOCl solution and individually plated on 1% water agar in the test tubes, which were. The entire set up was incubated under alternate cycles of 12/12-h light and darkness for a period of 2 weeks. The seeds were examined carefully for the occurrence of colonies of *F. oxysporum* f.sp. *carthami* and seedlings showing symptoms were recorded. The symptoms developed on the seedlings were compared with the other set in which the seeds were inoculated with the spore suspension of *F. oxysporum* f.sp.*carthami*.

### Incidence of the *F. oxysporum* f.sp. *carthami* in water agar method

In addition to the water agar seedling symptom test, both uninoculated and *F. oxysporum* f.sp.*carthami* inoculated seeds were sown in thoroughly washed sand bed and were incubated for a prolonged period of 30 days. During incubation, sand bed was kept moist to prevent desiccation. The seedlings which showed symptoms were identified and such seedlings were finally excised and cut into pieces of 1 cm and incubated on wet blotters. Such components were observed for the occurrence of *F. oxysporum* f.sp. *carthami* and its incidence was recorded.

### Fungicide treatment

In order to find out an effective treating agent for the management of wilt disease a few chemical fungicides viz., Anucop, Bavistin, Captan, Mancozeb M-45, Milzim, Vitavax and Volzim were used at the concentration of 0.1, 0.2 and 0.3%. In this case each fungicide was separately dusted on the seeds and was smeared in a clean polyethylene pouch.

### Isolation, mass culture and formulation preparation of biocontrol agents for seed treatment

For the purpose of utilizing bioagents, a few microbes that included a fungus *Trichoderma harzianum*, bacteria like *Bacillus subtilis* and *Pseudomonas fluorescens* were isolated from safflower rhizosphere soil obtained from the experimental plots of Safflower Research Station, Annigeri, Dharwad district of Karnataka by serial dilution technique (Crossan, 1967).

*T. harzianum* was grown on potato dextrose agar (PDA) in Petri plates and spores were harvested from 12 days old sporulating colonies. 10 ml of sterilized distilled water was added to each of the culture plate and was gently agitated with the help of a fine sterilized camel hairbrush. The spore suspension was collected and filtered through muslin cloth and centrifuged at 10,000 rpm. The sediment was collected, further air-dried and mixed with purified talc powder in the ratio of 1:10 (w/w).

Bacterial biocontrol agents, *B. subtilis* and *P. fluorescens* were cultured on nutrient broth and King's broth, respectively. Two-day-old cultures of *B. subtilis* and *P. fluorescens* were centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and the pellets were washed in sterilized distilled water repeatedly thrice and finally suspended in sterile distilled water. Using spectrophotometer, the optical density of the solution was adjusted to 0.45 at 610 nm, which gave the concentration of $4 \times 10^8$ and $9 \times 10^8$ cfu/ml, respectively. Each of the bacterial formulation was prepared under aseptic condition by mixing 400 ml of bacterial suspension with one kg of purified talc powder. It was air dried and stored in polyethylene bags at 4°C. The bacterial formulations were separately dusted to seeds at three different concentrations of 5, 10 and 15 g/kg of seeds.

### Preparation of the plant extracts

Plant materials such as fresh leaves of *Adathoda vasica* and *Becopa monniera* were harvested and thoroughly washed in tap water. 100g of leaves of each plant were macerated to thick paste with the help of a mortar and pestle. It was extracted with 100 ml of distilled water and filtered through the muslin cloth. The extract was then centrifuged at 5,000 rpm for 15 min. The supernatant obtained was collected and stored at $5^0$C for further use. Seeds were separately soaked in 25, 50 and 75 % leaf extract for 24 h at $28\pm2^0$ C. The treated seeds were then air-dried and used for the assessment of *F. oxysporum* f.sp. *carthami* both in laboratory and field conditions.

### Efficacy of different treating agents against *F.oxysporum* f.sp. *carthami* under laboratory conditions

In all the cases treated seeds were plated on wet blotters and incubated following standard procedures (Anonymous, 1996). The incubated seeds were further carefully evaluated for the occurrence of mycoflora. Similarly treated seeds were subjected to paper towel method, and their percent germination was recorded. The root-shoot length of the seedlings was also measured after 7 days; vigour index was calculated based on mean root length × mean shoot length/ no. of germinated seeds and tabulated. For comparison, untreated seeds maintained under similar conditions served as corresponding control.

### Effect of different treating agents on the incidence of *Fusarium* wilt under greenhouse condition

To evaluate the efficacy of different treatments, treated seeds were sown in soil and separately maintained under greenhouse and field conditions. In greenhouse conditions seeds were sown in the earthen pots (22-cm diameter at 10 seeds/pot containing soil, sand and manure in the ratio of 1:1:1). The germination and the disease incidence were recorded on 7th and 60th days of sowing, respectively.

