Global Scientific JOURNALS

GSJ: Volume 10, Issue 4, April 2022, Online: ISSN 2320-9186

www.globalscientificjournal.com

Evaluation of different plant extracts and essential oils in-vitro conditions against *Fusarium oxysporium*, a causal agent of Sunflower Wilt disease.

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Abstract: Evaluation of 21 different plant extracts and 11 essential oils were tested for their anti-fungal activity in-vitro condition against *Fusarium oxysporium*, pathogen of Sunflower wilt disease. The plants selected for the study were *Azardirachta indica, Allium cepa, Allium sativum, Zingiber officinale, Lawsonia inermis, Lantana camara, Parthenium hysterophorus, Citrus limon, Eucalyptus globulus, Psidium guajava, Mangifera indica, Annona squamosa, Aegle marmelos, Nerium oleander, Ricinus communis, Calotropis procera, Vitex negundo, Catharanthus roseus, Phyllanthus emblica, Moringa oleifera, Cymbopogon citratus.* Among all these Plant extracts at 20% Eucalyptus (74.44%), Mehendi (63.33%) Mango (53.66%), Indian bael (53%), Amla (53%), Guava (52.55%), Nerium (50%), custard apple (47.77%), Pink periwinkle (45.55%) were efficient in controlling the growth of the pathogen. At 50% concentration of the plant extracts Garlic, Mehendi, Eucalyptus, Amla, Ginger, Mango, Guava, Nerium, Indian Bael, Periwinkle, Lantana, Parthenium, and Vitex were efficient in controlling the pathogen to 100%, 96.66%, 87.44%, 76.66%, 60%, 60%, 56.66%, 55.555, 55.22%, 53.66%, 42.2%, 39.66% and 32.22% respectively.

Essential oils like *Eucalyptus oil (Eucalyptus globulus), Tea tree oil (Melaleuca alternifolia), Peppermint oil (Mentha piperita), Lemon grass oil (Cymbopogon flexuosus), Lemon oil (Citrus limon), Cinnamon oil (Cinnamomum verum), Rosemary oil (Rosemarinus officinalis), Ylang ylang oil (Cananga odorata), Frankin Cense oil (Boswellia carteri), Citronella oil (Cymbopogon winterianus), Orange (Citrus sinensis) oil.* All essential oils were effective in controlling the growth of Fusarium. But high degree of antifungal activity was exhibited by Tea tree oil, Peppermint, Lemon grass, Lemon, Cinnamon, Rosemary, Ylang ylang and Orange.

Key words: Sunflower wilt, Fusarium wilt, Plant extracts, essential oils, Anti- fungal activity.

Introduction:

Sunflower (*Helianthus annuus*) member of Asteraceae family is an important oilseed crop which was introduced in India in early 1960's. Mostly Sunflower is grown in countries of the world like Ukraine, Russia, Argentina, China, and United states (NDSU, 2007). The oil of sunflower has gained significance due to its light colour, flavour, Low levels of saturated fatty acids and presence of Poly unsaturated fatty acids like Linoleic acid, Oleic acid, and the remainder consists of saturated fatty acids stearic acid and palmitic acid. (Mukhtar, 2009). The sunflower oil is also used in making Candles, soaps, Lubricants etc., Sunflower seeds are available in the market both in raw as well as roasted forms which are edible, due to high percent of Oils and

proteins. They also possess many nutrients such as vitamins A, B, E, selenium, copper, Zinc (Gonzalez et al., 2002). But every year there are yield losses due to many infections caused by Microorganisms, which decrease quality and quantity of the sunflower crop remarkably (Mirza and beg, 1983).

Many diseases are caused by fungi and Bacteria but of few which cause great loss to the crop are Leaf spot, Rust, Powdery mildew, Downy mildew, Wilt, Rot diseases. Many Fusarium spp are reported to cause sunflower wilt (Nahar et al., 2005). The most devastating disease of sunflower is caused by particularly *Fusarium oxysporium* among all other species *F. solani, F. moniliforme, F. helianthi* and *F. equesti* (Masirevic and Jasnic, 2006).

