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Research Article

Sensitivity of *Botrytis cinerea* isolate collected from gladiolus against selected fungicides, plant oils and botanicals in North India

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Abstract

Integrated disease management is the best and most environmentally friendly technique for managing of a plant disease. Among the nine fungicides tested *in vitro* against *Botrytis cinerea*, which causes grey mould in *Gladiolus*, hexaconazole and a combination of iprodione+carbendazim exhibited complete inhibition of the mycelia growth at all the tested concentrations. In the evaluation of 15 different plant oils against *B. cinerea*, oils of *Mentha piperita, Cymbopogon martini, Pelargonium graveolens, Cymbopogon* sp., and *Oreganum vulgare* resulted in 100% mycelial growth inhibition of *B. cinerea* at all concentrations ranging from 200 to 1000 ppm. Aqueous extract of cloves of *Allium sativum* resulted best among the different botanicals and bioproducts tested *in vitro* with a maximum average mycelial growth inhibition of 57.39%, followed by the leaves of *Azadirachta indica* (45.47%) at 20, 50, 75, and 100 ppm. In integrated management of the grey mould of gladiolus under *in vivo* conditions, the combination of quintal + *A. sativum* + neem oil exhibited the maximum reduction in disease (94.40 %), followed by the combination of contaf + *A.sativum* + neem oil, which showed 93.19% disease reduction. The treatments viz., quintal +*A.sativum* + neem oil also resulted in the most superior treatment in enhancing growth and yield parameters of gladiolus with significantly improved plant height (70.34 cm), yield of corms (38.00), spike length (53.17 cm), number of florets (15.65), and number of flowers (32.67). These results indicate that the integrated management approach by using fungicides, botanicals and essential oils could be used for the control of grey mould diseases caused by *Botrytis* pathogens.

Keywords: *Botrytis*; botanicals; bioproducts; management

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Introduction

Botrytis, named as botrytis grey mould (BGM) is an anamorphic fungus belonging to the hyphomycetes group and containing approximately 28 pathogen species (Dewey and Grant-Downton, 2016). Among these species, *Botrytis cinerea,* has been noted to infect over 200 species of hosts (Choquer *et al.*, 2007; Williamson *et al.*, 2007; Walker *et al.,* 2015) that belong to more than 170 families of plants of agricultural and agri-food importance (Elad *et al.*, 2016). This pathogen enters into the host at the beginning of the disease cycle through a short asymptomatic and biotrophic phase (Veloso and Van Kan, 2018). The infection mechanism of *Botrytis cinerea* has been studied as a model organism because of high quality references genome sequences (Van Kan *et al.,* 2017; Saats and Van Kan, 2012). The worldwide effect of *Botrytis cinerea* on flowers, vegetables, and products of plants is noticeable because of the wide and severe pre-harvest and post-harvest losses. A novel approach to the management of *B. cinerea,* pathogen, while maintaining quality of fruit, has been carried out by the application of essential oils. This strategy eliminates the necessity for synthetic fungicides, thereby in accordance with the consumer preferences and lessen the environmental pollution (du Plooy *et al.,* 2009).

In this era, negative effects of chemicals were seen on the human health and environment (Epstein 2014; Komárek *et al.,* 2010), as well as the population of *B. cinerea* shown resistance against most of the chemical fungicides (Fernández-Ortuño *et al*., 2016), hence there is magnified interest in the environmentally safe and friendly alternatives for the control of botrytis gray mold (Tracy, 2014). To conquer these limitations, an integrated management strategies is used, in which the combination of different fungicides, botanicals and plant oils can reduce the potential *B. cinerea* infection risk (González-Domínguez *et al*., 2020).