### Efficacy of different treating agents against *Fusarium* wilt under field conditions

In the experimental plots, field trails were conducted during rabi (2002-2003) season, in randomized block design. The seeds of different treatments were separately sown in the plots of size 5×6 $m^2$ with an inter row spacing of 30 cm. Untreated seeds sown under similar conditions served as control. Along with this, seeds were dressed with target pathogen *F. oxysporum* f.sp.*carthami* and sown for comparison. The pots and the field were applied with known

**Table 1.** Incidence of *F. oxysporum* f.sp. *carthami* in safflower seedlings Under different laboratory conditions.

| Seedling symptom test | Seed germination (%) | % incidence of *Fusarium oxysporum* f.sp. *carthami* on seeds/ seedlings |
|---|---|---|
| Water agar method | 66 | 18.33±0.33[b] |
| Growing-on test | 66 | 21.33±0.33[a] |

Values are mean of four replicates of 100 seeds each. According to Duncan's Multiple Range Test (DMRT), values followed by different superscripts are significantly different at $P \leq 0.05$.

**Table 2.** Effect of *F. oxysporum* f.sp. *cathami* on seed germination , seedling vigour, plant height of safflower in paper towel method.

| Seed treatment | Variation in the seedling quality of safflower due to *Fusarium oxysporum* f.sp. *carthami* | | | | |
|---|---|---|---|---|---|
| | Seed germination (%) | MRL±SE | MSL±SE | VI | Fresh weight (gm) |
| Control | 64 | 8.5±0.03[a] | 4.1±0.03[a] | 806 | 0.31±0.29[a] |
| Control+ pathogen | 58 | 7.4±0.03[b] | 4.0±0.08[a] | 661 | 0.27±0.20[b] |

Values are mean of four replicates of 100 seeds each. Control=seed without *F.oxysporum* inoculation, pathogen=*F. oxysporum* f.sp.*carthami,* MRL-mean root length, MSL-mean shoot length, VI-vigour index. According to Duncan's Multiple Range Test (DMRT), values followed by different superscripts are significantly different at $P \leq 0.05$.

quantity of fertilizers (180-300 kg N+P+K $ha^{-1}$). Apart from the rate of seedling emergence, plant height, number of branches, number of capitula, number of leaves, average leaf area and size of the stem were also recoded at flowering stage. Sequentially disease incidence was also recorded from emergence till seed setting. Percent disease incidence, variation in plant growth and yield were calculated using following formula described by Mukherjee et al. (2001).

% disease incidence = [(Wilt in treated plots - wilt in check plots) / Wilt in check plots] x 100

% Increase in seedling emergence= [(Seedling emergence in treated plots - Seedling emergence in check plots) / Seedling emergence in check plots] x 100

% Increase in yield = [(Yield in treated plots - Yield in check plots) / Yield in check plots] x 100

% Increase in plant height = [(Plant height in treated plots - Plant height in check plots) / Plant height in check plots] x 100

% Increase in branches = [(No. of branches in treated plots - No. of branches in check plots) / No. of branches in check plots] x 100

% Increase in capitula = [(No. of capitula in treated plots - No. of capitula in check plots) / No. of capitula in check plots] x 100

% Increase in leaves = [(No. of leaves in treated plots - No. of leaves in in check plots) / No. of leaves in check plots] x 100

% Increase in size of stem = [(Average stem girth in treated plots - Average stem girth in check plots) / Average stem girth in check plots] x 100

The resultant data from repeated experiments were combined and analyzed statistically based on ANOVA followed by Duncan's Multiple Range Test.

## RESULTS AND DISCUSSION

*F. oxysporum* f.sp. *carthami* infected seeds of safflower and seedlings showed less germination correspondingly in water agar and growing on test was high incidence of the fungus (recast statement) (Table 1). This pathogen also affected the seedling quality in paper towel method (Table 2).

The results depict that there was reduction in the incidence of *F. oxysporum* f.sp. *carthami* at different concentrations of fungicides. Compared to control among the fungicides tested, incidence of the pathogen was inhibited significantly with Captan (0.2%) followed by Anucop, Bavistin, Blitox, Vitavax, Catafol Milzim (0.3%) Mancozeb M-45 and Volzim (Figure 1). Besides, the fungicide treated seeds enhanced the germination compared to control. Sugha et al. (1995) and Sugha (2001) have reported the effectiveness of Bavistin, Captan and Vitavax on population dynamics of white rot fungus (*Fusarium* sp.) of pea. According to Gupta and Aneja (2001) have reported considerable decline in the pathogen population due to treatment with Captan and Mancozeb M-45 in soybean. Singh et al. (2002) have made a comparative *in vitro* study with some fungicides like Captan, Dithane M-45, Vitavax, Bavistin for their efficacy in controlling *Fusarium* species on mungbean. Dubey and Patel (2001) also tested different concentrations of fungicides against various pathogens. Dithane M-45 and Bavistin were reported to be effective in reducing seed-borne infection of *Fusarium* sp. on maize seeds (Kumar and Agarwal, 1998). These fungicides might have paralyzed the fungus at varied level depending upon their dose and type.