The wilted plant shows the symptoms of reduction of growth as compared to the healthy plants. The leaves of the infected plants become pale yellow and eventually fall down and death of the leaves occur (Wu *et al*. 2009).

Many chemical fungicides are used to control the pathogen. Sultana and Abdul (2013) used Fungicides and Microbiological antagonists and oil cakes in-vitro and in-vivo to control *Fusarium oxysporium.* Aliette, benlate, carbendazim, Mancozeb, Ridomil, Topsin-M, Vitavax completely inhibit the colony growth of *F. oxysporium* at 100 ppm.

Fungicides like Nativo, Cabrio@top, Dragon, Romeo proved to control the pathogen growth completely (Azizullah Keerio *et al.,* 2017).

Very little research work was having been done on plant extracts efficacy in controlling the growth of Pathogen in-vitro conditions. Plant extracts of Neem, Bitter apple, Apple of Sodom, Tobacco and Jimson weed proved to have antifungal activity in controlling the linear growth of *Fusarium oxysporium* (Azizullah keerio *et.al.*, 2017)

So, the present work was taken up to test the efficacy of the different plant extracts in controlling the growth of the Pathogen in-vitro conditions in laboratory of Government Degree college, Armoor.

Materials and Methods:

Isolation and culture of *Fusarium oxysporium* from diseased leaf of Sunflower:

The pathogen causing Wilt disease in Sunflower is isolated from infected leaves collected from Plants grown in college campus for research work and the Fields in Nyavanandi and Dharpally villages of Nizamabad District of Telangana state. To obtain a pure culture of the pathogen, infected leaves were cut into one-cm sections and surface sterilised for one minute with 5% sodium hypochlorite solution before being rinsed 3 times with sterile distilled water. The sterilised leaf discs were placed in petri plates containing Potato dextrose agar medium. These petri plates were incubated at 25 ± 2℃ for seven days. After 7 days, the fungal inoculum was sub cultured again and the plates were incubated for one more week at 25 ± 2 ℃. This fully grown pathogen was utilized for research purpose.

Preparation of aqueous plant extracts:

Fresh leaves were used to make aqueous

plant extracts. This in-vitro study included 21 distinct plants. The plant material was cleaned twice with tap water and once with distilled water, air dried, then grinded separately in distilled water. 100 gm of fresh plant material was ground in a mixer grinder with an equal amount of distilled water (1:1 w/v). Two layers of cheese cloth was used to filter the extracts. As a result, the filtrate obtained is regarded as a 100 percent concentrated plant extract that is stored in the refrigerator for further research.

Table-1: List of plant extracts used to control pathogen causing wilt on Sunflower. Common name of the plant, Botanical name, Plant part used for experiment

S.No	Name of Plant	Botanical name	Part used
1	*Neem*	*Azardirachta indica*	**Leaves**
2	Onion	*Allium cepa*	**Bulb**
3	Garlic	*Allium sativum*	**Bulb**
4	Ginger	*Zingiber officinale*	**Rhizome**
5	Mehendi	*Lawsonia inermis*	**Leaves**
6	Lantana	*Lantana camara*	**Leaves**
7	Parthenium	*Parthenium hysterophorus*	**Leaves**
8	Lemon	*Citrus limon*	**Leaves**
9	Eucalyptus	*Eucalyptus globulus*	**Leaves**
10	Guava	*Psidium guajava*	**Leaves**
11	Mango	*Mangifera indica*	**Leaves**
12	Custard apple	*Annona squamosa*	**Leaves**
13	Golden apple (Indian Bael)	*Aegle marmelos*	**Leaves**
14	Oleander plant	*Nerium oleander*	**Leaves**
15	Castor oil plant	*Ricinus communis*	**Leaves**
16	Apple of sodon	*Calotropis procera*	**Leaves**
17	Chinese chaste tree	*Vitex negundo*	**Leaves**
18	Pink Periwinkle	*Catharanthus roseus*	**Leaves**
19	Indian Gooseberry	*Phyllanthus emblica*	**Leaves**
20	Drumstick tree	*Moringa oleifera*	**Leaves**
21	Lemon grass	*Cymbopogon citratus*	**Leaves**

