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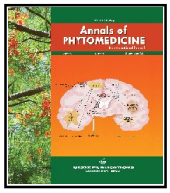

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Homeopathic medicines as new strategy against plant pathogenic fungi

Dheer Kumar, Samir K. Biswas◆, Shivam Kumar, Girijesh Kumar Jaisval, Deepoo Singh, Ravi Kumar, Rahul Kumar Tiwari*, Milan Kumar Lal* and Ravinder Kumar*

Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, 208002, Uttar Pradesh, India
** ICAR-Central Potato Research Institute, Shimla, 171001, Himanchal Pradesh, India*

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Abstract

Nowadays, homeopathy is being rapidly practiced almost all over the world for preventing or curing human and plant diseases. In the current study, homeopathic medicine, *i.e.*, Arnica montana, Thuja occidentalis, sulfur, and silicea were applied against plant disease causing pathogens, *i.e.*, *Fusarium oxysporum* f. sp. *lycopersici, Ascochyta rabiei*, *Alternaria solani*, *Septoria lycopersici*, and *Phomopsis vexans*. The radial mycelium growth of these species was found to be significantly decreased with the increased concentrations of homeopathic medicine under *in vitro* condition. At 8 days after inoculation, the highest inhibition of mycelial growth of *F. oxysporum* f. sp. *lycopersici*, *A. solani*, *S. lycopersici*, *A. rabiei*, and *P. vexans* over control was observed with *T. occidental* at 200 ppm (73.73%), *T. occidentalis* at 200 ppm (76.73%), *A. montana* at 200 ppm (64.22%), silicea at 200 ppm (59.77%), and sulfur at 200 ppm (53.82%), respectively. Accordingly, these fungal species minimal radial growth was as follows: 22.80 mm, 18.10 mm, 31.66 mm, 34.91 mm, and 38.12 mm. Such findings outline the potential of homeopathic medicine treatment in the reduction of mycelial growth of several fungal species affecting consumed crops.

1. Introduction

Homeopathy simply means treating diseases with remedies, prescribed in minute doses. It is based on the natural law of healing "similar similibus curantur", which means "likes are cured by likes" and was given a scientific basis in the early 19th century. Homeopathy postulates that suppressive activities, that go against the vital principle, are to blame for the onset of disease in living beings (Bellavite *et al*., 2007). Nowadays, homeopathy is being rapidly practiced almost all over the world. In India, it has become a household name due to its safety and the gentleness of its cure. It has been stated that about 10 per cent of the Indian population slowly depends on homeopathy for healthcare needs which are considered the second most popular system of medicine in the country. Nearly 70 per cent of homeopathy medicines are derived from plant parts, *i.e.,* fruit, stem, bark, flower, leaf, stigma, or root, as well as a non-woody plant and some other herbs. Most homeopathic medicines are prepared from natural herbs. Generally, they are non-hazardous and eco-friendly target-specific modes of action with least side effects, which prevent or cure human diseases. Diseases in plants are caused by an imbalance of vital energy, which can also lead to the plants demise if their production is drastically reduced (Nazarov *et al*., 2020). However, homoeopathic medicine helps to mitigating negative effects on vital energy and restore equilibrium by boosting the plant's defensive mechanism (Biswas *et al*., 2002). To eliminate white mold in bean plants, growers turned to homoeopathic remedies containing phosphorus and *Calcarea carbonica* (Rissato *et al*., 2016). Recently, homeopathic medicine, *e.g*., *A. montana*, *T. occidentalis*, sulfur, and silicea were applied against plant disease-causing pathogens, *i.e., F. oxysporum* f. sp. *lycopersici*, *A. solani*, *S. lycopersici*, *A.rabiei*, and *P. vexans*. *A. montana* is a beautiful mountain daisy mostly found in acidic soils and mountain pastures. It exhibits anti-inflammatory, antiseptic, antifungal, and antibacterial activities (Conforti *et al.,* 1997). Hanif and Dawar (2015), reported that the mycelial growth of *F. oxysporum*, *Rhizoctonia solani*, and *Macrophomina phaseolina* was significantly inhibited by *A. montana*. *T. occidentalis* is an evergreen coniferous tree used intensively in the homeopathic system of medicine (Hulten and Mistry, 1986; Alam *et al.,* 2022). It possesses several medicinal values including antiviral, antifungal, antidiarrheal, and antioxidant properties (Caruntu *et al*., 2020). Khanna and Chandra (1989), obtained positive results in the control of tomato (*Solanum lycopersicum* L.) rot caused by *F. roseum*, after the application of *T. occidentalis*. To combat soil-borne infections, researchers have experimented a variety of formulations, including bacterial suspensions, fungal spores, and powdery preparations of fungal mycelium. The population of soil-borne plant pathogens and plant parasitic nematodes has been reduced in several experiments where microbial antagonist was added to the soil (Topalobic *et al.,* 2020). Root rot pathogen colonization can be reduced and plant growth can be enhanced using microbial antagonists such as *Trichoderma* spp., *Paecilomyces lilacinus*, *Verticillium chlamydosporium*, *Bacillus* spp., *Stachybotrys atra*, *Pseudomonas aeruginosa*, and *Gliocladium virens* (Brotman, 2013; Loser *et al*., 2021). By releasing chitinolytic enzymes and generating mycoparasitism inhibitory chemicals, antagonistic bacteria can regulate soil-borne diseases (Sharp *et al*., 2013). Therefore,

Corresponding author: Dr. Samir Kumar Biswas
Professor Head, Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur-208002, Uttar Pradesh, India
E-mail: samirkrbiswas@rediffmail.com
Tel.: +91-9452522504

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mycoparasitism inhibitors may be responsible for the suppression of soil-borne diseases (Lahlali *et al*., 2022). Chitinase enzyme is responsible for the breakdown of β-glucan, chitin, and polysaccharides in fungal cells, hence killing the pathogen (Veliz *et al*., 2017). Rex *et al.* (2019), reported that the antifungal activity of crude plant extracts of ten plant species, *viz.,* Neem (*Azadiracta indica*), Prosopis (*Prosopis juliflora*), Onion (*Allium cepa*), Eucalyptus (*Eucalyptus obliqua*), Pungam (*Pongamia pinnata*), Curry leaf (*Murrya koenigii*), Garlic (*Allium sativum*), Henna (*Lawsonia inermis*), Turmeric (*Curcuma longa*) and Ginger (*Zingiber officinale*) were evaluated against *A. solani* (Ellis and Martin) by using poisoned food technique under *in vitro* conditions and found that turmeric extract recorded minimum radial mycelial growth of 9.50 mm with maximum per cent inhibition of 89.44 over control, followed by garlic observed (12.80 mm, 85.77 per cent) and eucalyptus (24.27 mm, 73.03 per cent), respectively. Volatile organic compound (VOC) from plants known to have varied potential sources for antimicrobial activity against soil-borne pathogens and evaluate *in vitro* antifungal activity of volatile organic compounds of carvone and citronellol against spore germination of *F. oxysporum* f.sp. *lycopercisi* (Praveen *et al.,*2021). Akshaya *et al.* (2021), reported that the ethyl acetate and methanolic fractions of cell-free culture filtrate (18 days old) of *O. sinensis* at a concentration of 3000 ppm showed maximum inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* (43.10 and 46.6 per cent, respectively), followed by *Fusarium oxysporum* f. sp. *cubense* (42.20 and 41.10 per cent, respectively), when tested by poisoned food technique. Gayathiri *et al.* (2021), reported the standard samples of papaverine tested against mycelial growth and conidial germination of *C. gloeosporioides* exhibited 100 per cent inhibition of mycelial growth of *C. gloeosporioides* at 1500 ppm and 3000 ppm in agar well diffusion assay and paper disc assay which confirmed the presence of antimicrobial metabolites from culture filtrates of *G. lucidum*. Maurya *et al.* (2021), was carried out to check inhibitory effect of botanicals against *Ustilaginoidea virens*. Among botanicals, neem oil at both concentration, *i.e.,* 5 per cent and 10 per cent was found highly effective and inhibit the growth by 66.97% and 70.14%, respectively followed by onion (62.89%, 67.64%), garlic (58.82%, 64.25%), ginger (55.88%, 63.57%) and tulsi (46.40%, 54.98%). Hence, plant roots can be protected from soil-borne pathogens *via* the barrier created by the microbial antagonists. The use of homeopathic medicine to control various pathogens affecting fruits was found highly effective and environmentally safe. For instance, the inhibitory effects of *T. occidentalis* against *Alternaria alternata*, *Fusarium moniliforme*, *Gloeosporium psidii*, *Colletotrichum gloeospoioides*, *Pestalotia* spp., and other fruit rot pathogens have been previously reported (Chandra *et al*., 1981; Wilson *et al*., 1991; Khanna *et al*., 1992; Baviskar *et al*., 2015). Arie (2019), reported that sulfur is a very effective homeopathic medicine against some plant pathogenic fungi such as *Fusarium* wilt (caused by *F. oxysporum*), *Septoria* leaf spot (caused by *S. lycopersici*), and *Phomopsis* blight (caused by *P. vexans*). Sinha and Singh (1983), found that sulfur fully inhibited the growth of *Aspergillus parasiticus*. Saxena *et al*. (1988), used 200 ppm sulfur and observed a growth inhibition of 22 fungi genera. Toledo *et al*. (2009), reported an inhibited mycelial growth of *A. solani* under the silicea influence. The current study aimed to evaluate the *in vitro* efficacy of homeopathic medicine, *i.e., A. montana, T. occidentalis,* sulfur, and silicea at different concentrations (50, 100, 150, and 200 ppm) against (a) *F. oxysporum* f. sp. *lycopersici*, (b) *A. solani*, (c) *A. rabiei*, (d) *S. lycopersici*, and (e) *P. vexans*.