Among the bioagents, seeds treated with *T. harzianum* (1%) showed maximum reduction in the incidence of wilt pathogen. Application of *B. subtilis* (1%) and *P. fluorescens* (1.5%) also reduced the incidence of the pathogen compared to control (Figure 1). Bioagents treated seeds also enhanced the germination compared to control. These results are in support of the findings of

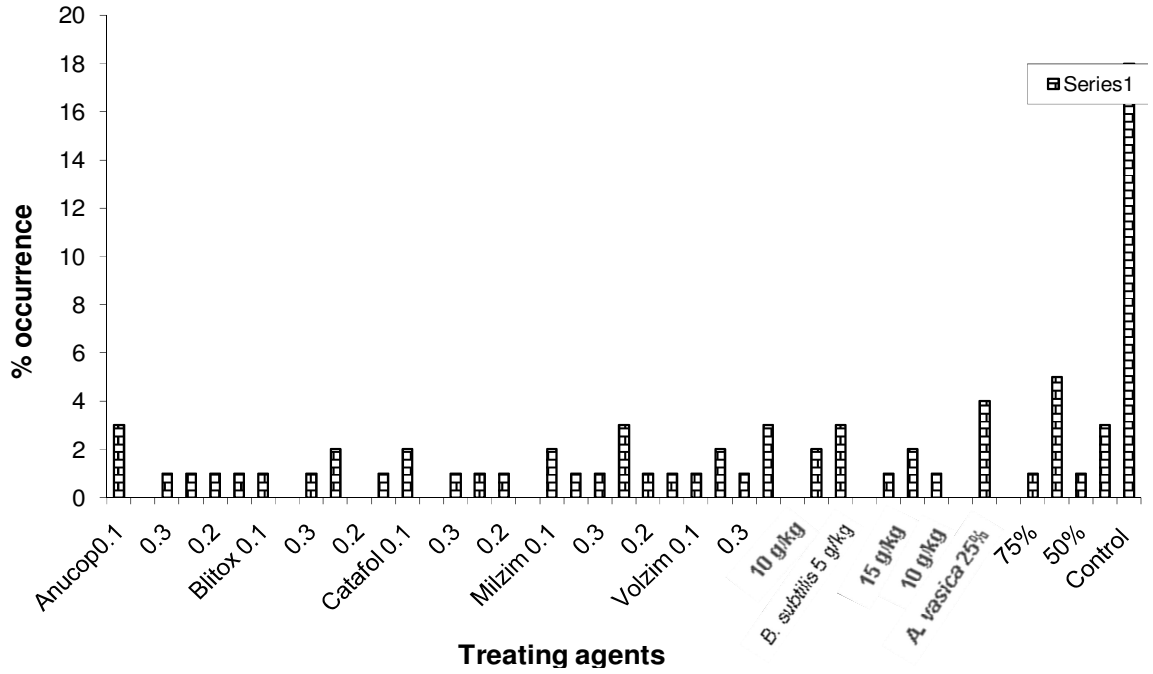


**Figure 1.** Effect of different treatments on the incidence of wilt fungus *Fusarium oxysporum* f.sp. *carthami* in safflower seeds.

Jeyalakshmi et al. (1998) who reported the biological control potential of *T. harzianum*. *Trichoderma* spp. And bacterial bioagents produce mycolytic enzymes, thus playing an important role in the degradation of target pathogens (Elad et al., 1982; Aziz et al., 1993; Baker and Dickman, 1993; Sivakumar and Narayanaswamy, 1998). Among plant extract treatments, 50% extract of *B. monniera* increased the germination, root-shoot length and seedling vigour in paper towel method and decreased the incidence of *F. oxysporum* f.sp. *carthami* which was followed by *A. vasica* compared to control (Figure 1 and Table 3). Many plant extracts are reported to increase seed germination through decreasing *F. oxysporum* incidence (Wegrazyka, 1984; Bansal and Gupta, 2000).

Nine fungicide enhanced seed germination, root-shoot length and seedling vigour. Among them Captan (0.2%) and Mancozeb M-45 (0.3%) followed by Vitavax, Bavistin, Catafol, Milzim (0.2%) and Volzim (0.3%) were more effective (Table 3). Present findings are in support of the reports of several workers (Dey et al., 1989; Chakrabarty and Rao, 1992; Laxminaryan et al., 1996). Bioagents treated seeds showed beneficial effects on germination which resulted in increased root-shoot length and seedling vigour. *T. harzianum* treated seeds were more efficient than *B. subtilis* and *P. fluorescens* treated samples (Table 3). Bunker and Mathur (2000) also reported the similar results with usage of *T. harzianum*.