Materials and Methods

Isolation and purification of causal pathogen

For the isolation and purification of the *Botrytis cinerea* pathogen, the diseased portions from the infected leaves were cut into small pieces. The pieces were washed thoroughly with tap water, then air dried and surface sterilized by soaking it in a 0.1% sodium hypochlorite for 30 seconds and rinsed twice in sterile distilled water, dried on the sterile blotting paper, and placed on potato dextrose agar (PDA) plates supplemented with streptomycin sulphate (100 $\mu g\ ml^{-1}$) to avoid the bacterial contamination The plates were incubated at 24±1 °C and examined for the pathogen growth. After 4 days, the young growing hyphal tips were transferred to freshly prepared PDA plates by hyphal tip technique according to Karr and Albersheim (1970). The slants of *Botrytis* cultures were stored in a refrigerator at 4 °C and utilized for further study.

In vitro assessment of fungicides

The antifungal activity of both systemic as well as non-systemic fungicides viz. Score 25 EC (difenoconazole), Amistar 250 SC (azoxystrobin), Contaf 5 EC (hexaconazole), Bavistin 50 WP (carbendazim), Hilnate 70 WP (thiophanate methyl) at 50, 100, 150 and 200 ppm and pyraclostrobin (5%) + metiram (55%) (Cabrio Top 60 WG), Zineb (75% WP) + hexaconazole (5 EC) (Avtar 15% WP), Propineb (Antracol 70 WP) and carbendazim (25%) + iprodione (25%) (Quintal 50 WP) at 150, 500, 750, 1000 ppm were evaluated against *B. cinerea* with the use of Poisoned Food Technique specified by Falck (1907). The desired amount of test fungicides was added to the flask containing double strength potato dextrose agar (PDA) medium. Then this PDA medium was dispensed into the disinfected Petri Plates, which inoculated in the three replications, and incubation was done at 22 ±1 °C. The diameter of the colony of the trial pathogen was documented until the control plates became full with the growth of mycelium in 8 days. The calculation of

percent inhibition in the growth of the mycelium of the pathogen was done as per the formula specified by Vincent (1947).

$$I = (C - T/ C) \times 100$$

Where:
C = average fungal colony diameter (mm) in control
T = average fungal colony diameter (mm) in antifungal treated PDA medium.

In vitro assessment of plant oils

The evaluation of various types of oils selected against the test pathogen (*Botrytis cinerea*) was performed for the evaluation of activities against fungus (Table 1). Four different concentrations, i.e. 100, 500, 700, and 1000 ppm of the above-mentioned oils were assessed for their activity against *B. cinerea* by the use of the Poisoned Food Technique. For making the dilutions of these plant oils, Tween 20 (1:10 ml) as an organic solvent was taken and poured in disinfected molten agar media, accompanied by the requisite concentrations of plant oils separately and thoroughly assorted in PDA medium, and then poured into disinfected petri plates. A 4 mm bit from pure culture of *B. cinerea* isolate was placed in the center of Petri plates center after its solidification, whereas the control was reserved without using the oil. Then the calculation of the data was done on the percent inhibition of growth (Hosen *et al.,* 2010).

Table 1. List of oils tested for antifungal activity against the pathogen

Sr No.	Common Name	Botanical Name	Family
1	Palmarosa	*Cymbopogon martini*	Poaceae
2	Geranium	*Pelargonium graveolens*	Geraniaceae
3	Menthol	*Mentha arvensis*	Lamiaceae
4	Citronella	*Cinnamomum aromaticum*	Lauraceae
5	Neem	*Azadirachta indica*	Meliaceae
6	Clove	*Eugenia caryophyllata*	Myrtaceae
7	Eucalyptus	*Eucalyptus globules*	Myrtaceae
8	Tulsi	*Ocimum sanctum*	Lamiaceae
9	Oregano	*Origanum vulgare*	Lamiaceae
10	Rosemerry	*Rosmarinus officinalis*	Lamiaceae
11	Lavender	*Lavandula angustifolia.*	Lamiaceae
12	Almond	*Prunus dulcis*	Rosacea
13	Mustard	*Brassica juncea*	Brasicaceae
14	Cedarwood	*Cedrus deodara*	Pinaceae
15.	Pippermint	*Mentha piperita*	Lamiaceae

In vitro assessment of bioresources (botanicals and bio products)

The water extracts of various plants, such as leaves of *Vitex nigundo* (bana), *Eucalyptus globulus* (Eucalyptus), *Adathoda vesica* (Basuti), cloves of *Allium sativum* (garlic), seeds of *Melia azedarach* (darek), *Murraya koengii* (karipatta), *Azadirachta indica* (neem), milk of *Euphorbia halioscopia*, rind of *Spindus laurifolia* (ritha), vermiwash, cow urine, and sourbutter milk were individually assessed under *in vitro* conditions against the test pathogen at concentrations of 20, 50, 75, and 100 % using the Poisoned Food Technique.