2. Materials and Methods

2.1 Experimental site

All experiments were conducted in the laboratory of Department of Plant Pathology, C.S.A., University of Agriculture and Technology, Kanpur during the 2020-2022 periods.

2.2 Isolation and purification of the pathogen

F. oxysporum f. sp. *lycopersici, A. rabiei, A. solani*, *S. lycopersici,* and *P. vexans* were isolated from soil and infected plant parts, *i.e.,* leaves, root, stem, and fruit. The selected samples were rinsed in tap water to remove dust particles, and surface contaminants. Subsequently, the young diseased parts of the leaves, stems, roots, and fruits were cut into small bits along with some healthy portions using a sterilized blade. The cut pieces were then dipped and sterilized with 0.1% sodium hypochlorite solution for 20-30 sec using sterilized forceps. They were then thoroughly rinsed thrice in sterilized water to remove any remaining trace of sodium hypochlorite solution. The excessive moisture was removed by placing samples in the fold of pre-sterilized blotting paper. Petri-plates were sterilized at 160-180 ^{o}C for 2 h in a hot air oven. The surface of the pieces was transferred in petri-dishes using sterilized forceps. Petri-dishes used in the experiment were previously poured with sterilized 20 ml PDA medium under laminar flow. The surface sterilization of three to four pieces of the diseased part of tomato leaves and roots, chickpea stem and root, and brinjal fruit were placed in each petri-dish at an equal distance fields (Synder and Hansen, 1940; Dhingra and Sinclair, 1995). These petri-dishes were properly marked with glass marking pencils indicating the date of isolation. They were finally sealed with parafilm tape, incubated at 25-28^{o}C in a BOD incubator, and observed periodically for fungal growth on 24 h intervals. Once the mycelial growth occurred, the hyphal tips for the advancing mycelium were transferred aseptically into the sterilized culture tubes containing 2% potato dextrose agar (PDA) medium for further purification, identification, and maintenance of culture. The purification of fungi isolates was processed by adopting a single spore isolate technique (Choi *et al.,*1999).

2.3 Identification of the pathogen

The pathogen was identified based on its morphological and cultural characteristics, as well as its pathogenic behavior toward the host. The *A. solani* stands distinct from other genera by its transversely and longitudinally septate (muriform) conidia. The conidia show a distinct beak which may be short or very long. The conidia are usually formed in chains, they were in the current case dark brown and typically obclavate (Alhussaen, 2012). The identification of *F. oxysporus* f. sp. *lycopersici* was possible from its hyphae which are hyaline branched and septate. The conidia are slimy and the microconidia are one celled, oval or comma shaped, pyriform or elongated, hyaline or pale, spindale shaped, and pointed (Correll *et al.,* 1986). The identification of *A. rabiei* was possible through the pycnidia which were brown, thick walled and immersed in blight. Conidia are bicelled, obovoid, hyaline to palebrown. Ascochyta blight is characterized by their spore size (Chen *et al.,* 2004). The identification of *P. vexans* was possible through its conidia which are hyaline, single celled, and globose to ovoid. These conidia were of two types: alpha conidia avoid to fusoid, and beta conidia (stylospores), filiform, curved at the apex, like a walking stick, and biguttulate (Dhingra and Sinclair, 1985). The identification of *S.*

lycopersici was possible through its conidia which are hyaline, filiform and multiseptated. Its pycnidia were dark, separate, globose, immersed or papillate (da Costa *et al.,* 2022).

2.4 Maintenance of the cultures

The fungal pathogen was subcultured on PDA slants and allowed to grow at 25 ± 1°C for 10 days. Samples were stored in the refrigerator (at 4°C) and subcultured once at regular intervals of a month.

2.5 Effect of different homeopathic medicine against major plant pathogenic fungi

The efficacy of *A. montana*, *T. occidentalis,* sulfur, and silicea at different concentrations of 50, 100, 150, and 200 ppm was evaluated in PDA medium using poisoned food technique against different pathogens in the laboratory. The homeopathic medicine was tested with three replications per treatment. The fungus was grown on PDA medium for 12 days before setting up the experiment. The PDA medium was prepared and melted, and homeopathic medicine was added to the melted medium at the required concentrations. 20 ml of poisoned medium was poured into sterilized petri-plates. The suitable check was maintained without the addition of homeopathic medicine (control). A mycelial disc of 5 mm was taken from the periphery of the old colony. The actively growing hyphal tip was removed by cork borer and placed in the center of poisoned petri-plates incubated at 25 ± 1°C until the control plate was full. The radial growth of the fungus on the poisoned medium was measured on a daily basis up to 8 days of inoculation. The diameter of the colony was measured in two directions and the average was recorded to find out the growth of concerning pathogens. All experiments were carried out in triplicate and the per cent reduction of mycelial growth over control was calculated using the following formula of Vincent (1927):

$$\text{Per cent decrease over control} = \frac{D_c - D_t}{D_c} \times 100$$

where, D_c is the average diameter of fungal growth in control and D_t is the average diameter of fungal growth in treatment.

Treatments were as follows: T1: Sulfur 50 ppm; T2: Sulfur 100 ppm; T3: Sulfur 150 ppm; T4: Sulfur 200 ppm; T5: *T. occidentalis* 50 ppm; T6: *T. occidentalis* 100 ppm; T7: *T. occidentalis* 150 ppm; T8: *T. occidentalis* 200 ppm; T9: *A. montana* 50 ppm; T10: *A. montana* 100 ppm; T11: *A. montana* 150 ppm; T12: *A. montana* 200 ppm; T13: Silicea 50 ppm; T14: Silicea 100 ppm; T15: Silicea 150 ppm; T16: Silicea 200 ppm; T17: Control.

2.6 Statistical analysis

Each treatment was replicated thrice and values were means ± SE. The data were computed using SPSS software version 21.

3. Results

3.1 Efficacy of homeopathic medicine against *F. oxysporum* f. sp. *lycopersici* (*in vitro*)

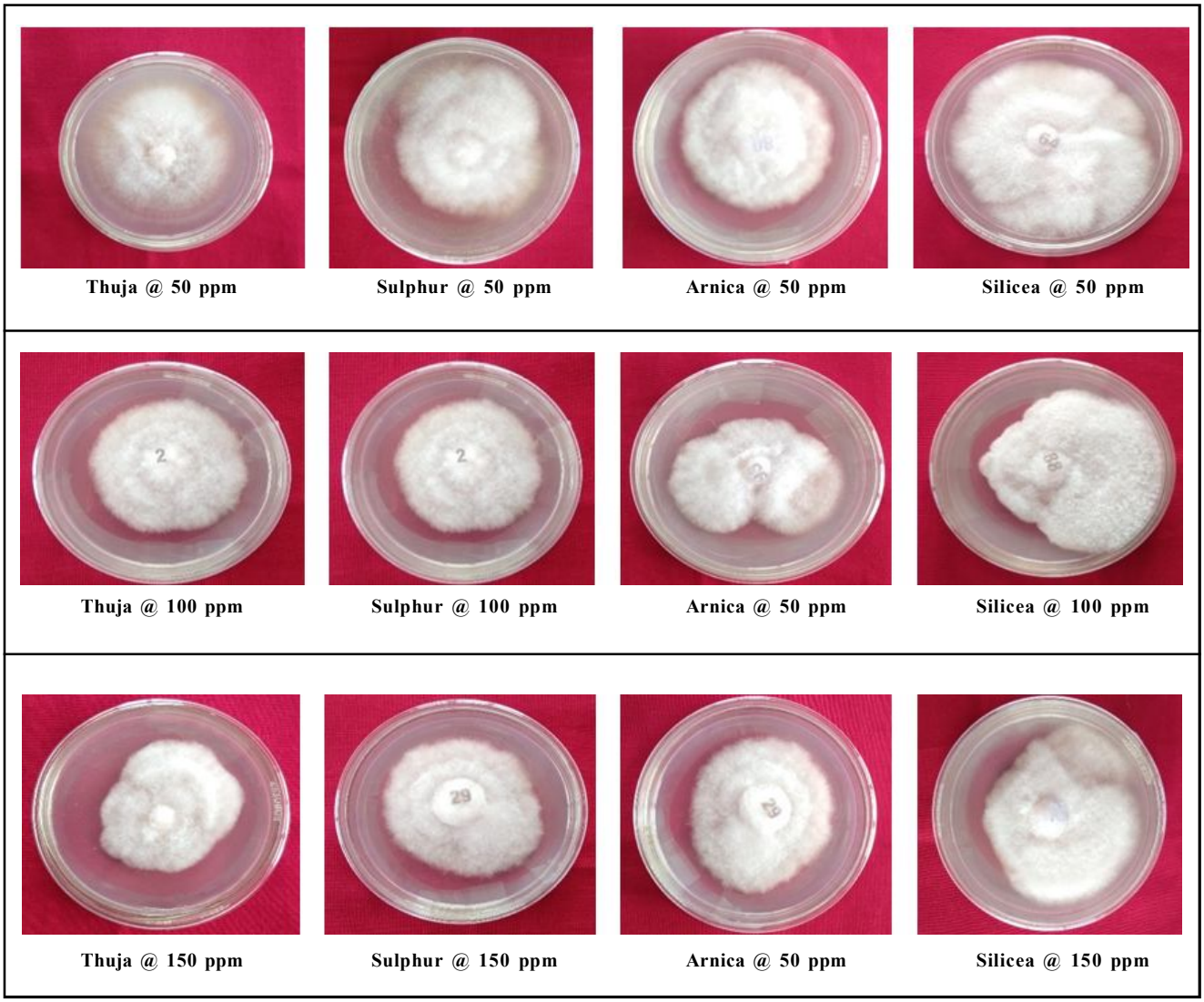
The effect of four homeopathic medicine, *i.e., A. montana, T. occidentalis,* sulfur, and silicea against *F. oxysporum* f. sp. *lycopersici* was evaluated at different concentrations (50, 100, 150, and 200 ppm) under *in vitro* condition. Results showed a significant inhibition of *F. oxysporum* f. sp. *lycopersici* mycelial growth in all four tested concentrations over control (Table 1, Figure1). Among all treatments, the minimal radial growth of mycelium was recorded in plates treated with *T. occidentalis* at 8 days after inoculation (DAI). The highest mycelial growth inhibition over control was observed with *T. occidentalis* at 200 ppm (73.73%), followed by sulfur at 200 ppm (72.35%), and *T. occidentalis* at 150 ppm (70.91%), corresponding, respectively, to 22.80 mm, 23.00 mm, and 25.25 mm mycelial growth at 8 DAI. The lowest mycelial growth inhibition was attributed to silicea at 50 and 100 ppm (29.60% and 36.12%, respectively), corresponding, respectively, to 61.10 mm and 55.44 mm mycelial growth at 8 DAI compared to the control. Promising inhibition percentages of *F. oxysporum* f. sp. *lycopersici* mycelial growth were observable with *A. montana* at 150 and 200 ppm compared to the control (61.46 and 68.66%, corresponding to 33.45 and 27.20 mm-mycelial growth at 8 DAI, respectively). Hanif and Dawar (2015), reported that *F. oxysporum*, *R. solani* and *M. phaseolina* was significantly inhibited by *A. montana* and *T. occidentalis*, and the growth parameters and yield of crops were improved. 200 ppm *Arsenicum album* reduced by 88% the leaf rot of betelvine disease caused by *Phytophthora* spp. Hanif and Dawar (2016). Dogra (2006), observed the antifungal activity of panchgavya against major soil-borne fungi. He found that mycelial bits, dipped for 12 h in panchgavya, caused more than 90% inhibition of *F. oxysporum* f. sp. *pisi* and *R. solani* f. sp. *pisi*, and 100% inhibition of *S. rolfsii*, *S. sclerotiorum* and *R. solani* seeds treated with homeopathic drugs. Besides that, plant growth parameters were improved.