Plant extracts in PDA media have been modified in the following ways:

Concentrations of 20% and 50% were prepared from a 100 % stock plant extract. After thoroughly mixing, separate extracts of 20% and 50% (v/v) were added to double strength potato dextrose agar, which was autoclaved at 121℃ for 15 minutes. The medium was sterilised before being put into 90mm sterile petri plates to solidify. Using a sterile cork borer, a 5mm disc of 7 -day old pathogen culture was sliced and inoculated onto the solidified surface of PDA media. Three replications were maintained and the plates were incubated at 25 ± 2 ℃. The medium without any plant extract served as control. The mycelial growth of Fusarium spp in different plates were taken on 3rd, 5th and 7th day. The percent inhibition of fungal

growth was calculated using the formula given by Vincent (1947).

I= C – T/ C * 100
Where I = Percent inhibition of growth
C = growth of pathogen in control petri plate (cm)
T = growth of pathogen in plant extract amended petri plate (cm)

Antifungal assays were carried out invitro according to Saikia et.al (2001).

The anti-fungal activity of essential oils like Eucalyptus (*Eucalyptus globulus)*, Tea tree *(Melaleuca alternifolia)*, peppermint *(Mentha piperita)*, Lemon grass *(Cymbopogon flexuosus)*, Lemon *(Citrus limon)*, Cinnamon *(Cinnamomum verum)*, Rosemary *(Rosemarinus officinalis)*, Ylang ylang *(Cananga odorata)*, Frankincense *(Boswellia carteri)*, Citronella *(Cymbopogon winterianus)*, Orange *(Citrus sinensis)* was also studied.

A 5 mm diameter sterilized filter paper disc was placed in the centre of the petri plate with PDA and loaded with 5 µl of essential oil. Four discs (5mm diameter) of Mycelial plugs were cross placed into each petri plate. Petri plates were kept at 25 ± 2 ℃ for 8 days. The zone of inhibitions around the oil containing sterile disc was measured after 8 days as the distance from the margins of the colony to the filter paper disc. The experiment was performed in four replications.

Results and Discussions:

21 different plant extracts were evaluated for their efficiency in inhibiting the growth of the pathogen by poisoned food technique against Fusarium spp. Mycelial growth of inhibition was obviously different for different plant extracts at 20 and 50 % concentrations. At 20% concentration of plant extracts maximum growth inhibition of the pathogen was observed in Eucalyptus (74.44%), Mehendi (63.33%) Mango (53.66%), Indian bael (53%), Amla (53%), Guava (52.55%), Nerium (50%), custard apple (47.77%), Pink periwinkle (45.55%), Garlic(37.77%) and Ginger (32.22%) as compared to control.

At 50% concentration of the plant extracts maximum growth inhibition of the pathogen was recorded in Garlic (100%), Mehendi (96.66%), Eucalyptus (87.44%), Amla (76.66%), Ginger (60%), Mango (60%), Guava (56.66%), Nerium (55.55%), Indian Bael (55.22%), Periwinkle (53.66%), Lantana (42.2%), Parthenium (39.66%) and Vitex (32.22%) respectively when compared to control.

All essential oils were effective in controlling the growth of Fusarium. But high degree of antifungal activity was exhibited by Tea tree oil, Peppermint, Lemon grass, Lemon, Cinnamon, Rosemary, Ylang ylang and Orange compared to other essential oils.

Table-2 Effect of different plant extracts at 20% concentration on mycelial growth of *Fusarium oxysporium*

S.No	Plant extracts @ 20 %	**T1(20%) Mean on Day-3**	**I = % of Inhibition I=C-T/ CX100 on Day-3**	**T1(20%) Mean on Day-5**	**I = % of Inhibition I=C-T/ CX100 on Day-5**	**T1(20%) Mean on Day-7**	**I = % of Inhibition I=C-T/ CX100 on Day-7**
1	*Neem (Azardirachta indica)*	2.3	50%	6.9	4.16%	8.6	4.44%
2	Onion (*Allium cepa)*	4.53	1.50%	7.63	-5.97%	8.33	7.44%
3	Garlic (*Allium*	1.1	76.08%	4	41.66%	5.6	37.77%