Plant extracts preparation

Fresh leaves of *Vitex nigundo* (bana), *Eucalyptus globulus* (Eucalyptus), *Adathoda vesica* (Basuti), cloves of *Allium sativum* (garlic), seeds of *Melia azedarach* (darek), *Murraya koengii* (karipatta), *Azadirachta indica* (neem), milk of *Euphorbia halioscopia* and rind of *Spindus laurifolia* (ritha), each weighing 200 g, were taken, washed with tap water, and used blender for 5 minutes. Ultimately, these were ground with the addition of a small amount of distilled water. The distilled water, about 100 mL, was added after grinding, and it was homogenized using the orbital shaker at 2000 rpm for about 30 minutes in order to produce an extract of 100% concentration. Then, using double layered muslin cloth, the plant material was filtered, and then the autoclave was used at a pressure of 15 psi (pound per square inch) for about an hour to sterilize the various plant extracts which were stored in a refrigerator for further studies.

In vitro efficiency of botanicals against the pathogen grey mould

The extracts were evaluated at the concentration of 50% with the inclusion of 50 ml of botanical, (100 %) extracts in 50 mL of disinfected (autoclaved for 20 minutes at 1.05 kg/cm^2) double strength PDA medium and under, aseptic conditions, cooled and dispensed in the disinfected petri plates. The inoculation of petri plates with bit a 4 mm diameter pathogen culture is 7 days old. Also, the extracts at 75 and 100% concentrations were assessed similarly with an adjustment on a v/v basis, while no adjustment was needed at 100% concentration. For comparison, as a control, the petri plates with 50 mL of double strength sterilized PDA medium mixed with disinfected distilled water were used. The replication of each treatment was done three times, and the petri plates were incubated in a biochemical oxygen demand (BOD) incubator at 22 ±1 °C. The daily observation of inoculated petri plates was done, and the diameter of the colony of the test pathogen was recorded until the control petri plates became packed with the mycelium of the test pathogen. The percentage inhibition due to various treatments in the growth of the mycelium of the pathogen was calculated earlier. Similarly, the evaluation of fresh cow urine from a local hill cow breed and the vermi wash was done at 20, 50, 75, and 100% concentrations to get a decrease in the growth of mycelium. For statistical analysis of the work a completely randomized design (CRD) factorial design was used.

Grey mould management was investigated using a combination of botanicals, fungicides and plant oils

The field evaluation of the most effective fungicides, botanicals, and plant oils viz., Quintal, Contaf, and Avtar; botanicals viz., *Murraya koengii, Azadirachta indica, Allium sativum*, and neem oil were evaluated for utilization on field trials. All treatment doses were thoroughly mixed with water before foliar spraying the plants after two months of planting, when the diseases first appeared in the field, and every 15 days until crop harvesting. On the commencement of disease, one fungicide spray was applied, followed by two sprays of a combination of plant oils and botanicals. However, no treatment was implemented for control; but simply water was sprayed.

Statistical analysis

The documented data from different *in vitro* and field trials were statistically analyzed. The differences shown by the treatments in different trials were verified for their significance at 5 percent with the use of the standard protocol described by Gomez and Gomez (1986).

Results and Discussion

Identification of the isolated pathogen

To identify the pathogen, the pure culture was tentatively identified on the basis of macroscopic and microscopic characters mentioned in the literature as given by Hennebert (1973) and Elad *et al*. (1994). The

molecular characterization of the botrytis grey mould fungus revealed that the associated pathogen is *Botrytis cinerea* with its aligned sequence data (700 bp) and based on nucleotides homology and phylogenetic analysis of fungus was detected to be *Botrytis cinerea* (GenBank Accession Number: KP-141790).