In greenhouse experiments, it was observed that all fungicides reduced the disease incidence from 18 to 4% (Table 4). Comparatively it was noticed that the reduction in disease incidence was maximum in Anucop, Bavistin, Captan (0.2%) and Mancozeb M-45 (0.3%). Captan and Mancozeb M-45 stood superior over Anucop (0.2%), Bavistin (0.2%), Blitox (0.2%), Milzim (0.2%). Fungicides acted possibly by inducing metabolic changes leading to development of toxic factors, resulting in the internal environment unfavorable for pathogens growth and activity, ultimately inducing the resistance and protection against infection. These results are confirmatory with the observations of Prakash and Ganesan (1998), Parimala et al. (1998), Padmavathi et al. (1998), Sandrou et al. (1998) and Kumar and Dubey (2001) in cowpea, blackgram, brinjal and sunflower, respectively. Reports of De and Chaudhary (1999) are also in confirmation with the present findings, who observed the minimization of wilt disease due to Bavistin, Mancozeb M-45 and Vitavax. Bhatti et al. (1985), Pedgoanker and Mayee (1989) and Cicero et al. (1992) have reported the effectiveness of Thiram, Cabendazim against safflower wilt pathogen *F. oxysporum* f.sp. *carthami*. All the fungicidal seed treatments increased germination, plant height which correlated with the findings of Pathak et al. (2001). Bioagents formulations gave significant results controlling the wilt disease, and also enhanced seed germination and plant height (Table 4). *T. harzianum* was more effective than other bioagents which minimized the wilt followed by *B. subtilis* and *P. fluorescens*. Application of biocontrol agents as a seed treatment may induce the accumulation of lytic enzymes in safflower. The increased

**Table 3.** Effect of treatments on seed germination, seedling growth and vigour of safflower var. manjira.

| Treatments | Dose (%) | Germination (%) | MRL±S.E. | MSL±S.E | Vigour index |
|---|---|---|---|---|---|
| **Fungicides** | | | | | |
| Anucop | 0.1 | 70 | 8.8±0.03$^{b}$ | 6.2±0.03$^{cd}$ | 1057 |
| | 0.2 | 88 | 9.5±0.08$^{a}$ | 6.8±0.05$^{c}$ | 1447 |
| | 0.3 | 72 | 8.8±0.05$^{b}$ | 6.4±0.04$^{c}$ | 1094 |
| Bavistin | 0.1 | 72 | 8.7±0.03$^{b}$ | 6.8±0.03$^{c}$ | 1140 |
| | 0.2 | 81 | 9.4±0.03$^{a}$ | 6.7±0.03$^{c}$ | 1309 |
| | 0.3 | 69 | 8.5±0.03$^{bc}$ | 6.6±0.12$^{c}$ | 1049 |
| Blitox | 0.1 | 70 | 8.1±0.03$^{c}$ | 6.2±0.03$^{cd}$ | 1001 |
| | 0.2 | 84 | 8.8±0.03$^{b}$ | 6.3±0.04$^{cd}$ | 1274 |
| | 0.3 | 78 | 8.2±0.03$^{c}$ | 6.2±0.05$^{cd}$ | 1123 |
| Captan | 0.1 | 70 | 9.1±0.03$^{a}$ | 8.6±0.05$^{a}$ | 1128 |
| | 0.2 | 92 | 9.8±0.03$^{a}$ | 8.8±0.05$^{a}$ | 1629 |
| | 0.3 | 77 | 8.9±0.03$^{b}$ | 6.8±0.02$^{c}$ | 1217 |
| Catafol | 0.1 | 68 | 9.0±0.08$^{a}$ | 5.8±0.01$^{e}$ | 1000 |
| | 0.2 | 72 | 8.5±0.05$^{bc}$ | 5.3±0.03$^{d}$ | 1066 |
| | 0.3 | 69 | 8.8±0.05$^{b}$ | 5.6±0.03$^{d}$ | 1001 |
| Dithane M-45 | 0.1 | 86 | 8.6±0.03$^{b}$ | 6.6±0.05$^{c}$ | 1309 |
| | 0.2 | 84 | 8.8±0.03$^{b}$ | 7.3±0.06$^{b}$ | 1361 |
| | 0.3 | 92 | 9.0±0.03$^{a}$ | 8.4±0.00$^{ab}$ | 1595 |
| Milzim | 0.1 | 68 | 8.1±0.03$^{c}$ | 5.7±0.03$^{e}$ | 0945 |
| | 0.2 | 90 | 8.7±0.03$^{b}$ | 7.3±0.06$^{b}$ | 1440 |
| | 0.3 | 80 | 8.7±0.03$^{b}$ | 6.7±0.04$^{c}$ | 1224 |
| Vitavax | 0.1 | 80 | 8.5±0.03$^{bc}$ | 6.6±0.01$^{c}$ | 1208 |
| | 0.2 | 96 | 8.6±0.03$^{bc}$ | 6.8±0.03$^{c}$ | 1498 |
| | 0.3 | 90 | 8.4±0.08$^{bc}$ | 7.1±0.03$^{b}$ | 1395 |
| Volzim | 0.1 | 80 | 8.4±0.03$^{bc}$ | 7.3±0.05$^{b}$ | 1353 |
| | 0.2 | 78 | 8.6±0.03$^{bc}$ | 6.7±0.03$^{c}$ | 1201 |
| | 0.3 | 88 | 9.1±0.03$^{a}$ | 6.9±0.03$^{c}$ | 1408 |
| **Bioagents formulations** | | | | | |
| *Trichoderma harzianum* | 0.5 | 78 | 8.8±0.35$^{b}$ | 7.4±0.03$^{c}$ | 1272 |
| | 1.0 | 90 | 9.2±0.01$^{a}$ | 6.8±0.03$^{d}$ | 1443 |
| | 1.5 | 79 | 8.7±0.05$^{b}$ | 6.6±0.03$^{d}$ | 1209 |
| *Bacillus subtilis* | 0.5 | 70 | 8.9±0.06$^{b}$ | 7.9±0.05$^{ab}$ | 1176 |
| | 1.0 | 81 | 9.2±0.01$^{a}$ | 8.0±0.03$^{a}$ | 1401 |
| | 1.5 | 70 | 8.8±0.04$^{b}$ | 7.8±0.03$^{b}$ | 1162 |
| *Pseudomonas fluorescens* | 0.5 | 62 | 7.8±0.03$^{d}$ | 7.7±0.03$^{b}$ | 1023 |
| | 1.0 | 68 | 8.1±0.02$^{c}$ | 7.8±0.03$^{b}$ | 1081 |
| | 1.5 | 78 | 8.8±0.04$^{b}$ | 8.3±0.03$^{a}$ | |