Table 1: Effect of homeopathic medicine at the different concentrations on radial mycelial growth against *F. oxysporum* f. sp. *lycopersici*

Treatments	Radial mycelial growth (mm) at different days				Inhibition (%) over control
	2 days	4 days	6 days	8 days	
T 1	10.12	19.30	30.40	41.74	51.92
T 2	8.90	16.70	23.27	39.10	54.95
T 3	5.60	13.46	20.50	31.38	63.84
T 4	3.20	7.50	13.05	23.00	72.35
T 5	7.06	15.40	25.66	37.60	56.68
T 6	6.03	11.06	19.62	30.87	64.43
T 7	5.80	10.50	18.76	25.25	70.91

T8	4.30	8.92	17.40	22.80	73.73
T9	12.40	24.90	39.70	53.50	38.36
T10	10.70	20.35	30.05	51.03	41.20
T11	6.10	11.60	25.76	33.45	61.46
T12	4.75	9.15	18.20	27.20	68.66
T13	14.70	29.40	46.05	61.10	29.60
T14	13.65	25.05	40.40	55.44	36.12
T15	6.25	15.75	26.44	35.40	59.21
T16	4.80	9.55	19.90	29.70	65.78
T17	21.10	40.50	63.05	86.80	-
SE	0.368	0.714	1.142	1.612	-
CD at 1 %	1.420	2.747	4.407	6.211	-

T1: Sulfur 50 ppm; T2: Sulfur 100 ppm; T3: Sulfur 150 ppm; T4: Sulfur 200 ppm; T5: *T. occidentalis* 50 ppm; T6: *T. occidentalis* 100 ppm; T7: *T. occidentalis* 150 ppm; T8: *T. occidentalis* 200 ppm; T9: *A. montana* 50 ppm; T10: *A. montana* 100 ppm; T11: *A. montana* 150 ppm; T12: *A. montana* 200 ppm; T13: Silicea 50 ppm; T14: Silicea 100 ppm; T15: Silicea 150 ppm; T16: Silicea 200 ppm; T17: Control.



Thuja @ 50 ppm **Sulphur @ 50 ppm** **Arnica @ 50 ppm** **Silicea @ 50 ppm**

Thuja @ 100 ppm **Sulphur @ 100 ppm** **Arnica @ 50 ppm** **Silicea @ 100 ppm**

Thuja @ 150 ppm **Sulphur @ 150 ppm** **Arnica @ 50 ppm** **Silicea @ 150 ppm**

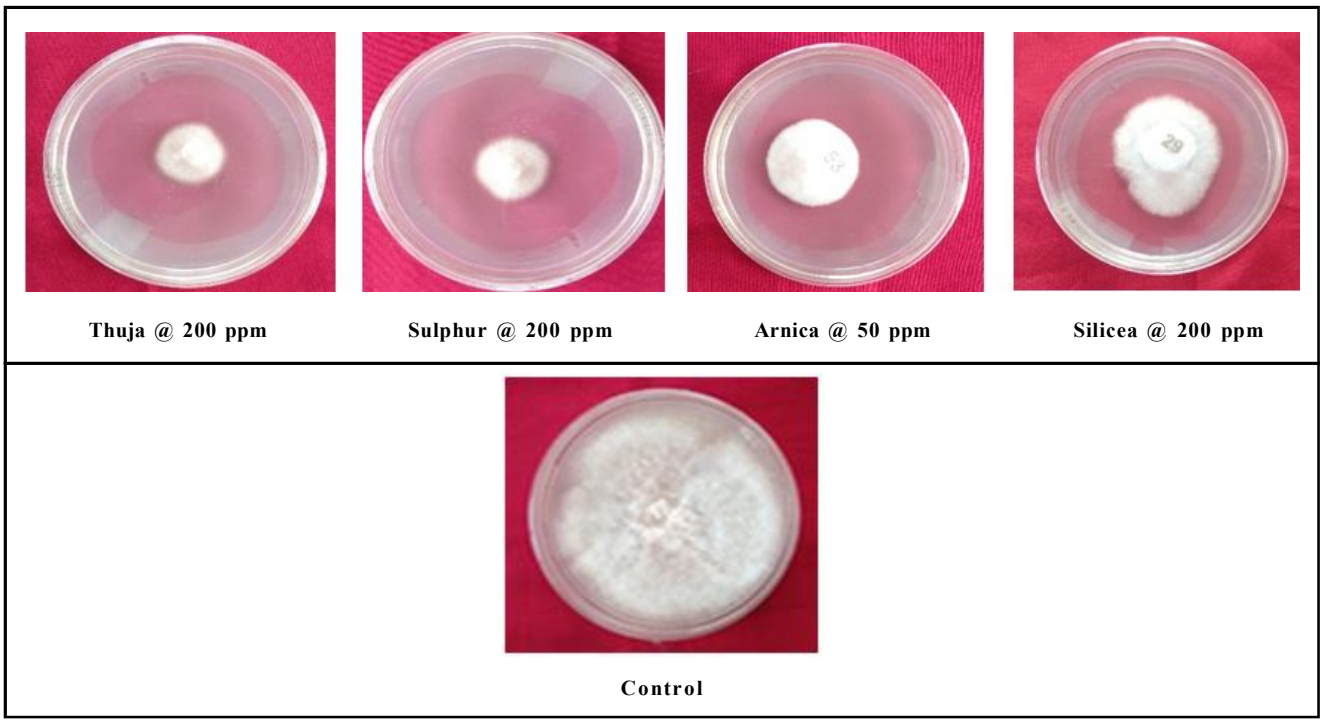


Figure 1: Effect of homeopathic medicine at different concentrations on radial mycelial growth of Fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici*

3.2 Efficacy of homeopathic medicine against *A. solani* (*in vitro*)

The effect of four homeopathic medicine, *i.e., T. occidentalis,* sulfur, *A. montana,* and silicea against *A. solani* was evaluated at different concentrations (50, 100, 150, and 200 ppm) under *in vitro* condition. Results showed a significant inhibition of the mycelial growth of *A. solani* in all four tested concentrations over control (Table 2, Figure 2). Among all treatments, the minimal radial growth of the mycelium was recorded in plates treated with *T. occidentalis* at 8 DAI. The highest mycelial growth inhibition over control was observed with *T. occidentalis* at 200 ppm (76.73%), followed by *A. montana* at 200 ppm (75.35%), sulfur at 200 ppm (74.29%), and *T. occidentalis* at 150 ppm (74.03%), corresponding, respectively, to 18.10 mm, 19.17 mm, 20.00 mm, and 20.20 mm mycelial growth at 8 DAI. The lowest mycelial growth inhibition compared to the control was attributed to silicea at 50 ppm (39.42%), corresponding to a 47.13 mm mycelial growth at 8 DAI.