	sativum)						
4	Ginger *(Zingiber officinale*)	3.1	32.60%	4.5	37.50%	6.1	32.22%
5	Mehendi (*Lawsonia inermis*)	1.53	66.74%	2.67	62.91%	3.3	63.33%
6	Lantana (*Lantana camara)*	2.2	52.17%	4.3	40.27%	6.63	26.33%
7	Parthenium (*Parthenium hysterophorus*)	2.53	45%	3.73	48.19%	7.27	19.22%
8	Lemon (*Citrus limon*)	3.63	21.08%	6.1	16.66%	8.1	10%
9	Eucalyptus (*Eucalyptus globulus*)	1.73	62.39%	2	72.22%	2.3	74.44%
10	Guava (*Psidium guajava*)	2.47	46.30%	3.4	52.77%	4.27	52.55%
11	Mango (*Mangifera indica*)	2.27	50.65%	3.67	49.02%	4.17	53.66%
12	Custard apple (*Annona squamosa*)	2.7	41.30%	4.43	38.47%	4.7	47.77%
13	Indian Bael (*Aegle marmelos)*	2.73	40.65%	3.8	47.22%	4.23	53%
14	Oleander plant (*Nerium oleander*)	2.9	36.95%	4.2	41.66%	4.5	50%
15	Castor oil plant (*Ricinus communis)*	4.6	0%	6.9	4.16%	8.4	6.66%
16	Apple of sodon (*Calotropis procera)*	5.1	-10.86%	8.1	-12.5%	8.6	4.44%
17	Chinese chaste tree (*Vitex negundo)*	3	34.78%	6.1	15.27%	7.6	15.55%
18	Pink Periwinkle (*Catharanthus roseus*)	2.87	37.60%	4.6	36.11%	4.9	45.55%
19	Indian Gooseberry *(Phyllanthus emblica*)	1.93	58.04%	2.8	61.11%	4.23	53%
20	Drumstick tree (*Moringa oleifera*)	5.03	-9.34%	7.7	-6.94%	8.4	6.66%
21	Lemon grass (*Cymbopogon citratus)*	3.4	26.08%	5.4	25%	7.4	17.77%
22	Control	4.6	0%	7.2	0%	9	0%

