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Efficacy of fungicides, plant extracts and bioagents against *Alternaria alternata* on coriander under laboratory condition

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Abstract
A study was conducted at the Department of Plant Pathology, N. M. College of Agriculture, NAU, Navsari during 2021-22 to find out the efficacy of different fungicides, plant extracts and bioagents against *A. alternata* caused alternaria blight of coriander under *in vitro* condition. In laboratory study among non-systemic fungicides, maximum mycelial growth inhibition was observed in copper oxychloride 50 WP at 2000 ppm (80.37%) followed by mancozeb 75 WP at 2000 ppm (75.93%). Whereas, among systemic fungicides, Propiconazole 25 EC at both concentrations completely inhibited the mycelial growth followed by azoxystrobin 23 SC at 500 ppm (33.33%). *A. indica* (Neem) at 10 percent concentration gave 51.85 percent inhibition and proved significantly superior over rest of the plant extracts tested. The maximum mycelial growth inhibition was recorded in *T. harzianum* Rifai. NAU isolate 77.50 percent.

Keywords: Coriander, *Alternaria alternata*, alternaria blight, fungicides, plant extracts, bioagents

Introduction
Coriander (*Coriandrum sativum* L.) is an annual herbaceous plant (2n = 22), which belongs to the family *Umbelliferae* or *Apiaceae* and generally grown in winter season as main crop in India (Singh and Verma, 2015) [17]. In India, spices are considered the highest quality in the world and are known as "Home of Spices". It is also known by various names like Dhania (Hindi), Dhane, Dhania (Bengali) and Kothmiri, Konphir, Libdhane (Gujarati). The coriander plant suffers from several diseases caused by fungi and other microorganisms. Important diseases incited by fungi are stem galls or tumours (*Protomyces macrospores*), stem rot (*Sclerotinia sclerotiorum*), wilt (*Fusarium oxysporum f. sp. coriandri*), powdery mildew (*Erysiphe polygoni*), root and stem rot (*Rhizoctonia solani* and *Macrophomina phaseolina*) and alternaria blight caused by *Alternaria alternata* (Khan *et al.*, 1984) [9]. Out of these diseases, alternaria blight of coriander is emerging as a major and wide spread problem in India as well as in Gujarat. A major disease of the *Alternaria* genus is the blight disease, which causes yield losses ranging from 32-57 percent on an average (Conn and Tewari, 1990). Therefore, it is very necessary to have some information on the inhibitory effect of fungicides, plant extracts and antagonistic effect of bioagents under laboratory condition. Keeping in view the importance of coriander and the severity of the disease, the present work was carried out to know the effective fungicides, plant extracts and bioagents *in vitro* and results so obtained were documented here under.

Materials and Methods
Efficacy of different fungicides against *Alternaria alternata in vitro*
Efficacy of different fungicides were tested in the Laboratory of Department of Plant Pathology, N. M. College of Agriculture, NAU, Navsari using Completely Randomized Design with three repetitions. Different non-systemic and systemic fungicides were selected and evaluated against the test pathogen. Among them, four non-systemic fungicides *viz.*, Mancozeb 75 WP, Propineb 70 WP, Zineb 75 WP and Copper oxychloride 50 WP with two concentrations (1000 and 2000 ppm) and systemic fungicides Propiconazole 25 EC, Carbendazim 50 WP, Azoxystrobin 23 SC and Thiophanate methyl 70 WP with two concentrations (250 and 500 ppm) against mycelial growth of *A. alternata* was tested by Poisoned Food Technique (PFT) suggested by Nene and Thapliyal (1979) [12]. The required quantity of each fungicide was added separately to sterilized medium mixed thoroughly and

poured in sterilized Petri plates and allowed to solidify for 12 hours. Each plate was inoculated with 5 mm disc of seven days old culture of *A. alternata* with the help of sterilized cork borer and incubated at 25±1 °C for seven days. A control was also maintained where medium was not supplemented with any fungicides. The mycelial growth of the test fungus was recorded and percent growth inhibition was calculated by Vincent's (1947) [21] formula given below.