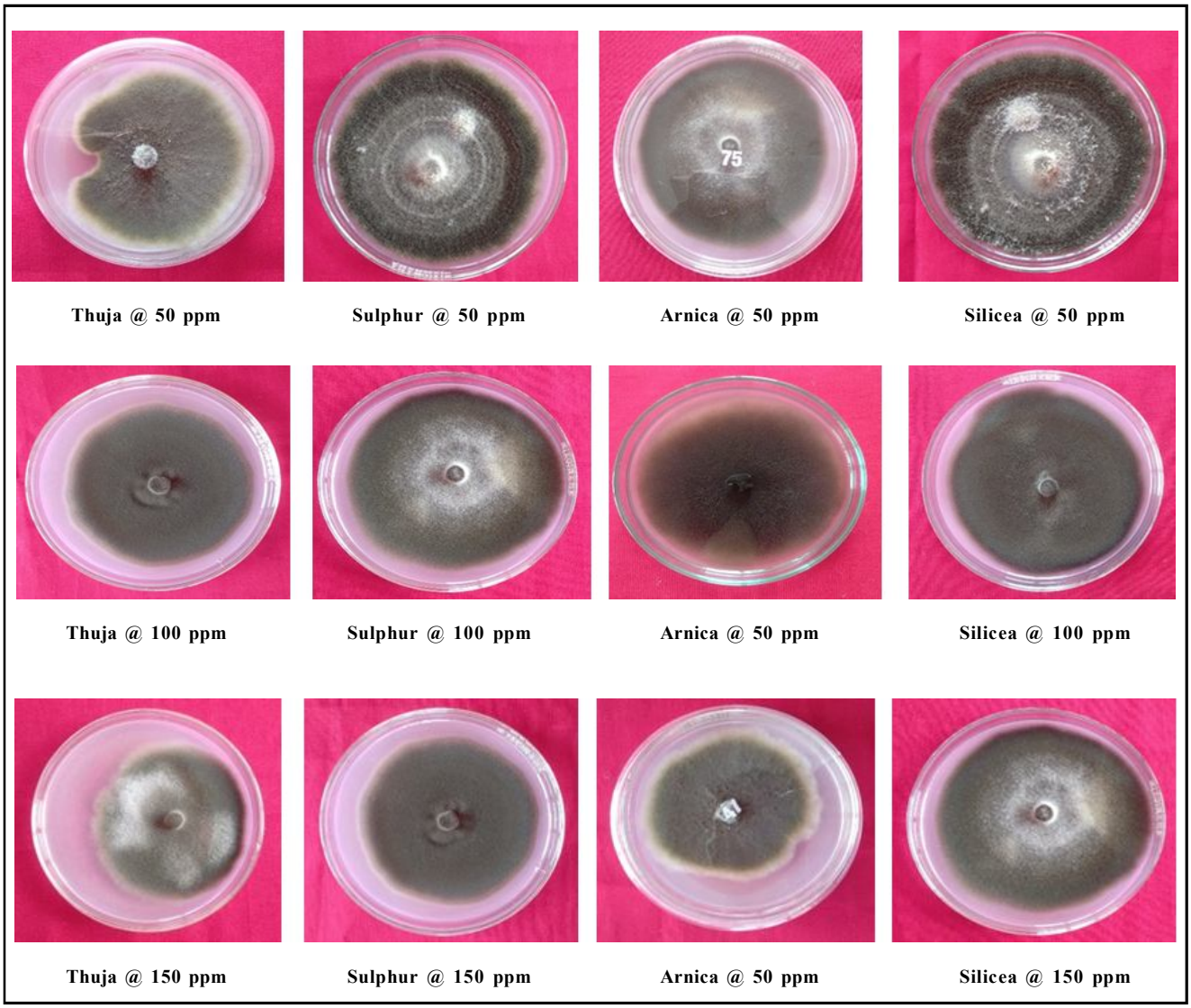
Dahiwale and Suryawanshi (2010), reported the antifungal activities of homeopathic medicines, *i.e., T. occidentalis,* sulfur, *A. montana, A. phosphoricum, Spongia tosta*, and *Chelidonium majus*. These treatments were tested individually and in mixture with mancozeb against the mycelial growth of *A. alternata* using potato dextrose agar (PDA) medium *via* food poisoning method. Dua and Atri (2004), reported the antifungal effect of homeopathic medicine, *i.e., T. occidentalis* and *Lycopodium* spp. against *A. solani*, the main reason of tomato and potato early blight disease. Patil and Suryawanshi (2014), noticed the potential control of strawberry fruit rot caused by *A. alternata* using different homeopathic medicines with promising effects on vegetative and reproductive growth.

Table 2: Effect of homeopathic medicine at different concentrations on radial mycelial growth against early blight disease of tomato caused by *A. solani*

Treatments	Radial mycelial growth (mm) at different days				Inhibition (%) over control
	2 days	4 days	6 days	8 days	
T1	12.40	17.24	29.06	36.00	53.72
T2	10.44	13.90	22.30	29.30	62.33
T3	8.93	12.29	18.95	22.50	71.07
T4	6.06	10.22	15.63	20.00	74.29
T5	8.12	12.10	19.10	26.66	65.73
T6	7.26	11.06	18.78	25.63	67.05
T7	6.93	10.10	16.77	20.20	74.03
T8	4.70	8.90	13.50	18.10	76.73
T9	11.29	16.78	26.42	32.44	58.30

T10	9.70	12.92	20.97	27.90	64.13
T11	7.72	11,23	17.90	22.00	71.12
T12	5.95	9.82	14.44	19.17	75.35
T13	16.30	28.66	38.72	47.13	39.42
T14	14.50	18.75	36.07	37.50	51.79
T15	9.95	11.77	26.33	24.40	69.92
T16	6.90	11.41	17.08	21.47	72.40
T17	24.50	39.70	56.50	77.80	-
SE	0.411	0.642	0.961	1.206	-
CD at 1 %	1.587	2.653	4.475	6.180	-

T1: Sulfur 50 ppm; T2: Sulfur 100 ppm; T3: Sulfur 150 ppm; T4: Sulfur 200 ppm; T5: *T. occidentalis* 50 ppm; T6: *T. occidentalis* 100 ppm; T7: *T. occidentalis* 150 ppm; T8: *T. occidentalis* 200 ppm; T9: *A. montana* 50 ppm; T10: *A. montana* 100 ppm; T11: *A. montana* 150 ppm; T12: *A. montana* 200 ppm; T13: Silicea 50 ppm; T14: Silicea 100 ppm; T15: Silicea 150 ppm; T16: Silicea 200 ppm; T17: Control.



Thuja @ 50 ppm **Sulphur @ 50 ppm** **Arnica @ 50 ppm** **Silicea @ 50 ppm**

Thuja @ 100 ppm **Sulphur @ 100 ppm** **Arnica @ 50 ppm** **Silicea @ 100 ppm**

Thuja @ 150 ppm **Sulphur @ 150 ppm** **Arnica @ 50 ppm** **Silicea @ 150 ppm**

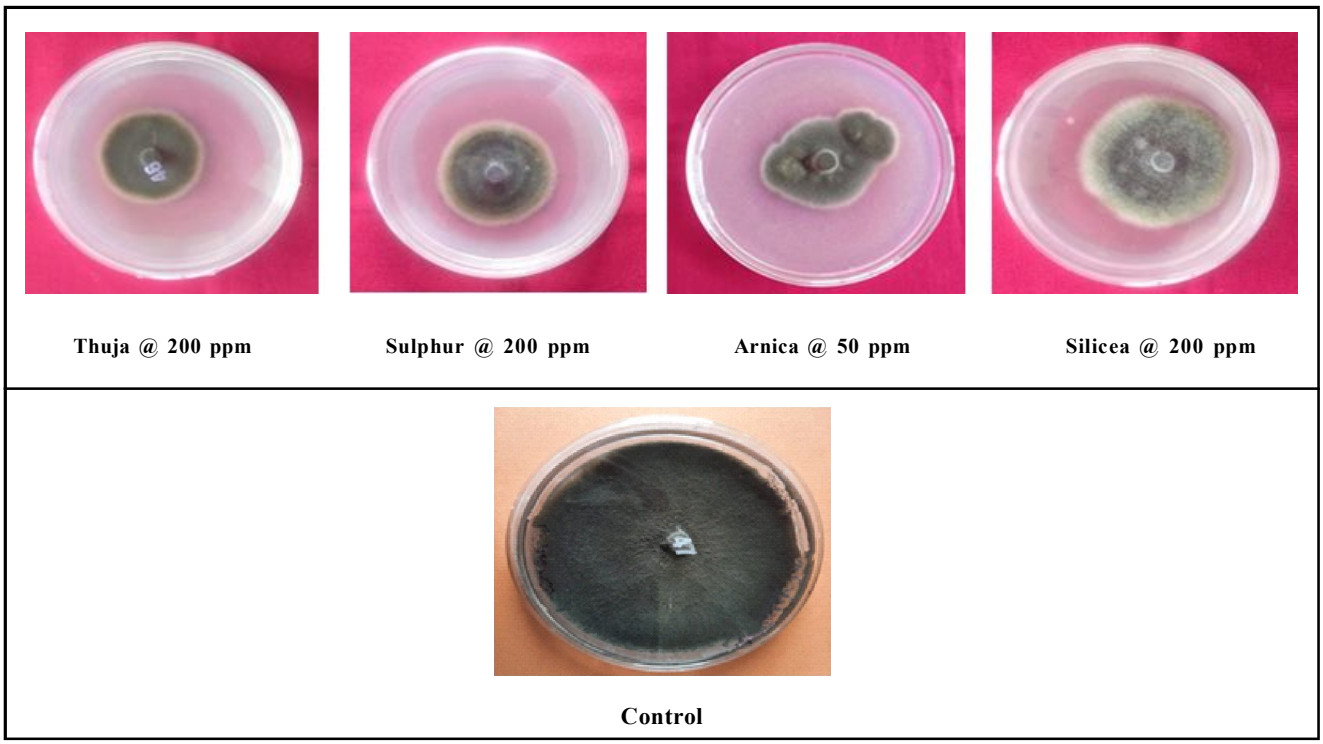


Figure 2: Effect of homeopathic medicine at different concentrations on radial mycelial growth of early blight disease of tomato caused by *A. solani*