**Table 3.** Contd.

| | | | | | |
|---|---|---|---|---|---|
| **Plant extracts** | | | | | |
| *Becopa monniera* | 25 | 76 | 9.1±0.03[b] | 7.3±0.03[a] | 1246 |
| | 50 | 83 | 9.7±0.03[a] | 7.6±0.01[a] | 1436 |
| | 75 | 80 | 8.7±0.03[c] | 7.5±0.03[a] | 1304 |
| *Adathoda vasica* | 25 | 76 | 9.1±0.01[b] | 7.3±0.05[a] | 1239 |
| | 50 | 79 | 9.4±0.02[a] | 7.4±0.03[a] | 1339 |
| | 75 | 77 | 9.1±0.03[b] | 7.3±0.03[a] | 1299 |
| Control | | 64 | 8.5±0.08[c] | 4.1±0.03[b] | 0813 |
| Control + Pathogen | | 58 | 7.4+0.16[d] | 4.0±0.05[b] | 0661 |

MRL-mean root length, MSL-mean shoot length, SE-standard error. Values are mean of four replications. According to Duncan's Multiple Range Test (DMRT), values followed by different superscripts are significantly different at P≤ 0.05.

**Table 4.** Effect of Seed treatment on seedling emergence, plant height, disease incidence under greenhouse conditions.

| Treatments | Dose (%) | Germination (%) | Plant height (cm) | Incidence of the wilt (%) |
|---|---|---|---|---|
| | | **Fungicides** | | |
| | 0.1 | 70 | 58.1±00.8[j] | 4+0.57[cd] |
| Anucop | 0.2 | 88 | 61.3±0.11[c] | - |
| | 0.3 | 72 | 59.1±0.57[ef] | 3+0.57[de] |
| | 0.1 | 72 | 57.8±0.57[jk] | 1+0.57[fg] |
| Bavistin | 0.2 | 81 | 62.1±00.8[bc] | - |
| | 0.3 | 69 | 56.7±0.11[lmn] | 2+0.57[ef] |
| | 0.1 | 70 | 57.4±0.23[jkl] | 3+0.57[de] |
| Blitox | 0.2 | 84 | 62.2±0.57[b] | 1+0.57[fg] |
| | 0.3 | 78 | 58.9±0.57[ghi] | 3+0.57[de] |
| | 0.1 | 70 | 57.2±1.66[pq] | 1+0.57[fg] |
| Captan | 0.2 | 92 | 64.1±0.57[a] | - |
| | 0.3 | 77 | 59.3±0.57[ef] | 1+0.57[g] |
| | 0.1 | 68 | 51.1±0.57[s] | 2+0.57[fg] |
| Catafol | 0.2 | 72 | 54.3±0.57[p] | 1+0.57[ef] |
| | 0.3 | 69 | 51.4±1.00[pqr] | 1+0.57[fg] |
| | 0.1 | 86 | 59.7±0.57[q] | 1+0.57[fg] |
| Dithane M-45 | 0.2 | 84 | 60.3±0.57[d] | 1+0.57[fg] |
| | 0.3 | 92 | 64.0±0.57[a] | - |
| | 0.1 | 68 | 53.1±0.57[qr] | 2+0.57[ef] |
| Milzim | 0.2 | 90 | 56.2±0.57[o] | 1+0.57[fg] |
| | 0.3 | 80 | 52.9±0.57[r] | 2+0.88[ef] |
| | 0.1 | 80 | 57.9±0.57[j] | 4+1.15[cd] |
| Vitavax | 0.2 | 96 | 60.0±0.57[de] | 4+0.57[cd] |
| | 0.3 | 90 | 59.7±0.57[def] | 2+0.57[ef] |
| Volzim | 0.1 | 80 | 56.3±0.57[o] | 2+0.57[ef] |