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

Where,
I = Percent inhibition of fungal growth
C = Diameter of colony in check (Average of both diagonals)
T = Diameter of colony in treatment (Average of both diagonals)

Efficacy of plant extracts against *Alternaria alternata in vitro*

The efficacy of seven plant extract, *viz.*, Neem, Datura, Custard apple, Onion, Aak, Marigold and Tulsi was tested at 10 percent concentration with Completely Randomized Design with three repetitions against mycelial growth of *A. alternata* was tested by Poisoned Food Technique (PFT). To get these, the required plant leaf was thoroughly washed with sterilized water. Each sample was homogenized in sterilized distilled water at the rate of 1 ml/g of tissues (1:1 V/W) with a pestle and mortar and filtered through fine muslin cloth. The filtrate thus obtained was centrifuged at 5000 rpm for 20 minutes and the supernatant was filtered with sterilized funnel having the pore size of 1-2 µ, which was consider as 100 percent standard plant extract solution. Required concentrations of respective plant extracts were prepared accordingly (Ansari, 1995) [3]. Required quantity of each plant extracts were mixed thoroughly in melted PDA, to get desired concentration, just before pouring in sterilized Petri plates and was allowed to solidify for 12 hours. Each plate was inoculated with 5 mm disc of seven days old culture of *A. alternata* with the help of sterilized cork borer. The inoculated Petri plates were incubated at 25+1 °C for seven days. A control was also maintained where medium was not supplemented with any of the plant extracts. Colony diameter (two diagonals) was measured after seven days of incubation. The percent growth inhibition was calculated according to fungicides.

Efficacy of Bioagents against *Alternaria alternata in vitro*

The antagonistic effects of five different fungal and bacterial bioagents, *viz.*, *Trichoderma harzianum* Rifai. NAU isolate, *Trichoderma koningii*, JAU isolate, *Trichoderma viride* Pers, Ex. Grey, NAU isolate, *Bacillus subtilis* Ell NAU isolate and *Pseudomonas fluorescens* Migula NAU isolate were tested against *A. alternata* by using dual culture technique (Morton and Stroube, 1955) [11] in Completely Randomized Design with four repetitions. Twenty millilitre of sterilized melted PDA medium poured aseptically in each 90 mm Petri plates and allowed to solidify, for bacterial bioagents melted PDA (50%) + NA (50%) media were used. Mycelial disc of 5 mm diameter of each antagonist and test fungus were cut with the help of sterilized cork borer from the edges of actively growing culture and placed on the PDA medium in the same Petri plates, on opposite corners by keeping 1 cm distance from distal ends of Petri plates. Inoculated Petri plates were incubated at 25+1 °C in BOD incubator for seven days. The plates were observed for growth of antagonist and test fungus after seven days of inoculation. The growth of antagonist and test fungus was measured by linear measurement. Control plate was also maintained by placing pathogens opposite corner in the same plate. The radial growth of the test pathogen was measured when control plate pathogen contacted to each other.

Results and Discussion

Efficacy of Non-systemic fungicides against *Alternaria alternata in vitro*

Four non-systemic fungicides were tested against *A. alternata in vitro* along with control. The fungicides were tested at two different concentrations (1000 and 2000 ppm) with control by poisoned food technique and observations on mycelial growth and percent growth inhibition were recorded after seven days of inoculation. The results obtained are communicated here under (Table 1 and Plate 1).

All the two concentrations of non-systemic fungicides tested found significantly superior in inhibiting the mycelial growth of *A. alternata* over control. As the concentration of fungicides increases, the mycelial growth inhibition also increases.

The least mycelial growth was observed in copper oxychloride 50 WP at 2000 ppm (17.67 mm) followed by mancozeb 75 WP at 2000 ppm (21.67 mm), zineb 75 WP at 2000 ppm (24.33 mm). However, propineb 70 WP recorded maximum mycelial growth (53.33 mm) at 1000 ppm concentration over control (90.00 mm) and found least effective as compared to other fungicides.