3.3 Efficacy of homeopathic medicine against *A. rabiei* (*in vitro*)

The effect of four homeopathic medicine, *i.e., T. occidentalis,* sulfur, *A. montana,* and silicea was evaluated against *A. rabiei* at different concentrations (50, 100, 150, and 200 ppm) under *in vitro* condition. Results showed a significant inhibition of the mycelial growth of *A. rabiei* in all four tested concentrations over control (Table 3, Figure 3). Among all treatments, the minimal radial growth of mycelium was recorded in plates treated with *A. montana* at 8 DAI. The highest mycelial growth inhibition over control was observed with *A. montana* at 200 ppm (64.22%) followed by *T. occidentalis* at 200 ppm (63.77%), *A. montana* at 150 ppm (62.18%), and *T. occidentalis* at 150 ppm (61.33%), corresponding, respectively, to 31.66 mm, 32.06 mm, 33.47 mm, and 34.22 mm mycelial growth at 8 DAI. The lowest mycelial growth inhibition over control was attributed to silicea at 50 ppm (34.99%), corresponding to a 57.73 mm mycelial growth at 8 DAI.

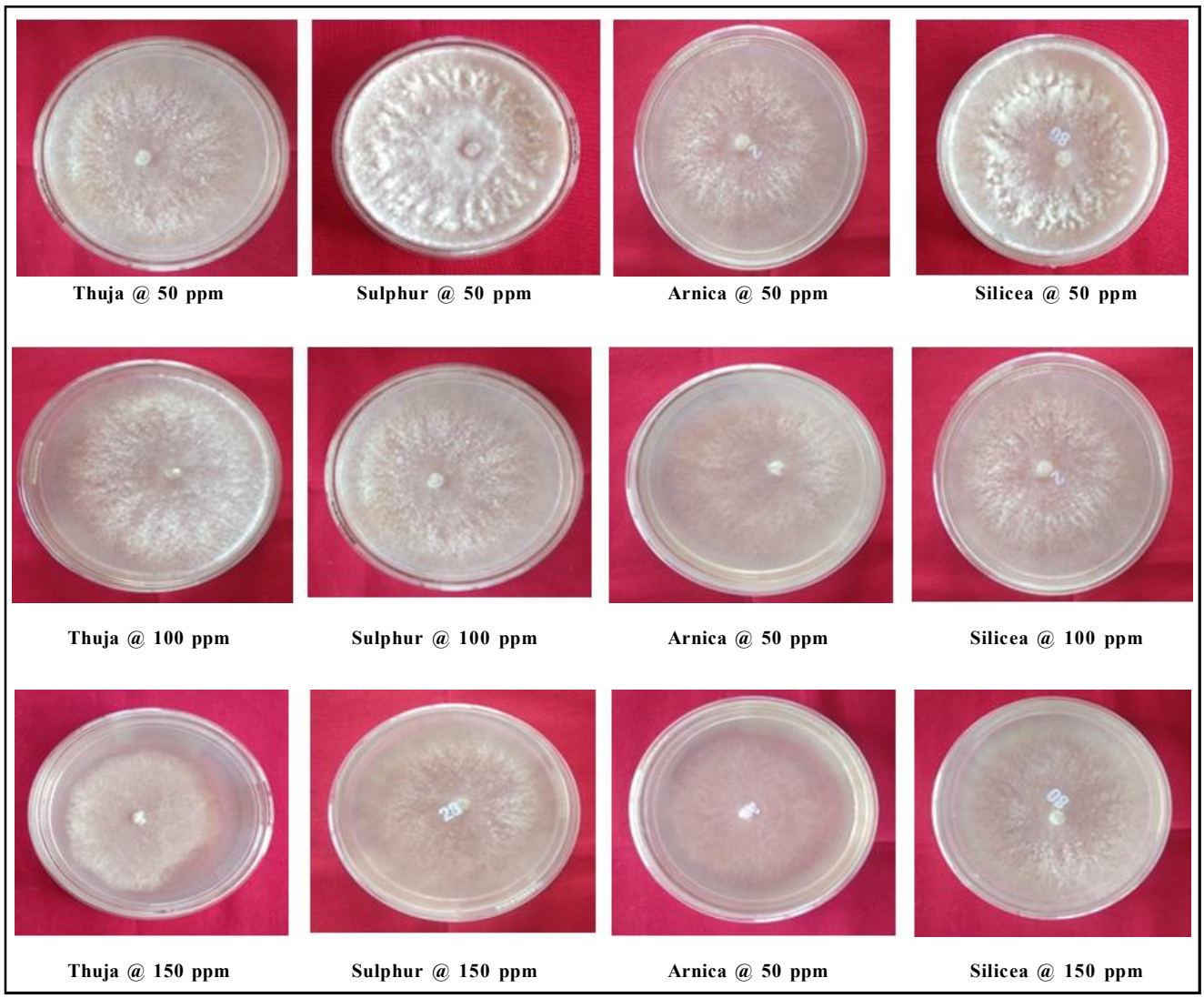
Ganzera *et al*. (2008) and Sugier *et al*. (2009), reported that *Arnica* spp. contain essential oils, terpenoids, flavonoids, and phenolic acids, especially pseudoguaianolide sesquiterpenes (0.2-0.8%) initially located in the flower head of *A. montana*, which exhibits antioxidant and antifungal activities against plant pathogens. Arie (2019), reported the effect of four homeopathic drugs, *i.e.*, *Thuja* spp., *Calcarea carbonica*, nitric acid and sulfur against *A. alternata*, *Helminthosporium ramera*, and *F. oxysparum*. The author outlined that *Thuja* spp., nitric acid and sulfur completely inhibited the growth of all fungi.

Table 3: Effect of homeopathic medicine at different concentrations on radial mycelial growth against Ascochyta blight of chickpea caused by *A. rabiei*

Treatments	Radial mycelial growth (mm) at different days				Inhibition (%) over control
	2 days	4 days	6 days	8 days	
T 1	13.70	26.23	41.16	57.73	34.99
T 2	12.08	21.43	33.43	49.58	43.97
T 3	11.53	20.66	32.62	47.52	46.30
T 4	9.62	16.57	27.19	38.47	56.53
T 5	10.10	18.17	28.06	43.41	50.94
T 6	9.88	17.70	26.50	39.57	55.28
T 7	8.55	15.42	25.06	34.22	61.33

T 8	7.40	13.87	23.80	32.06	63.77
T 9	9.98	17.78	27.87	40.38	54.37
T10	8.05	15.02	25.77	35.50	59.88
T11	7.84	14.82	24.50	33.47	62.18
T12	6.08	12.16	22.20	31.66	64.22
T13	12.50	24.67	38.87	50.60	42.82
T14	11.20	19.60	31.44	46.16	47.84
T15	10.46	16.05	28.32	44.54	49.67
T16	8.15	15.04	25.76	36.36	58.91
T17	32.45	48.64	69.10	88.50	-
SE	0.436	0.751	1.168	1.605	-
CD at 1 %	1.738	2.629	4.572	6.134	-

T1: Sulfur 50 ppm; T2: Sulfur 100 ppm; T3: Sulfur 150 ppm; T4: Sulfur 200 ppm; T5: *T. occidentalis* 50 ppm; T6: *T. occidentalis* 100 ppm; T7: *T. occidentalis* 150 ppm; T8: *T. occidentalis* 200 ppm; T9: *A. montana* 50 ppm; T10: *A. montana* 100 ppm; T11: *A. montana* 150 ppm; T12: *A. montana* 200 ppm; T13: Silicea 50 ppm; T14: Silicea 100 ppm; T15: Silicea 150 ppm; T16: Silicea 200 ppm; T17: Control.