Antifungal activity of fungicides

Chemical control remains the most effective method for controlling grey mould. It was estimated that fungicides specifically targeting *B. cinerea* cost around 540 million 9 euros annually, representing 10% of the world fungicide market (Dean *et al.*, 2012). A total of nine different systemic and non-systemic fungicides viz., Bavistin, Contaf, Score, Hilnate and Amistar at 50, 100, 150, and 200 ppm and Cabrio top, Avtar, Antracol and Quintal at 150, 500, 750, 1000 ppm were assessed *in vitro* environments against *Botrytis* isolates using Poisoned Food Technique (Table 2). The data (Figure 1) showed that Contaf and Quintal at 50, 100, 150, and 200 ppm concentrations exhibited complete inhibition of mycelium growth of *B. cinerea* under *in vitro* environments followed by Avtar (91.16%) and Score (63.99%), respectively while Hilnate showed minimum effect (11.68%).

Table 2. *In vitro* evaluation of chemicals against *B. cinerea*

Chemicals	Mycelial growth (mm)				Mean	Mycelial growth inhibition (%)				Mean
	50 ppm	100 ppm	150 ppm	200 ppm		50 ppm	100 ppm	150 ppm	200 ppm	
Quintal (Iprodione + Carbendazim)	0 .00	0.00	0 .00	0.00	0.00	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
Contaf (Hexaconazole)	0 .00	0 .00	0.00	0 .00	0.00	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
Avtar (Zineb + Hexaconazole)	18.83	13.00	0 .00	0 .00	7.96	79.07 (62.75)	85.55 (67.63)	100.00 (89.96)	100.00 (89.96)	91.16 (77.58)
Score (Difenaconazole)	38.00	32.50	29.17	28.00	31.92	57.78 (49.47)	63.89 (53.06)	66.67 (56.09)	67.60 (54.72)	63.99 (53.33)
Cabrio Top (Pyraclostrobin + Metiram)	49.50	41.00	40.33	30.00	40.21	45.12 (42.18)	54.44 (47.54)	55.19 (50.10)	59.45 (54.91)	53.55 (48.68)
Antracol (propineb)	50.17	41.67	42.67	38.33	43.21	44.37 (41.75)	56.37 (48.64)	52.59 (49.46)	58.89 (49.24)	53.06 (47.27)
Bavistin (Carbendazim)	71.67	69.33	63.67	61.00	66.42	20.37 (26.73)	23.71 (29.12)	28.89 (34.56)	29.26 (31.79)	25.56 (30.55)
Amistar (Azoxystrobin)	77.00	70.67	69.00	63.67	70.08	14.45 (22.29)	21.48 (27.59)	23.34 (29.62)	30.00 (32.72)	22.32 (28.06)
Hilnate (Thiophanate methyl)	79.67	79.00	79.00	80.67	79.58	11.12 (18.78)	11.49 (19.77)	11.86 (20.10)	12.27 (21.41)	11.68 (19.97)
Control	90.00	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	42.77	38.57	35.98	34.23		52.53 (49.43)	56.87 (43.02)	57.48 (52.62)	60.08 (50.79)	
$CD_{0.05}$		Chemicals = 1.44 Concentration = 0.96 Chemicals × Concentration = 2.89								

Efficacy of carbendazim, thiophenate methyl and other fungicides against grey mould disease in number of crops well documented in literature by various workers (Stall 1965; Grewal 1982; Burcak 1998; Delen *et al.,* 2004; Zitter and Wilcox, 2006; Singh *et al.,* 2008; Koycu *et al.,* 2012). These results corroborate the findings of Hosen (2010) and Hosen (2011) that iprodione and carbendazim was effective in inhibition of spore germination *B. cinerea.* The reduction in disease severity by iprodione + carbendazim, difenaconazole and hexaconazole are in consonance with the findings of Madhu Meeta *et al.*, 1986; Agarwal and Tripathi, 1999.