Table 1: Efficacy of non-systemic fungicides against *A. alternata in vitro*

Tr. No.	Technical name/active ingredient	Concentration (ppm)	Mycelial growth (mm)	Mycelial growth inhibition (%)
T_1	Mancozeb 75% WP	1000	5.16* (26.67)	57.02** (70.37)
T_2		2000	4.65 (21.67)	60.63 (75.93)
T_3	Propineb 70% WP	1000	7.30 (53.33)	39.66 (40.74)
T_4		2000	6.58 (43.33)	46.06 (51.85)
T_5	Zineb 75% WP	1000	5.51 (30.33)	54.52 (66.30)
T_6		2000	5.06 (25.67)	57.75 (71.48)
T_7	Copper oxychloride 50% WP	1000	4.93 (24.33)	58.67 (72.96)
T_8		2000	4.20 (17.67)	63.70 (80.37)
T_9	Absolute control (without treatment)	-	9.49 (90.00)	0.57 (0.00)
S.Em. ±			0.08	0.56
CD (P=0.05%)			0.23	1.68
CV %			2.28	2.01

* Figures outside the parentheses are $\sqrt{x}$ transformation values
**Figures outside the parentheses are arcsine transformation values where in parentheses are original values

The maximum mycelial growth inhibition was observed in copper oxychloride 50 WP at 2000 ppm (80.37%) followed by mancozeb 75 WP at 2000 ppm (75.93%), zineb 75 WP at 2000 ppm (71.48%). However, propineb 70 WP found least effective as compared to other fungicides and had least mycelial growth inhibition (40.74%) at 1000 ppm concentration over control.

Thus, maximum mycelial growth inhibition was observed in copper oxychloride 50 WP at 2000 ppm and least effective fungicide was propineb 70 WP at 1000 ppm concentration against *A. alternata in vitro* condition.

The results obtained in present study is in close agreement with the results obtained by Thaware *et al.* (2010) [19]. They reported that copper oxychloride (0.2%) with 86.33 percent growth inhibition was found in *A. alternata* causing leaf blight of cowpea. Kadam *et al.* (2018) [6] observed that the leaf and fruit rot in pomegranate caused by *A. alternata* was evaluated with five non-systemic fungicides in *in vitro* condition. Among non-systemic fungicides the highest mycelial growth inhibition was with mancozeb (78.05%), copper oxychloride (70.13%) and copper hydroxide (66.79%).

Efficacy of Systemic fungicides against *Alternaria alternata in vitro*

Four systemic fungicides were tested against *A. alternata in vitro* along with control. The fungicides were tested at two different concentrations (250 and 500 ppm) with control by poisoned food technique and observations on mycelial growth and percent growth inhibition were recorded after seven days of inoculation. The results obtained are communicated here under (Table 2 and Plate 2).

The least mycelial growth was found in propiconazole 25 EC (0.00 mm) at both 250 and 500 ppm concentration over control (90.00 mm). This treatment was followed by azoxystrobin 23 SC at 500 ppm (60.00 mm) and carbendazim 50 WP at 500 ppm (70.67 mm). Maximum mycelial growth was observed in thiophanate methyl 70 WP at 250 ppm (81.67 mm).

Propiconazole 25 EC at both concentrations completely inhibited the mycelial growth and proved significantly superior over the rest of the treatments tried. The next best effective treatment in order of merit was azoxystrobin 23 SC at 500 ppm (33.33%) followed by carbendazim 50 WP at 500 ppm (21.48%) however, thiophanate methyl 70 WP found less effective as compared to other fungicides and had least mycelial growth inhibition (9.26%) at 250 ppm concentration over control.

All the two concentrations of systemic fungicides tested against *A. alternata* observed poor except propiconazole 25 EC inhibited completely mycelial growth of *A. alternata* significantly superior over control. As the concentration of fungicides increases, the mycelial growth inhibition also increases except propiconazole 25 EC, where inhibition remained the same in both lower and higher concentration of fungicide.