Thuja @ 50 ppm **Sulphur @ 50 ppm** **Arnica @ 50 ppm** **Silicea @ 50 ppm**

Thuja @ 100 ppm **Sulphur @ 100 ppm** **Arnica @ 50 ppm** **Silicea @ 100 ppm**

Thuja @ 150 ppm **Sulphur @ 150 ppm** **Arnica @ 50 ppm** **Silicea @ 150 ppm**

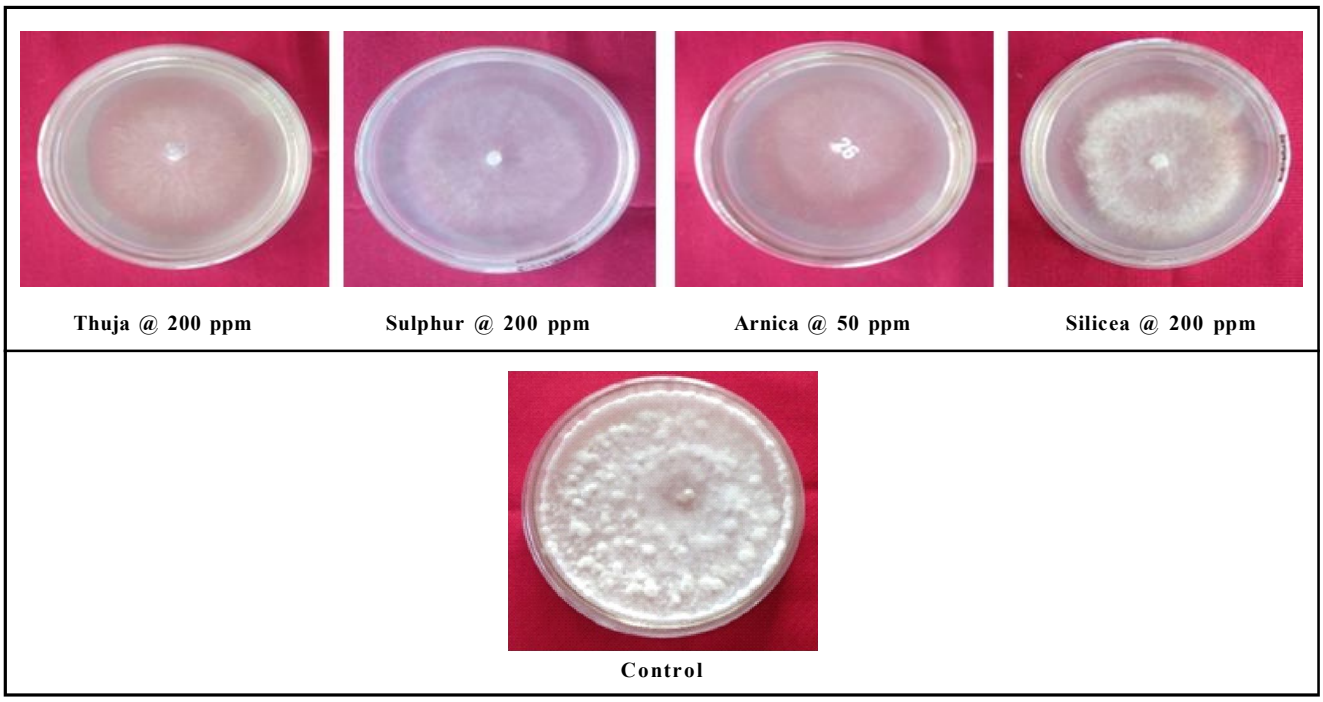


Figure 3: Effect of homeopathic medicine at different concentrations on radial mycelial growth of Ascochyta blight of chickpea caused by *A. rabiei.*

3.4 Efficacy of homeopathic medicine against *S. lycopersici* (*in vitro*)

The effect of four homeopathic medicine, *i.e.*, *T. occidentalis,* sulfur, *A. mintana,* and silicea was evaluated against *S. lycopersici* at different concentrations (50, 100, 150, and 200 ppm) under *in vitro* condition. Results showed a significant inhibition of the mycelial growth of *S. lycopersici* in all four tested concentrations over control (Table 4, Figure 4). Among all treatments, the minimal radial growth of mycelium was recorded in plates treated with silicea at 8 DAI. The highest mycelial growth inhibition over control was observed with silicea at 200 ppm (59.77%), followed by sulfur at 200 ppm (58.95%), silicea at 150 ppm (57.50%), and sulfur at 150 ppm (57.23%), corresponding, respectively, to 34.91 mm, 35.62 mm, 36.88 mm, and 37.11 mm mycelial growth at 8 DAI. The lowest mycelial growth inhibition over control was attributed to *A. montana* at 50 ppm (29.56%), corresponding to a 61.12 mm mycelial growth at 8 DAI.

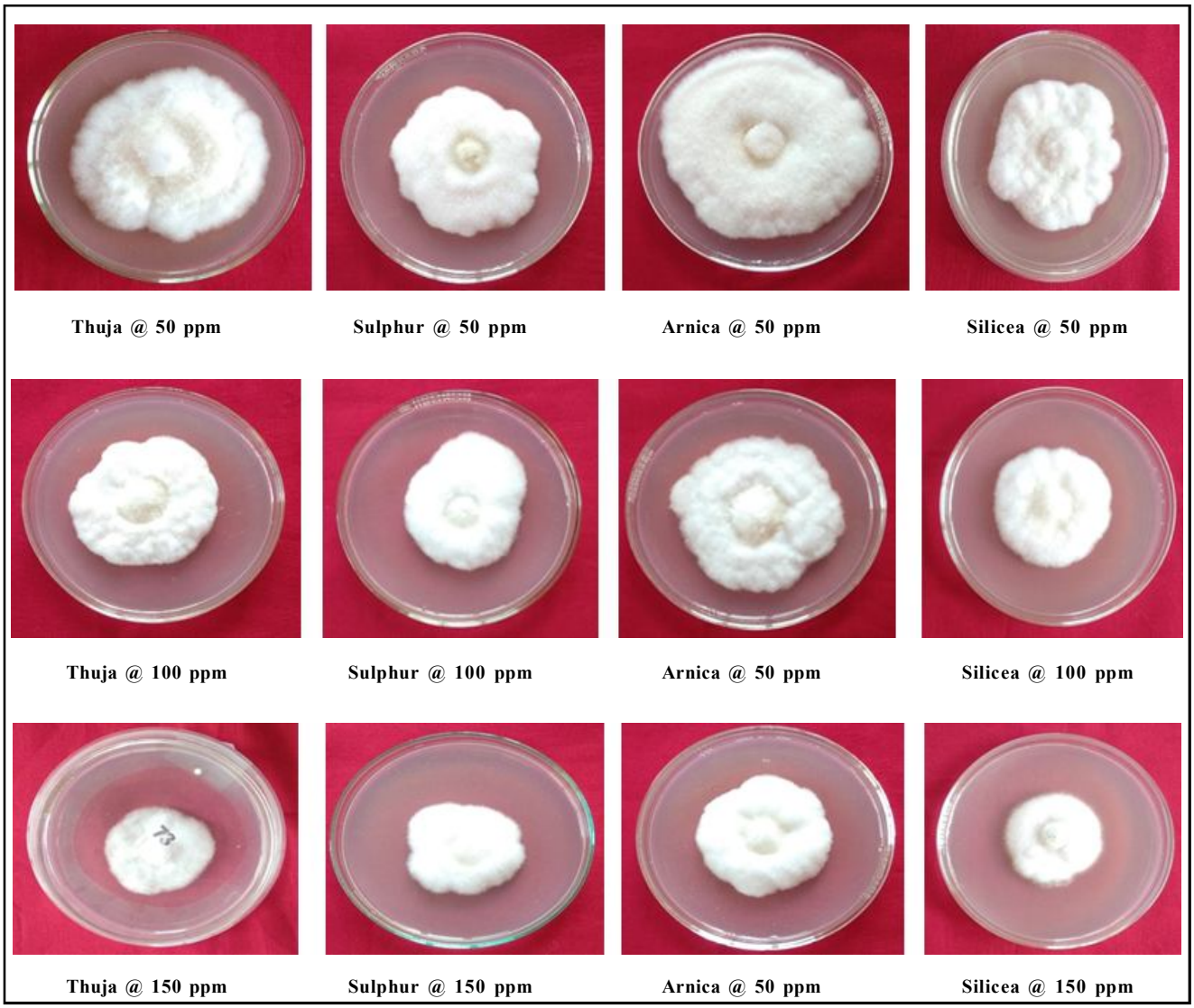
Hanif and Dawar (2015), evaluated the *in vitro* effect of homeopathic medicine, namely; *A. montana* and *T. occidentalis* against root rot fungi (*F. oxysporum, R. solani*, and *M. phaseolina*) and outlined promising values. Khanna and Chandra (1989), reported a positive effect of homeopathic medicines, *i.e.*, *Lycopodium* spp., *Thuja* spp., *Arsenicum* spp., and *Zincum* spp. against *F. moniliforme, A. alternata, Gloeosporium psidii, Colletotrichum gloeosporioides*, and *Pestalotia* spp. succeeding in the control of these fruit rot pathogens. Few researchers have reported the same aspects in controlling plant pathogens. For instance, Saxena *et al*. (1988) reported the antifungal activity of *T. occidentalis* used for okra seed treatment.

Table 4: Effect of homeopathic medicine at different concentrations on radial mycelial growth against Septoria leaf spot of tomato caused by *S. lycopersici*

Treatments	Radial mycelial growth (mm) at different days				Inhibition (%) over control
	2 days	4 days	6 days	8 days	
T 1	11.50	22.90	32.37	43.60	49.75
T 2	10.10	18.57	23.45	39.44	54.55
T 3	8.10	14.08	22.60	37.11	57.23
T 4	7.25	12.27	21.93	35.62	58.95
T 5	13.67	24.47	36.47	45.61	47.44
T 6	12.44	23.42	34.76	44.72	48.46
T 7	10.12	19.15	29.18	42.21	51.35
T 8	9.41	17.77	26.87	40.32	53.53
T 9	15.43	27.67	39.40	61.12	29.56

T10	13.12	25.70	36.31	45.63	47.41
T11	11.29	23.68	34.47	43.11	50.32
T12	10.53	13.10	28.77	41.22	52.50
T13	9.93	18.50	28.42	41.81	51.82
T14	8.15	16.60	22.58	38.40	55.75
T15	7.87	13.88	21.10	36.88	57.50
T16	6.72	12.66	20.23	34.91	59.77
T17	22.17	39.56	61.87	86.78	-
SE	0.407	0.750	1.124	1.632	-
CD at 1 %	1.634	2.256	4.214	6.381	-

T1: Sulfur 50 ppm; T2: Sulfur 100 ppm; T3: Sulfur 150 ppm; T4: Sulfur 200 ppm; T5: *T. occidentalis* 50 ppm; T6: *T. occidentalis* 100 ppm; T7: *T. occidentalis* 150 ppm; T8: *T. occidentalis* 200 ppm; T9: *A. montana* 50 ppm; T10: *A. montana* 100 ppm; T11: *A. montana* 150 ppm; T12: *A. montana* 200 ppm; T13: Silicea 50 ppm; T14: Silicea 100 ppm; T15: Silicea 150 ppm; T16: Silicea 200 ppm; T17: Control.