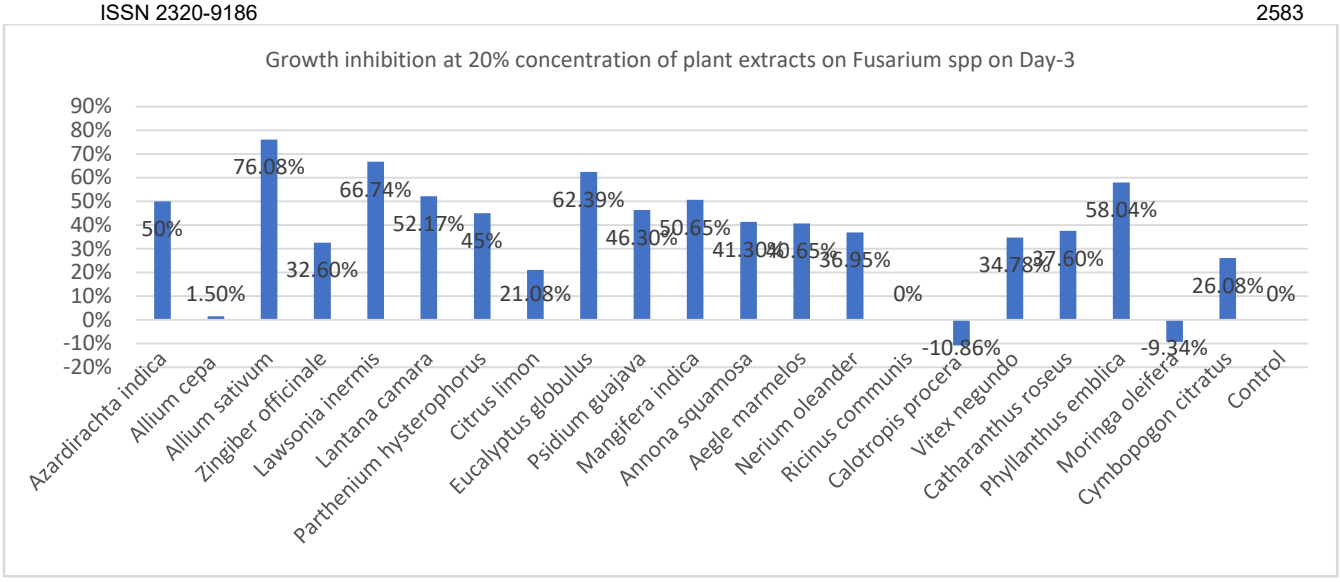

Figure 1

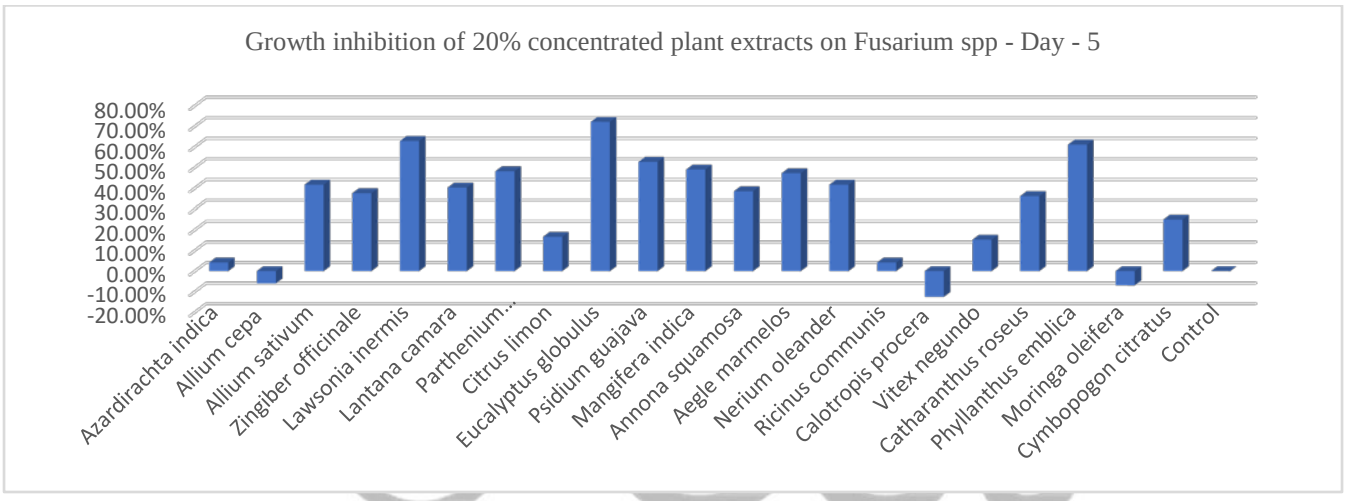

Figure 2

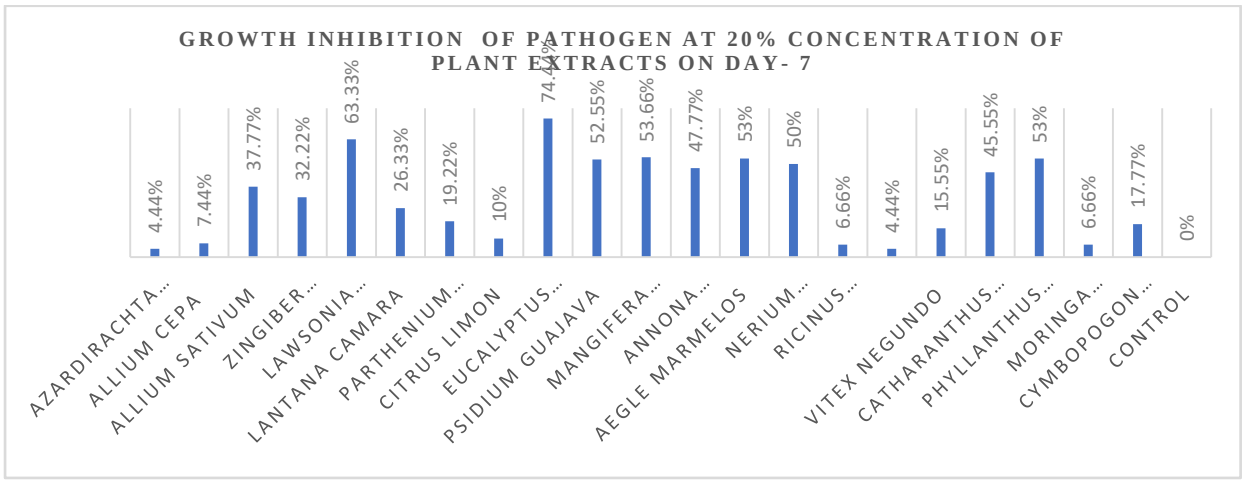

Figure 3

Table-3 Effect of different plant extracts at 50% concentration on mycelial growth of *Fusarium oxysporium*

S.No	Plant extracts @ 50 %	**T1(20%) Mean on Day-3**	**I = % of Inhibition I=C-T/ CX100 on Day-3**	**T1(20%) Mean on Day-5**	**I = % of Inhibition I=C-T/ CX100 on Day-5**	**T1(20%) Mean on Day-7**	**I = % of Inhibition I=C-T/ CX100 on Day-7**
1	*Neem (Azardirachta indica)*	2.3	50%	4.9	31.94%	6.6	26.66%
2	Onion (*Allium cepa*)	2.1	54.34%	4.6	36.11%	6.7	25.55%

3	Garlic (*Allium sativum*)	0	100%	0	100%	0	100%
4	Ginger *(Zingiber officinale*)	1.2	73.91%	2.6	63.88%	3.6	60%
5	Mehendi (*Lawsonia inermis*)	.03	93.47%	.03	95.83%	0.3	96.66%
6	Lantana (*Lantana camara)*	1.9	58.69%	4.06	43.61%	5.2	42.2%
7	Parthenium (*Parthenium hysterophorus*)	2.07	55%	3.5	51.38%	5.43	39.66%