These results are in accordance with the results of Thaware *et al*. (2010) [19]. They reported that propiconazole (0.05%) completely inhibited the growth of *A. alternata* causing leaf blight of cowpea. Pamrao (2017) [13] found cent percent mycelial growth inhibition of *A. alternata* infecting mungbean in treatment of propiconazole (0.1%). Rani *et al.* (2018) [15]. According to them, among all tested fungicides propiconazole at 0.1, 0.2 and 0.3 percent completely (100%) inhibited growth of *Alternaria* spp. (*A. tenuissima* and *A. alternata*) infecting pigeonpea.

Table 2: Efficacy of systemic fungicides against *A. alternata in vitro*

Tr. No.	Technical name/active ingredient	Concentration (ppm)	Mycelial growth (mm)	Mycelial growth inhibition (%)
T_1	Propiconazole 25% EC	250	0.71*(0.00)	89.48** (99.99)
T_2		500	0.71 (0.00)	89.48 (99.99)
T_3	Carbendazim 50% WP	250	8.63 (74.00)	24.92 (17.78)
T_4		500	8.44 (70.67)	27.60 (21.48)
T_5	Azoxystrobin 23% SC	250	7.99 (63.33)	32.96 (29.63)
T_6		500	7.78 (60.00)	35.26 (33.33)
T_7	Thiophanate methyl 70% WP	250	9.06 (81.67)	17.53 (9.26)
T_8		500	8.97 (80.00)	19.47 (11.11)
T_9	Absolute control (without treatment)	-	9.51 (90.00)	0.57 (0.00)
S.Em. ±			005	0.82
CD (P=0.05%)			0.15	2.44
CV %			1.31	3.79

* Figures outside the parentheses are $\sqrt{x}$ +0.5 transformation values

**Figures outside the parentheses are arcsine transformation values where in parentheses are original values

Efficacy of Plant Extracts against *Alternaria alternata in vitro*

Naturally available seven plant extracts were tested against *A. alternata in vitro* along with control. The plant extracts were tested at 10 percent concentration with control by poisoned food technique. Observation on mycelial growth and percent growth inhibition were recorded after seven days of inoculation. The results obtained are presented here under (Table 3 and Plate 3).

The least mycelial growth was found in neem (43.33 mm) followed by datura (51.67 mm), onion (53.33 mm), tulsi (55.33 mm), aak (61.33 mm), marigold (65.67 mm) and maximum mycelial growth was observed in custard apple (71.00 mm) at 10 percent concentration over control (90.00 mm).

A. indica (Neem) at 10 percent concentration gave 51.85 percent inhibition and proved significantly superior over rest of the plant extracts tested. The next best effective treatments were *D. stramonium* (Datura) 42.59 percent and at par with *A. cepa* (Onion) 40.74 percent. *O. sanctum* (Tulsi) 38.52 percent and *C. gigantean* (Aak) found moderately effective with 31.85 percent inhibition. However, *T. erecta* (Marigold) and *A. reticulata* (Custard apple) were found less effective as compared to other plant extracts and gave poor growth inhibition with 27.04 and 21.11 percent at 10 percent concentration, respectively over control.

Thus, maximum mycelial growth inhibition was found in *A. indica* (Neem) while the minimum mycelial growth inhibition was found in *A. reticulata* (Custard apple) at 10 percent concentration.