Thuja @ 50 ppm **Sulphur @ 50 ppm** **Arnica @ 50 ppm** **Silicea @ 50 ppm**

Thuja @ 100 ppm **Sulphur @ 100 ppm** **Arnica @ 50 ppm** **Silicea @ 100 ppm**

Thuja @ 150 ppm **Sulphur @ 150 ppm** **Arnica @ 50 ppm** **Silicea @ 150 ppm**

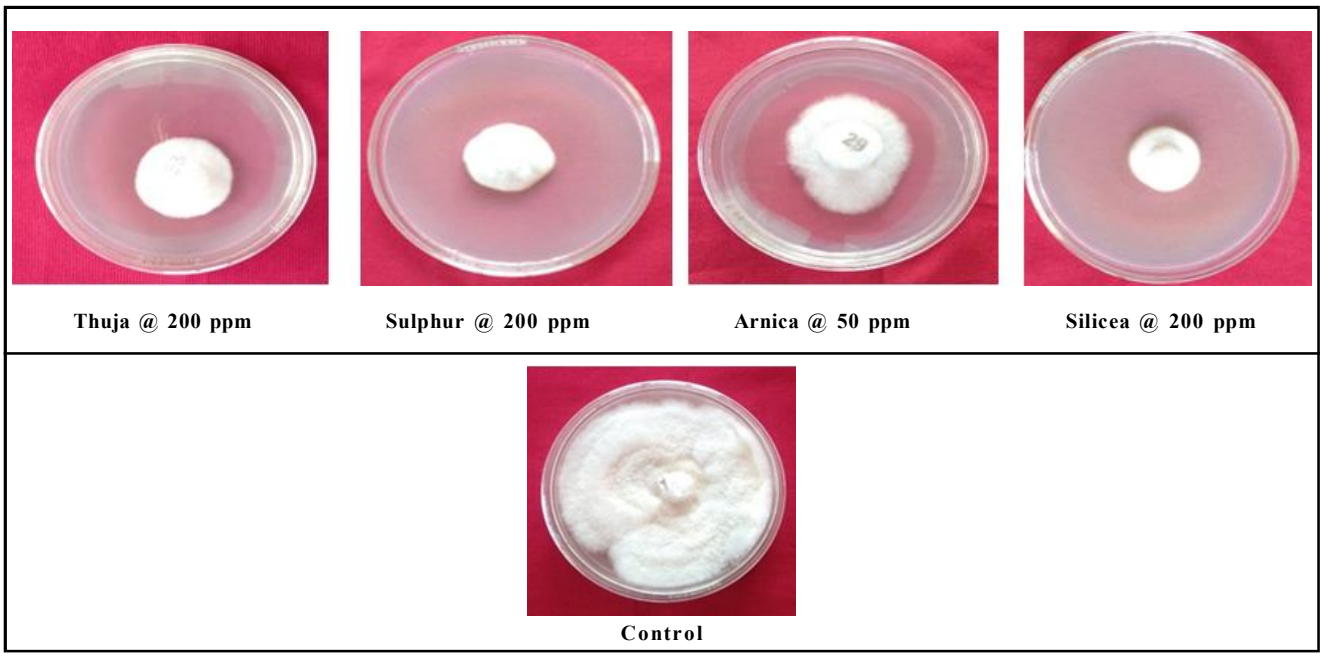


Figure 4: Effect of homeopathic medicine at different concentrations on radial mycelial growth of Septoria leaf spot of tomato caused by *S. lycopersici*.

3.5 Efficacy of homeopathic medicine against *P. vexans* (*in vitro*)

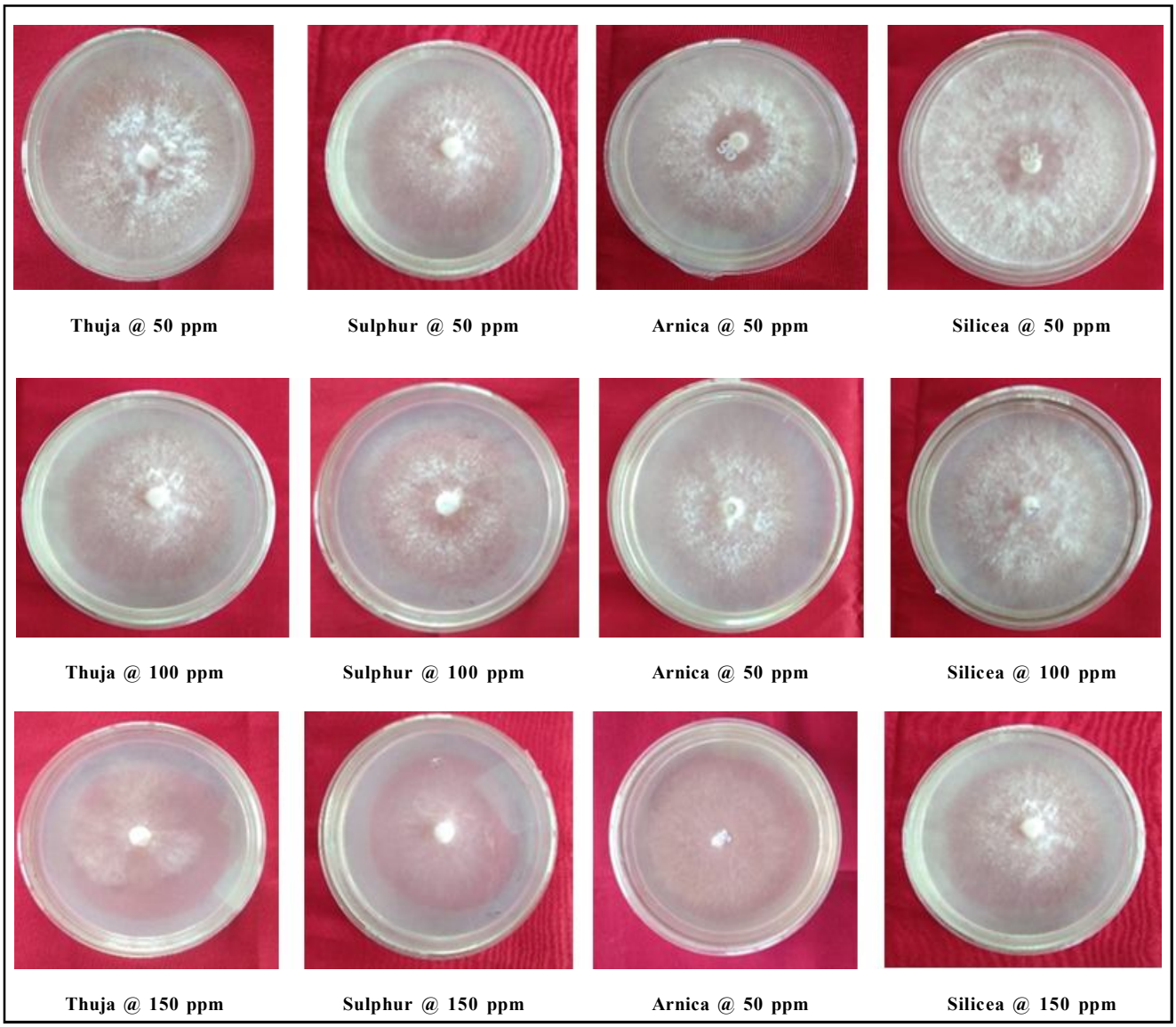
The effect of four homeopathic medicine, *i.e.*, *T. occidentalis*, sulfur, *A. montana*, and silicea was evaluated against *P. vexans* at different concentrations (50, 100, 150, and 200 ppm) under *in vitro* condition. Results showed a significant inhibition of the mycelial growth of *P. vexans* in all four tested concentrations over control (Table 5, Figure 5). Among all treatments, the minimal radial growth of mycelium was recorded in plates treated with sulfur at 8 DAI. The highest mycelial growth inhibition over control was observed with sulfur at 200 ppm (53.82%), followed by *T. occidentalis* at 200 ppm (51.66%), and Sulfur at 150 ppm (50.93%), corresponding respectively to 38.12 mm, 39.90 mm, and 40.50 mm mycelial growth at 8 DAI. The lowest mycelial growth inhibition over control was attributed to silicea at 50 ppm (38.82%), corresponding to a 50.50 mm mycelial growth at 8 DAI. Asha *et al*. (2014), reported the effective role of *T. occidentalis* against *A. solani*, *F. oxysporum* sp. *lycopersici*, and *P. vexans* fungal genera. Panda *et al*. (2013), treated cereal seeds with 0.1% *T. occidentalis*. They outlined an improvement in plant growth parameters, *i.e.*, shoot length and weight, and root length and weight of cereal crops. Particularly, the growth of millet and wheat root infecting fungi.