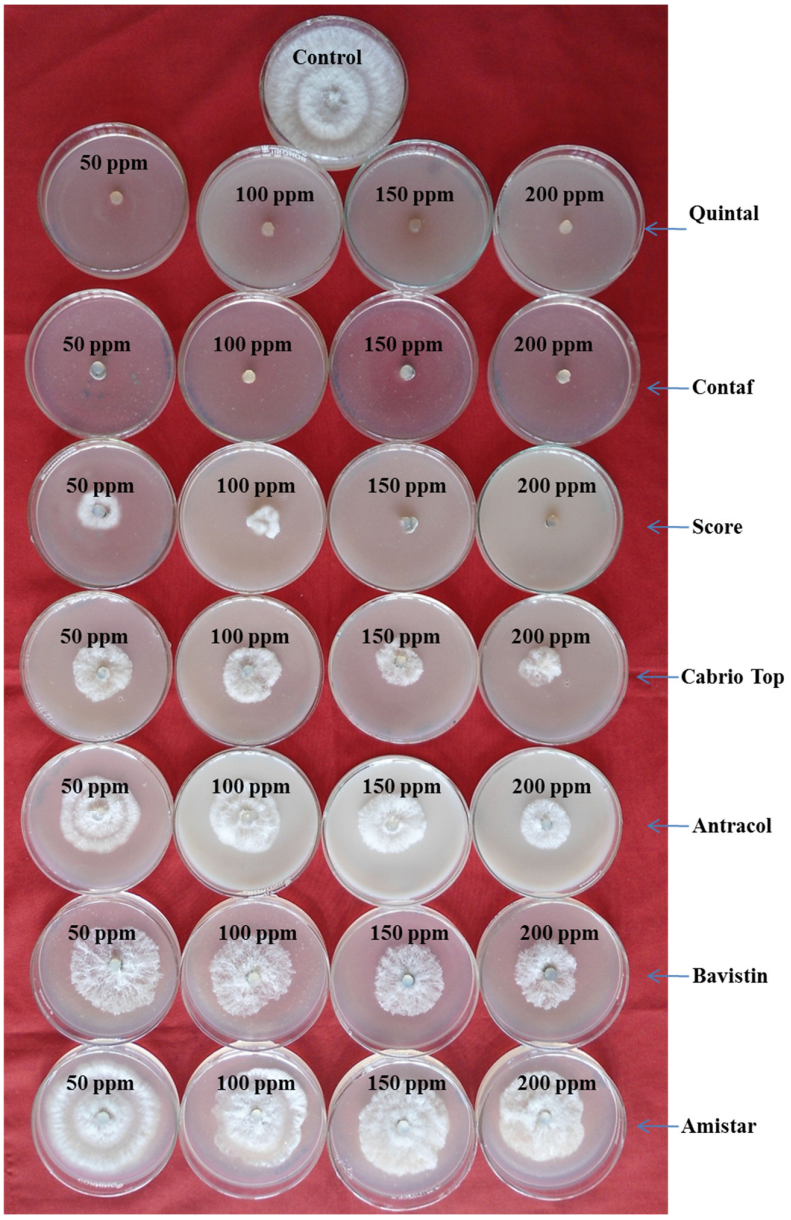

Figure 1. Mycelial growth of *B. cinerea* at different concentrations of chemicals

The effectiveness of combi product (systemic and non-systemic) can be explained as either or both of its constituents are from effective fungicide, and thus exhibiting excellent protective, curative, and eradicative, and post infection activity to restrict mycelial growth as well inhibition of the formation and conidial germination. Rashid *et al.* (2016) showed the superiority of combination formulations involving both protectants and

systemic fungicides over the sole application of either fungicide separately. Higher disease control in carbendazim comb products is attributed due to its effect on inhibiting the "two track" type of mitosis which is common in ascomycetes, and disrupting of mitosis, through the inactivation of spindle formation composed of microtubules (Hammerschlag and Sisler, 1972; Davidse, 1986).