Table 3: Efficacy of plant extracts against *A. alternata in vitro*

Tr. No.	Plant extracts	Mycelial growth (mm)	Mycelial growth inhibition (%)
T_1	*Azadirachta indica* (Neem)	6.58* (43.33)	46.06** (51.85)
T_2	*Datura stramonium* (Datura)	7.19 (51.67)	40.73 (42.59)
T_3	*Annona reticulata* (Custard apple)	8.43 (71.00)	27.33 (21.11)
T_4	*Allium cepa* (Onion)	7.30 (53.33)	39.65 (40.74)
T_5	*Calotropis gigantean* (Aak)	7.83 (61.33)	34.34 (31.85)
T_6	*Tagetes erecta* (Marigold)	8.10 (65.67)	31.32 (27.04)
T_7	*Ocimum sanctum* (Tulsi)	7.44 (55.33)	38.35 (38.52)
T_8	Absolute control (without treatment)	9.49 (90.00)	0.58 (0.00)
S.Em. ±		0.08	0.81
CD (P=0.05%)		0.25	2.42
CV %		1.82	4.33

* Figures outside the parentheses are $\sqrt{x}$ transformation values
**Figures outside the parentheses are arcsine transformation values where in parentheses are original values

More or less similar kind of trend was also observed by Kantwa *et al*. (2014) [7] studied the efficacy of seven plant extracts at five concentrations (50, 100, 200, 500 and 1000 ppm) through poisoned food technique against the mycelial growth and sporulation of *A. alternata* causing leaf blight of clusterbean. Among them, garlic clove extract was found most effective in inhibiting the mycelial growth (46.60%) of *A. alternata* followed by neem (43.30%) and datura (40.30%) leaf extract. Singh *et al.* (2018) [18] tested the efficacy of seven plant extracts at four concentrations *i.e.*, 5, 10, 15 and 20 and found that *A. indica* (Neem) was significantly inhibited the mycelial growth of pathogen at all concentrations followed by *D. strumarium* (Jimson weed). Similar kind of results were also obtained by Amina and Shamim (2016) [2], Pamrao (2017) [13] and Prasad *et al*. (2017) [14].

Efficacy of Bioagents against *Alternaria alternata in vitro*

Total five fungal and bacterial bio agents were evaluated for their antagonistic action against *A. alternata* by dual culture method *in vitro*. The observations on mycelial growth and percent growth inhibition were recorded when the growth of two pathogens in control plate contact to each others. The results obtained are presented here under (Table 4 and Plate 4).

The least mycelial growth was found in *T. harzianum* Rifai. NAU isolate (20.25 mm) followed by *T. viride* Pers, Ex. Grey, NAU isolate (23.25 mm), *T. koningii*, JAU isolate (27.50 mm), *P. fluorescens* Migula NAU isolate (41.75 mm), while, maximum mycelial growth was observed in *B. subtilis* Ell NAU isolate (45.25 mm) over control.

Table 4: Efficacy of bioagents against *A. alternata in vitro*

Tr. No.	Bio-control agents	Mycelial growth (mm)	Mycelial growth inhibition (%)
T_1	*Trichoderma harzianum* Rifai. NAU isolate	4.50* (20.25)	61.72** (77.50)
T_2	*Trichoderma koningii*, JAU isolate	5.24 (27.50)	56.45 (69.45)
T_3	*Trichoderma viride* Pers, Ex. Grey, NAU isolate	4.82 (23.25)	59.48 (74.17)
T_4	*Bacillus subtilis* Ell NAU isolate	6.73 (45.25)	44.83 (49.72)
T_5	*Pseudomonas fluorescens* Migula NAU isolate	6.46 (41.75)	47.07 (53.61)
T_6	Absolute control (without treatment)	9.49 (90.00)	0.58 (0.00)
S.Em. ±		0.07	0.88
CD (P=0.05%)		0.21	2.60
CV %		2.24	3.90

* Figures outside the parentheses are $\sqrt{x}$ transformation values
**Figures outside the parentheses are arcsine transformation values where in parentheses are original values

All the bio control agents significantly inhibited the mycelial growth of *A. alternata* over control. The maximum mycelial growth inhibition was recorded in *T. harzianum* Rifai. NAU isolate 77.50 percent. The next best effective treatment found was *T. viride* Pers, Ex. Grey, NAU isolate 74.17 percent followed by *T. koningii*, JAU isolate 69.45 percent and *P. fluorescens* Migula NAU isolate 53.61 percent while, minimum mycelial growth inhibition was recorded in *B. subtilis* Ell NAU isolate 49.72 percent.