8	Lemon (*Citrus limon*)	2.57	44.13%	5.1	29.16%	6.7	25.55%
9	Eucalyptus (*Eucalyptus globulus*)	0	100%	0	100%	1.13	87.44%
10	Guava (*Psidium guajava*)	2.17	52.82%	3.2	55.55%	3.9	56.66%
11	Mango (*Mangifera indica*)	2.07	55%	2.7	62.5%	3.6	60%
12	Indian Bael (*Aegle marmelos)*	1.52	66.95%	2.97	58.75%	4.03	55.22%
13	Oleander plant (*Nerium oleander*)	2.6	43.47%	4.2	41.66%	4	55.55%
14	Castor oil plant (*Ricinus communi)*	3.6	21.73%	5.8	19.44%	6.9	23.33%
15	Apple of sodon (*Calotropis procera)*	3.3	28.26%	5.1	29.16%	7.8	13.33%
16	Chinese chaste tree (*Vitex negundo)*	1.5	67.39%	3.8	47.22%	6.1	32.22%
17	Pink Periwinkle (*Catharanthus roseus*)	2.63	42.82%	3.63	49.58%	4.17	53.66%
18	Indian Gooseberry *(Phyllanthus emblica)*	.67	85.43%	1.63	77.36%	2.1	76.66%
19	Drumstick tree (*Moringa oleifera*)	4.13	10.21%	6.6	8.33%	7.53	16.33%
20	Lemon grass (*Cymbopogon citratus)*	3	34.78%	5.1	29.16%	6.5	27.77%
21	Control	4.6	0%	7.2	0%	9	0%