Table 5: Effect of homeopathic medicine at different concentrations on radial mycelial growth against Phomopsis blight of brinjal caused by *P. vexans*

Treatments	Radial mycelial growth (mm) at different days				Inhibition (%) over control
	2 days	4 days	6 days	8 days	
T1	13.77	22.50	33.20	46.77	43.34
T2	10.20	20.70	31.09	41.87	49.27
T3	8.95	19.06	30.96	40.50	50.93
T4	7.42	18.40	29.05	38.12	53.82
T5	14.60	26.78	38.88	47.50	42.45
T6	11.05	24.70	36.50	43.12	47.76
T7	9.15	22.15	34.10	41.50	49.72
T8	7.70	21.83	32.70	39.90	51.66
T9	16.90	28.50	40.50	48.20	41.61
T10	15.85	26.80	38.78	46.50	43.67

T11	11.90	24.95	36.30	45.44	44.95
T12	8.50	23.10	35.12	43.50	47.30
T13	17.30	30.06	41.34	50.50	38.82
T14	16.70	27.40	39.12	47.70	42.21
T15	12.40	25.12	37.10	46.21	44.22
T16	9.10	24.50	36.35	44.72	45.82
T17	23.20	41.50	64.30	82.55	-
SE	0.485	0.913	1.358	1.686	-
CD at 1 %	1.899	2.795	4.691	6.867	-

T1: Sulfur 50 ppm; T2: Sulfur 100 ppm; T3: Sulfur 150 ppm; T4: Sulfur 200 ppm; T5: *T. occidentalis* 50 ppm; T6: *T. occidentalis* 100 ppm; T7: *T. occidentalis* 150 ppm; T8: *T. occidentalis* 200 ppm; T9: *A. montana* 50 ppm; T10: *A. montana* 100 ppm; T11: *A. montana* 150 ppm; T12: *A. montana* 200 ppm; T13: Silicea 50 ppm; T14: Silicea 100 ppm; T15: Silicea 150 ppm; T16: Silicea 200 ppm; T17: Control.



Thuja @ 50 ppm **Sulphur @ 50 ppm** **Arnica @ 50 ppm** **Silicea @ 50 ppm**

Thuja @ 100 ppm **Sulphur @ 100 ppm** **Arnica @ 50 ppm** **Silicea @ 100 ppm**

Thuja @ 150 ppm **Sulphur @ 150 ppm** **Arnica @ 50 ppm** **Silicea @ 150 ppm**

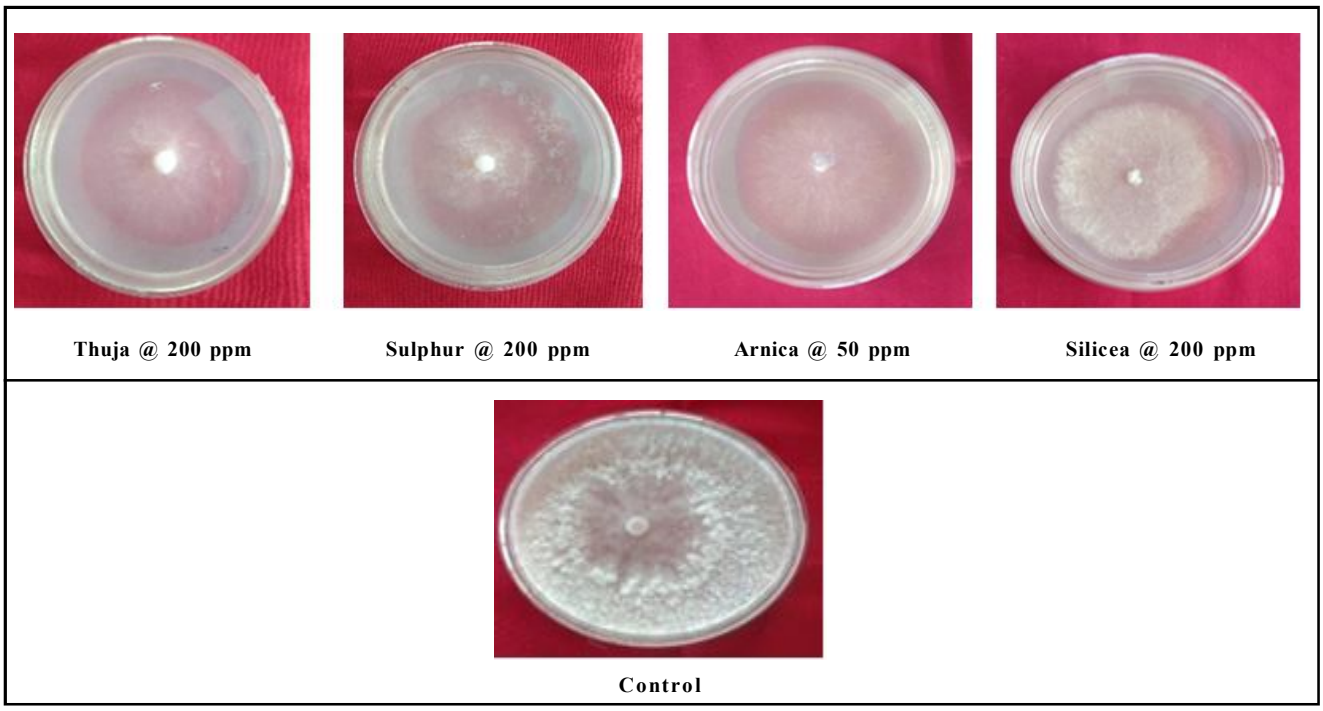


Figure 5: Effect of homeopathic medicine at different concentrations on radial mycelial growth of Phomopsis blight of brinjal caused by *P. vexans*.

4. Discussion

Effect of four homeopathic medicines, *viz., T. occidentalis,* sulphur, *A. mintana,* and silicea, at different concentrations (50, 100, 150 and 200 ppm) were evaluated *in vitro* condition and found that significantly inhibited mycelial growth of *F. oxysporum* f. sp. *lycopersici,* in all four tested concentrations over control (Table 1 and Figure1). Among all these treatments, the minimum radial growth of mycelium was recorded in plates treated with 50, 100, 150 and 200 ppm of *T. occidentalis,* representing as 37.60 mm, 30.87 mm, 25.25 mm and 22.80 mm, at 8 days after inoculation (DAI), which was highest (73.73%) inhibition of mycelial growth over control. Similarly, in *A. solani* (Table 2 and Figure 2), the maximum inhibition of radial growth of mycelium was observed in plates treated with *T. occidentalis* @ 50, 100, 150 and 200 ppm, representing as 26.66 mm, 25.63 mm, 20.20 mm and 18.10 mm,on 8 days after inoculation (DAI), which was highest inhibited 76.73% of mycelial growth over control.

In *A. rabiei,* effect of four homeopathic medicine (*T. occidentalis,* sulphur, *A. mintana,* and silicea) at different concentration (50, 100, 150 and 200 ppm) were evaluated *in vitro* condition and found that significantly inhibited mycelial growth of all four tested concentrations over control (Table 3 and Figure 3). Among all these treatments, the minimum radial growth of mycelium was recorded in plates treated with 50, 100, 150 and 200 ppm of *A. montana,* representing as 40.38 mm, 35.50 mm, 33.47 mm and 31.66 mm, at 8 days after inoculation (DAI), which was highest (64.22%) inhibition of mycelial growth over control.

Effect of four homeopathic medicine, *viz., T. occidentalis,* sulphur, *A. mintana,* and silicea, at different concentration (50, 100, 150 and 200 ppm) were evaluated *in vitro* condition and found that significantly inhibited mycelial growth of *S. lycopersici* and *P. vexans,* in all four tested concentrations over control (Table 4, Figure 4 and Table 5, Figure 5). The maximum inhibition of radial growth of *S. lycopersici* was recorded in Figure 4 treated with silicea @ 50, 100, 150 and 200 ppm, representing as 41.81 mm, 38.80 mm, 36.88 mm and 34.91 mm on 8 days after inoculation (DAI), which was inhibited 59.77% mycelial growth over control as well as in *Phomopsis vexans,* the minimum radial growth of mycelium was recorded in Figure 5 treated with 50, 100, 150 and 200 ppm of sulphur, representing as 46.77 mm, 41.87 mm, 40.50 mm and 38.12 mm, at 8 days after inoculation (DAI), which was highest (53.82%) inhibition of mycelial growth over control.

5. Conclusion

Plants produce secondary metabolites throughout the homoeopathic drug making process. These metabolites are non-toxic and non-residue forming, making them safe to the environment. This study investigated the synergistic effect of homoeopathic medications and microbial antagonist in preventing several fungal diseases, *i.e.*, *Fusarium* wilt, early blight of potato and Septoria leaf spot of tomato, Ascochyta blight of chickpea, and Phomopsis blight of brinjal.

Pathogens (*Fusarium*, *Phomopsis*, *Septoria*, *Alternaria*, and *Ascochyta*), responsible for plant diseases were treated using homoeopathic remedies including *A. montana*, *T. occidentalis*, sulfur, and silicea. The increased concentrations of homeopathic medicine were found to significantly reduce the radial mycelial growth of *F. oxysporum* f. sp. *lycopersici*, *A. solani*, *S. lycopersici*, *A. rabiei*, and *P. vexans* under *in vitro* condition. At 8 DAI, *T. occidentalis* at 200 ppm (73.73%), *T. occidentalis* at 200 ppm (76.73%), *A. montana* at 200 ppm (64.22%), silicea at 200 ppm (59.77%), and sulfur at 200 ppm (53.82%) showed the maximum mycelial growth inhibition

compared to the control. The minimal radial growth for these five fungal species was 22.80 mm, 18.10 mm, 31.66 mm, 34.91 mm, and 38.12 mm, respectively. These homeopathic remedies can, therefore, help plants defend themselves against fungus and other pathogen attack. Further, research should investigate the efficacy of used homeopathic remedies against other fungal species affecting horticultural crops.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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