The present study is in close conformity with the results obtained by Roopa *et al.* (2014) [16] studied the bio efficacy of various fungal bioagents and found that the *T. harzianum* found effective in inhibiting the mycelial growth (77.50%) followed by *T. viride* (75.14%), *T. koningii* (73.19%) and *T. virens* (71.53%). The least inhibition of the fungus was observed in *B. subtilis* (52.02%) and *P. fluorescens* (36.22%). Zakir *et al.* (2021) [22] investigated the efficacy of different bio agents against *A. alternata in vitro*. *T. harzianum* was found to be superior over all treatments with (83.73%) growth inhibition followed by *T. viride* (78.45%) and *P. fluorescens* (63.00%), while *B. subtilis* (51.21%) was found to be least effective. Vighe (2014) [20], Ahmad and Ashraf (2016) [1], Jakatimath *et al.* (2017) [5], Kayim *et al.* (2017) [8] and Marchande *et al.* (2020) [10] also reported same kind of results.

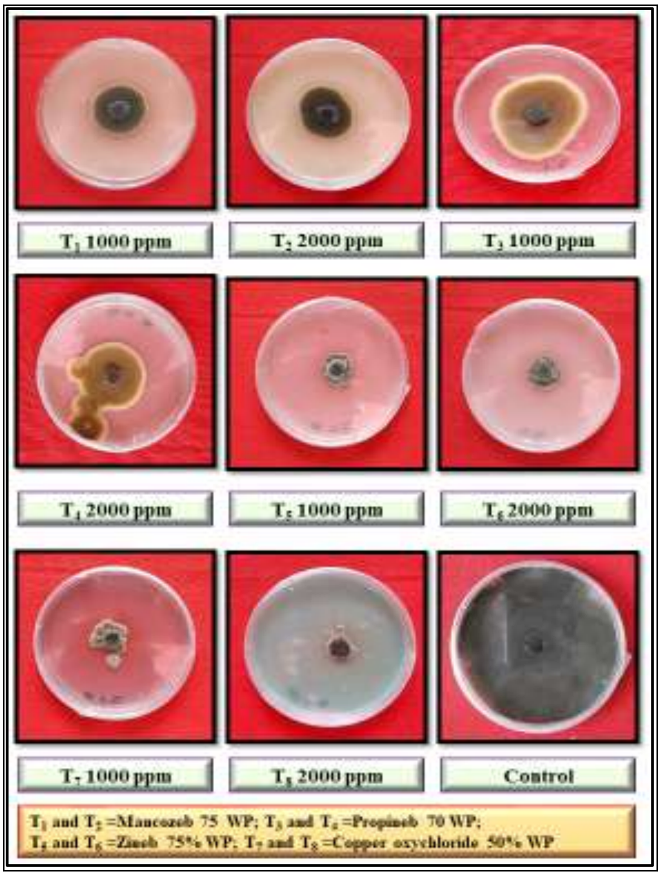

Plate 1: Evaluation of non-systemic fungicides (1000 ppm and 2000 ppm) against *A. alternata in vitro*