**Table 4.** Contd.

| | | | | |
|---|---|---|---|---|
| | 0.2 | 78 | 54.1±0.57 $^{p}$ | 4+0.57 $^{ef}$ |
| | 0.3 | 88 | 59.2±0.57 $^{gh}$ | 3+0.57 $^{cd}$ |
| | | **Bioagents formulations** | | |
| | 0.5 | 78 | 58.4±0.11 $^{hij}$ | 3+0.57 $^{de}$ |
| *Trichoderma harzianum* | 1.0 | 90 | 59.9±0.57 $^{def}$ | - |
| | 1.5 | 79 | 59.1±0.57 $^{f}$ | 2+0.57 $^{g}$ |
| | 0.5 | 70 | 56.9±0.57 $^{klm}$ | 3+0.57 $^{de}$ |
| *Bacillus subtilis* | 1.0 | 81 | 60.1±0.88 $^{de}$ | - |
| | 1.5 | 70 | 57.8±0.57 $^{jk}$ | 1+0.57 $^{fg}$ |
| | 0.5 | 62 | 55.8±0.57 $^{h}$ | 2+0.57 $^{ef}$ |
| *Pseudomonas fluorescens* | 1.0 | 68 | 56.4±0.57 $^{mn}$ | 1+0.57 $^{fg}$ |
| | 1.5 | 78 | 57.9±0.57 $^{j}$ | - |
| | | **Plant extracts** | | |
| | 25 | 76 | 56.1±0.00 $^{mn}$ | 4+0.57 $^{cd}$ |
| *Becopa monniera* | 50 | 83 | 59.3±0.57 $^{gh}$ | - |
| | 75 | 80 | 57.9±0.57 $^{j}$ | 1+0.57 $^{kg}$ |
| | 25 | 76 | 56.8±0.57 $^{lm}$ | 5+0.57 $^{c}$ |
| *Adathoda vasica* | 50 | 79 | 58.1±0.57 $^{ij}$ | 1+0.57 $^{fg}$ |
| | 75 | 77 | 55.4±0.57 $^{o}$ | 3+0.57 $^{de}$ |
| Control | | 64 | 40.4±0.11 $^{t}$ | 18±0.57 $^{b}$ |
| Control + Pathogen | | 58 | 38.8±0.57 $^{u}$ | 58 ±0.57 $^{a}$ |

Values are mean of four replications of 100 seeds each, According to Duncan's Multiple Range Test (DMRT), values followed by different superscripts are significantly different at $P \leq 0.05$.

activities of PAL, lytic enzymes and accumulation of phenolics in safflower in response to seed treatment with *T. harzianum* and *P. fluorescens* might have also contributed to increase resistance against pathogen. Effect of these bioagents in plant disease management have been reported by Chang et al. (1986), De et al. (1996), De and Chaudhary (1999), Prasad and Rangeswaran (1999). But *B. subtilis* enhanced the plant height compared to *T. harzianum* and *P. fluorescens*. In case of plant extracts, *B. monniera* (50%) reduced the disease incidence and increased the seed germination, plant height followed by *A. vasica* compared to control. Various workers (Natarajan and Lalithakumari, 1987) have also recorded similar results using plant extracts on rice.

Five fungicides, which were effective under laboratory conditions, were also tested under field conditions. They enhanced seed germination, plant height, number of branches, mean leaf area, number of leaves, girth of stem, number of capitula and number of seeds/capitula compared with untreated seeds (Table 5). Among them, Captan (0.2%) showed maximum increase in all the growth parameters, Mancozeb M-45 (0.3%), Anucop, Bavistin and Blitox (0.2%) were the next effective treatments, which increased the plant growth parameters over control. These fungicides also decreased the wilt incidence during different growth stages of the plants. The early occurrence of wilt was recorded at 30 days after sowing. The highest wilt incidence was in control and even in Blitox (0.2%) treated plants. Seed treatment improved field standing of the plants against the disease through increased plant growth. Seed treatment with fungicides ultimately enhanced the yield through the reduction of wilt disease. In various crops, Taylor and Harman (1990), Mishra and Dharam (1992), Mukhopadyay et al. (1992), De and Chaudhary (1999) and Kumar and Dubey (2001) have reported the similar findings with respect to yield and diseases.

Van Loon et al. (1998) and Laha and Venkataraman (2001) have suggested different fungicides treatment for

**Table 5.** Effect of seed treatments on different growth parameters of safflower against *Fusarium oxysporum* f.sp.*carthami* under field conditions.