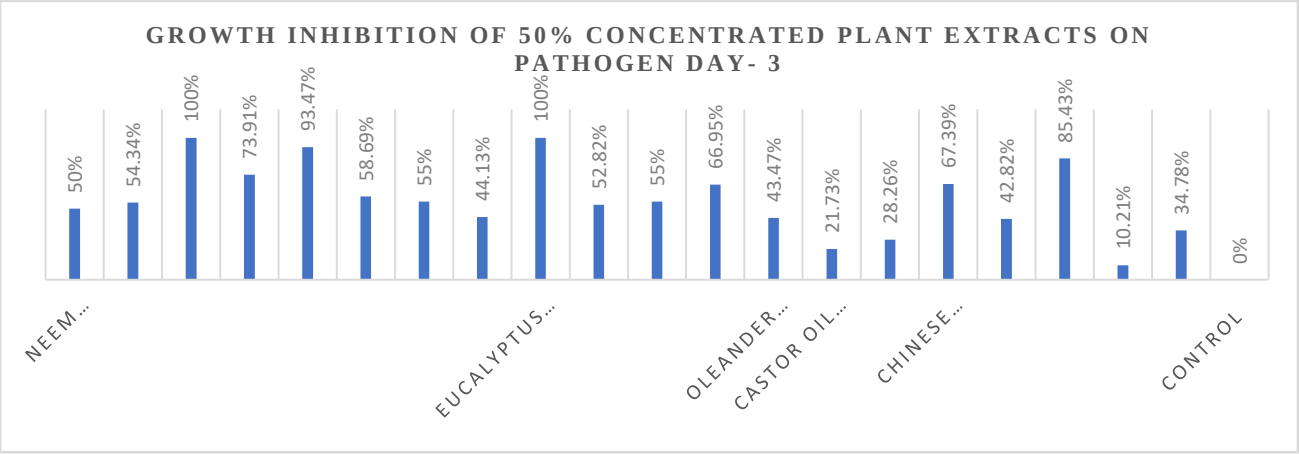

Figure 4

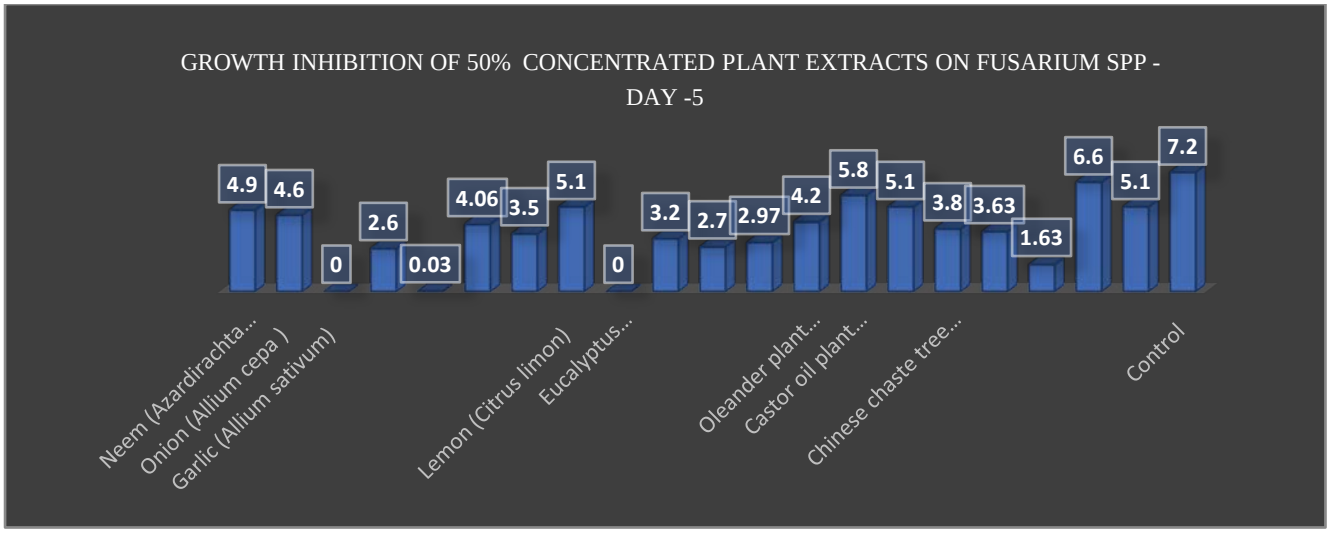

Figure 5

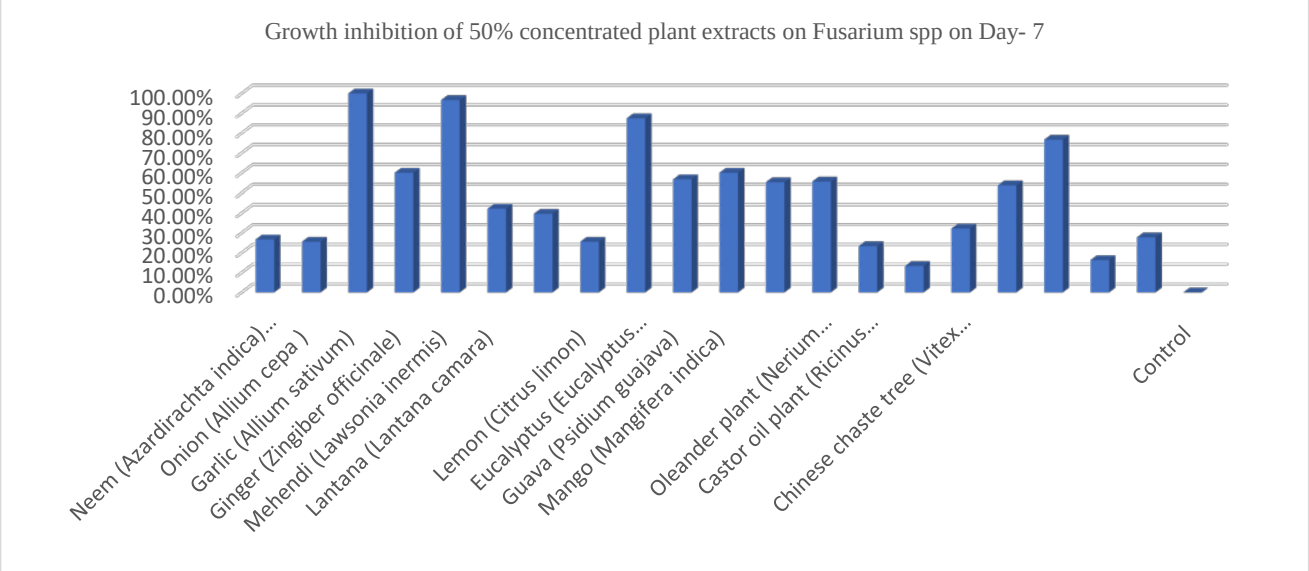

Figure 6

Figure 7 Growth of Fusarium in 20% and 50% treated PDA with Onion extract and Garlic extract on Day 3 and Day -7 respectively

Figure 8 Growth of inhibition of Fusarium spp on Henna and Eucalyptus plant extract on Day - 3 and Day – 7 respectively

The inhibitory effect of 11 essential oils against Fusarium spp is shown in Table-4

S.No	Name of the essential oil	Scientific name	Zone of inhibition
1	Eucalyptus	*Eucalyptus globulus*	0.5
2	Tea tree oil	*Melaleuca alternifolia*	1.5
3	Peppermint	*Mentha piperita*	1.32
4	Lemon grass	*Cymbopogon flexuosus*	1.05
5	Lemon	*Citrus limon*	0.87
6	Cinnamon	*Cinnamomum verum*	1.57
7	Rosemary	*Rosemarinus officinalis*	1.52
8	Ylang ylang	*Cananga odorata*	1.4
9	Frankincense	*Boswellia carteri*	0.97
10	Citronella	*Cymbopogon winterianus*	0.57
11	Orange	*Citrus sinensis*	1.3

Our research showed that all essential oils were effective in controlling the growth of Fuasrium. But high degree of antifungal activity was exhibited by Tea tree oil, Peppermint, Lemon grass, Lemon, Cinnamon, Rosemary, Ylang ylang and Orange.

Figure 9 Influence of Tea tree oil and Orange oil on Mycelial growth of Fusarium spp

Figure 10 Influence of Eucalyptus oil and Citronella oi on Mycelial growth of Fusarium oxysporiuml

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