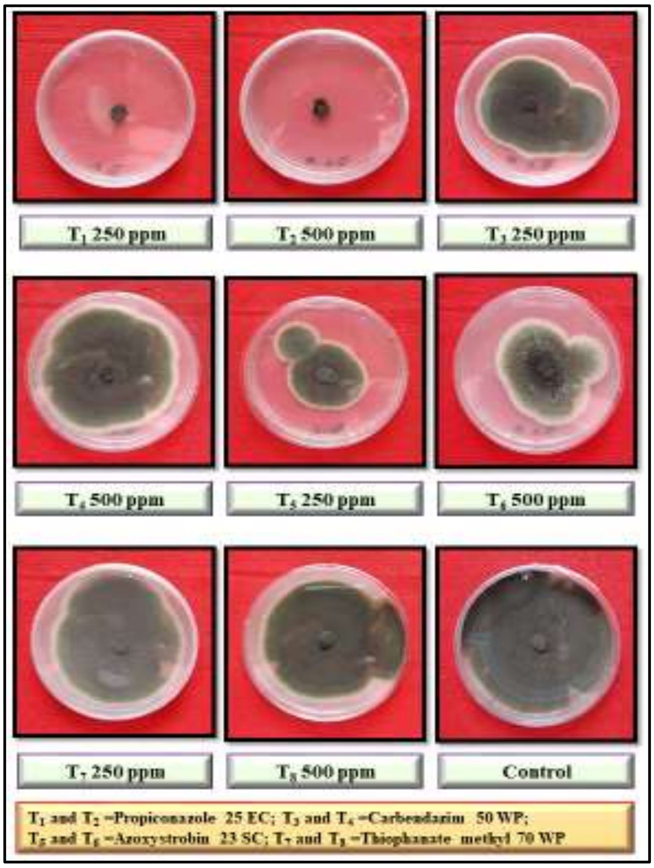

Plate 2: Evaluation of systemic fungicides (250 ppm and 500 ppm) against *A. alternata in vitro*

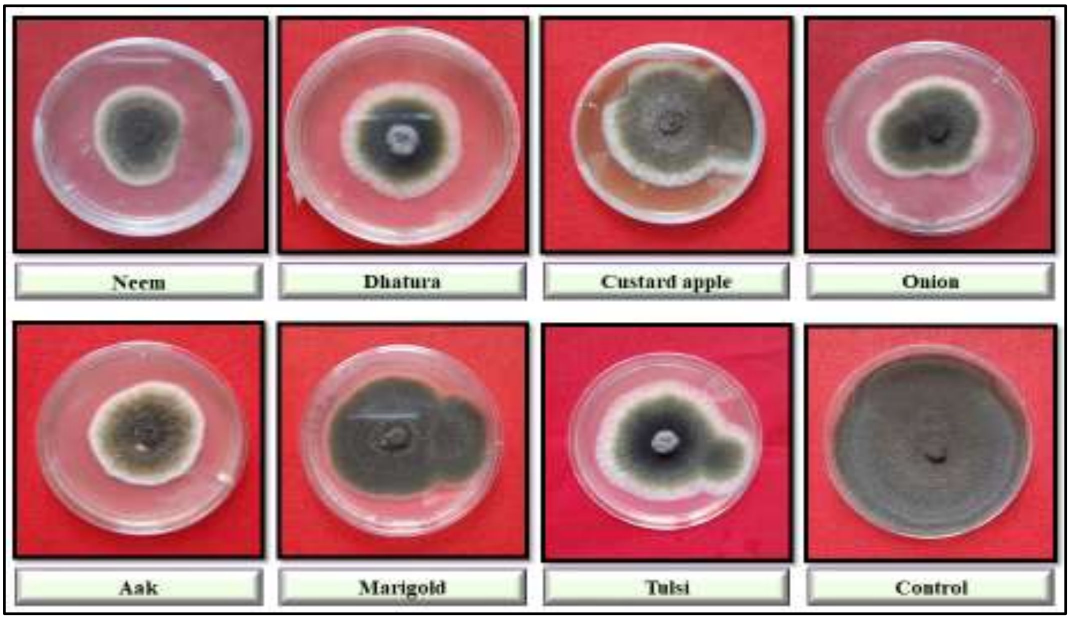

Plate 3: Evaluation of plant extracts against *A. alternata in vitro*

Conclusions

Based on present investigations, it is concluded that the lowest mycelial growth with maximum percent growth inhibition was recorded in copper oxychloride 50 WP significantly superior over control at 2000 ppm concentration among non-systemic fungicides while, propiconazole 25 EC cent percent mycelial growth inhibition at both concentrations among systemic fungicides similarly, neem leaf extract found as best among phytoextracts at 10 percent concentration and *T. harzianum* Rifai. NAU isolate was found as best among bio control agents against *A. alternata* caused alternaria leaf blight of coriander *in vitro* condition.

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