| Seed treatments | Dose (%) | Responses of different treatments on quality of safflower plants | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Seed germin. (%) | Plant height (cm) | Mean branches/ plant | Leaf area/ (cm) | Mean no.of leaves/ plant | Mean girth of stem/plant (mm) | Total no. of heads/plant | Mean no. of seeds/plant |
| **Fungicides** | | | | | | | | | |
| Anucop | 0.2 | 90 | 62.0±0.57 $^{c}$ (53.5) | 7.3±0.57$^{ab}$ (34.2) | 67±0.57$^{b}$ (26.4) | 74.4±0.57$^{d}$ (59.7) | 6.36±0.57$^{ab}$ (22.1) | 10.3±0.57$^{c}$ (212.1) | 19+0.57$^{c}$ |
| Bavistin | 0.2 | 88 | 63.2±0.57 $^{bc}$ (56.4) | 6.0 ±0.57$^{bc}$ (10.3) | 65±0.57$^{c}$ (22.6) | 68.1±0.57 $^{e}$(46.1) | 5.48±0.57$^{b}$ (5.2) | 7.8±0.57$^{d}$ (135.2) | 15+0.57$^{p}$ |
| Blitox | 0.2 | 84 | 62.4±0.57 $^{c}$ (54.4) | 5.6±0.57$^{bc}$ (3.0) | 62 ±0.57$^{d}$ (17.0) | 77.8±0.57$^{b}$ (67.0) | 6.46±0.57$^{b}$ (24.0) | 7.1±0.57$^{de}$ (114.8) | 13+0.57$^{ef}$ |
| Captan | 0.2 | 92 | 74.0 ±0.57 $^{a}$ (83.2) | 7.7±0.57$^{a}$ (41.6) | 75±0.57$^{a}$ (41.5) | 80.2±0.57$^{a}$ (72.1) | 7.43±0.57$^{a}$ (42.6) | 14.7±0.57$^{a}$ (345.5) | 24+0.57$^{a}$ |
| Mancozeb M-45 | 0.3 | 92 | 64.4 ±0.57 $^{b}$ (59.4) | 7.5±0.57$^{a}$ (37.9) | 74±0.57$^{a}$ (39.6) | 75.4±0.57$^{c}$(61.8) | 6.48±0.57$^{ab}$ (24.4) | 11.6±0.57$^{b}$ (252.7) | 21+0.57$^{b}$ |
| **Bioagents formulations** | | | | | | | | | |
| *Trichoderma harzianum* | 1.0 | 90 | 63.8±0.57 $^{a}$ (57.8) | 7.1±0.57$^{ab}$ (30.5) | 64±0.57$^{c}$ (20.8) | 72.2±0.57$^{d}$ (54.9) | 6.23±0.57$^{ab}$ (19.6) | 6.2±0.57$^{ef}$ (86.4) | 14+0.57$^{e}$ |
| *Bacillis subtilis* | 1.0 | 86 | 61.8±0.57 $^{c}$ (52.5) | 5.8±0.57$^{c}$ (6.6) | 57±0.57$^{e}$ (7.5) | 66.1±0.57$^{f}$ (41.8) | 5.39±0.57$^{b}$ (3.5) | 5.8±0.57$^{ef}$(77.6) | 12+0.57$^{f}$ |
| *Psuedomonas fluorescens* | 1.5 | 84 | 57.2±0.57 $^{e}$ (41.6) | 5.7±0.57$^{c}$ (4.8) | 55±0.57$^{e}$ (3.8) | 65.2±0.57$^{f}$ (39.9) | 5.31±0.57$^{a}$ (2.0) | 5.7±0.57$^{ef}$ (72.2) | 10+0.57$^{g}$ |
| **Plant extracts** | | | | | | | | | |
| *Becopa monniera* | 50 | 83 | 59.3±0.57 $^{d}$ (46.8) | 5.9±0.57$^{c}$ (8.4) | 56±0.57$^{f}$ (5.7) | 75.4±0.57$^{c}$ (61.8) | 6.39±0.57$^{ab}$ (22.6) | 5.4±0.57$^{f}$ (64.2) | 9+0.57$^{gh}$ |
| *Adathoda vasica* | 50 | 80 | 58.1±0.57 $^{de}$ (43.8) | 5.7±0.57$^{c}$ (4.77) | 54±0.57$^{g}$ (1.88) | 64.8±0.57$^{f}$ (39.1) | 5.84±0.57$^{ab}$ (12.1) | 5.1±0.57$^{f}$ (52.4) | 8+0.57$^{h}$ |
| Control | | 64 | 40.4+0.57 $^{f}$ | 5.44±0.57$^{c}$ | 53±0.57$^{g}$ | 46.6±0.57$^{g}$ | 5.21±0.57$^{b}$ | 3.3±0.57$^{g}$ | 4±0.57$^{i}$ |
| Control + pathogen | | 58 | 38.8+0.57 $^{f}$ | 5.20±0.57$^{c}$ | 50.4±0.57$^{h}$ | 42.1±0.57$^{h}$ | 2.13±0.57$^{c}$ | 1.8±0.57$^{c}$ | 2±0.57$^{j}$ |

Data given in parenthesis is refers to per cent increment over control. According to Duncan's Multiple Range Test (DMRT), values followed by different superscripts are significantly different at $P \leq 0.05$. Values are mean of four replications of 100 seeds each.

better suppression of various diseases. Seed treatments with fungicides were found effective against wilt pathogen (*F. oxysporum* f.sp. *phaseoli*) of French bean under field conditions. They also increased seed germination, plant height and grain yield (Mukherjee et al., 2001). Bioagents showed significant effect on seed germination and improved the quality of safflower plants such as plant height, branches/plant, leaf area and number of leaves, girth of stem, number of heads/plant and number of seeds/plant (Table 5). Among bioagents treatment *T. harzianum* showed maximum beneficial effects followed by *B. subtilis* and *P. fluorescens*. These bioagents also decreased the wilt incidence during different growth stages. The highest wilt incidence was recorded in *F. oxysporum* f.sp. *carthami* treated seeds and untreated control plots (18%). Bioagent formulations improved field standing of the plant against the disease and alsoimproved the plant growth parameters. Improvement in quality of safflower plants due to treatment with *Trichoderma* species, *B. subtilis* and *P. fluorescens* against *Fusarium* species has also been reported by Gehlot and Purohit (2002). Results with respect to efficacy of the plant extracts tested for the control of safflower wilt under field conditions revealed less incidence of wilt (2%) due to *B. monniera* over by *A. vasica* (3%). Both plants extracts showed moderate effect on germination, as well as increased the shoot length, number of leaves, plant height, number of heads and other growth parameters (Tables 5 and 6). Plant extracts with their antifungal properties may affectrespiration through the presence of inhibitors, carboxin, which

**Table 6.** Effect of different seed treatments on the incidence of wilt of safflower under field conditions.

| Treatment | Dose (%) | % variation in the development of wilt due to various treatments in safflower during different growth stages | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 30 DAS | | | 60 DAS | | 90DAS | |
| | | Seed germination (%) | DI (%) | DIR (%) | DI (%) | DIR (%) | DI | DIR (%) |
| **Fungicides** | | | | | | | | |
| Anucop | 0.2 | 90 | - | 90±0.57$^{a}$ | 1±0.57$^{c}$ | 84±0.57$^{c}$ | 1±0.57$^{de}$ | 84+0.57$^{c}$ |
| Bavistin | 0.2 | 88 | - | 88±0.57$^{c}$ | 1±0.57$^{c}$ | 83±0.57$^{d}$ | 1±0.57$^{de}$ | 83+0.57$^{c}$ |
| Blitox | 0.2 | 84 | 1±0.57$^{c}$ | 77±0.57$^{f}$ | 1±0.57$^{c}$ | 79±0.57$^{e}$ | 2±0.57$^{cd}$ | 75+0.57$^{f}$ |
| Captan | 0.2 | 92 | - | 92±0.57$^{a}$ | - | 92±0.57$^{a}$ | - | 92+0.57$^{a}$ |
| Mancozeb M-45 | 0.3 | 92 | - | 92±0.57$^{a}$ | - | 92±0.57$^{a}$ | - | 92+0.57$^{a}$ |
| **Bioagents formulations** | | | | | | | | |
| *Trichoderma harzianum* | 1.0 | 90 | - | 90±0.57$^{b}$ | - | 90±0.57$^{b}$ | - | 90+0.57$^{b}$ |
| *Bacillus subtilis* | 1.0 | 86 | - | 86±0.57$^{d}$ | 1±0.57$^{c}$ | 81±0.57$^{d}$ | 1±0.57$^{de}$ | 81+0.57$^{d}$ |
| *Pseudomonas fluorescens* | 1.5 | 84 | 1±0.57$^{c}$ | 78±0.57$^{f}$ | 1±0.57$^{c}$ | 79±0.57$^{e}$ | 1±0.57$^{de}$ | 79+0.57$^{e}$ |
| **Plant extracts** | | | | | | | | |
| *Becopa monnieri* | 50 | 83 | - | 83±0.57$^{e}$ | 1±0.57$^{c}$ | 78±0.57$^{e}$ | 2±0.57$^{cd}$ | 74+0.57$^{f}$ |
| *Adathoda vasica* | 50 | 80 | 1±0.57$^{c}$ | 74±0.57$^{g}$ | 1±0.57$^{c}$ | 75±0.57$^{f}$ | 3±0.57$^{c}$ | 67+0.88$^{g}$ |
| Control | | 64 | 14±0.57$^{b}$ | | 16±0.57$^{b}$ | | 18±0.57$^{b}$ | |
| Control + pathogen | | 58 | 58±0.57$^{a}$ | | 58±0.57$^{a}$ | | 58±0.57$^{a}$ | |

DI-disease incidence, DIR-disease incidence reduction. According to Duncan's Multiple Range Test (DMRT), values followed by different superscripts are significantly different at $P \leq 0.05$. Values are mean of four replications of 100 seeds each.

interfered with synthesis of protein, DNA and RNA in the rapidly metabolizing cells of the pathogens (Raysdale and Silser, 1970; Natarajan and Lalithakumari, 1987). Varshey (2001) evaluated the effect of some plant extracts on important diseases of plants on rice crop. Banasal and Gupta (2001) have used many plant extracts against *F. oxysporum* for its effective control.

Efficacy of fungicides, bioagents and plant extracts showed similar results in *in vitro* and *in vivo* in the management of *Fusarium* wilt of safflower. Among fungicides Captan (0.2%), Mancozeb M-45 (0.3%) and Anucop (0.2%), in bioagents *T. harzianum* (1.0%), plant extract *B. monniera* (50%) were found highly effective in controlling safflower diseases in turn enhancing the quality of plants under both greenhouse and field conditions. Thus, the present study highlight the importance of seed treatment with most effective fungicides, bioagents and plant extracts in suppression of safflower wilt disease.

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