Antifungal activity of plant oils

In vitro assessment of all 15 essential oils was done against *Botrytis cinerea* using the Poisoned Food Technique, and the data was documented and represented in Table 3. The data showed that significantly maximum (100%) percent growth inhibition was observed in *Mentha piperita, Cymbopogon martini, Pelargonium graveolens, Cymbopogon* sp., and *Oreganum vulgare,* respectively, with no growth of mycelium in all trialed concentrations. While, 89.67% growth inhibition was shown by *Syzygium aromaticum* plant oils, whereas no inhibition of mycelium growth was observed in *Olea europea, Cedrus deodara,* and *Brassica juncea* (Figure 2). Stavropoulao *et al.* (2014) and Soylu *et al.* (2010) confirmed the antifungal activity of essential oils derived from oregano (*O. vulgare* L. f. sp. *hirtum*), thyme (*T. vulgaris* L.), and lemon (*Citrus limon* L.) plants, against some important postharvest pathogens (*B. cinerea*, *Penicillium italicum*, and *Penicillium digitatum*).

Table 3. *In vitro* evaluation of plant oils against *B. cinerea*

Plant oils	Mycelial growth (mm)					Mycelial growth inhibition (%)				
	200 ppm	500 ppm	700 ppm	1000 ppm	Mean	200 ppm	500 ppm	700 ppm	1000 ppm	Mean
Mentha piperita L.	0.00	0.00	0.00	0.00	0.00	100.00 (89.69)	100.00 (89.69)	100.00 (89.69)	100.00 (89.69)	100.00 (89.69)
Cymbopogon martini Roxb.	0.00	0.00	0.00	0.00	0.00	100.00 (89.69)	100.00 (89.69)	100.00 (89.69)	100.00 (89.69)	100.00 (89.69)
Pelargonium graveolens L.	0.00	0.00	0.00	0.00	0.00	100.00 (89.69)	100.00 (89.69)	100.00 (89.69)	100.00 (89.69)	100.00 (89.69)
Cymbopogon sp. L.	0.00	0.00	0.00	0.00	0.00	100.00 (89.69)	100.00 (89.69)	100.00 (89.69)	100.00 (89.69)	100.00 (89.69)
Oreganum vulgare L.	0.00	0.00	0.00	0.00	0.00	100.00 (89.69)	100.00 (89.69)	100.00 (89.69)	100.00 (89.69)	100.00 (89.69)
Syzygium aromaticum (L.) Merrill & Perry	14.67	9.00	6.67	5.67	9.00	83.33 (65.88)	89.67 (71.25)	92.34 (73.90)	93.34 (75.04)	89.67 (71.51)
Rosemarinus officinale L.	74.67	47.00	43.33	23.67	47.17	15.33 (23.03)	46.00 (42.68)	51.67 (45.93)	73.33 (58.88)	46.58 (42.63)
Ocimum sanctum L.	82.67	67.00	54.00	43.67	61.84	8.73 (17.18)	25.33 (30.20)	40.00 (39.21)	53.00 (46.70)	31.77 (33.32)
Azadirachta indica A. Juss.	84.67	78.00	63.33	46.73	68.18	7.67 (16.06)	12.67 (20.83)	29.33 (32.76)	50.33 (45.17)	25.00 (28.70)
Mentha arvensis L.	88.67	84.33	81.67	78.33	83.33	1.33 (6.53)	5.67 (13.72)	8.33 (16.76)	12.67 (20.83)	7.00 (14.46)
Eucalyptus globulus Labill	89.00	86.00	83.33	80.00	84.60	1.00 (5.73)	4.00 (11.47)	7.33 (15.69)	10.00 (18.42)	5.58 (12.83)
Brassica juncea L.	90.00	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Cedrus deodara Trew	90.00	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

Prunus amygdalustimes (Mill.) D.A. Webb	90.00	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Olea europa Linn.	90.00	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Control	90.00	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	52.27	51.33	48.90	45.50		39.59 (22.40)	42.71 (31.69)	45.56 (37.37)	49.54 (44.17)	
CD $_{0.05}$	Plant oils (P.O) = 0.84 Concentration = 0.69 P.O. x C = 1.69									

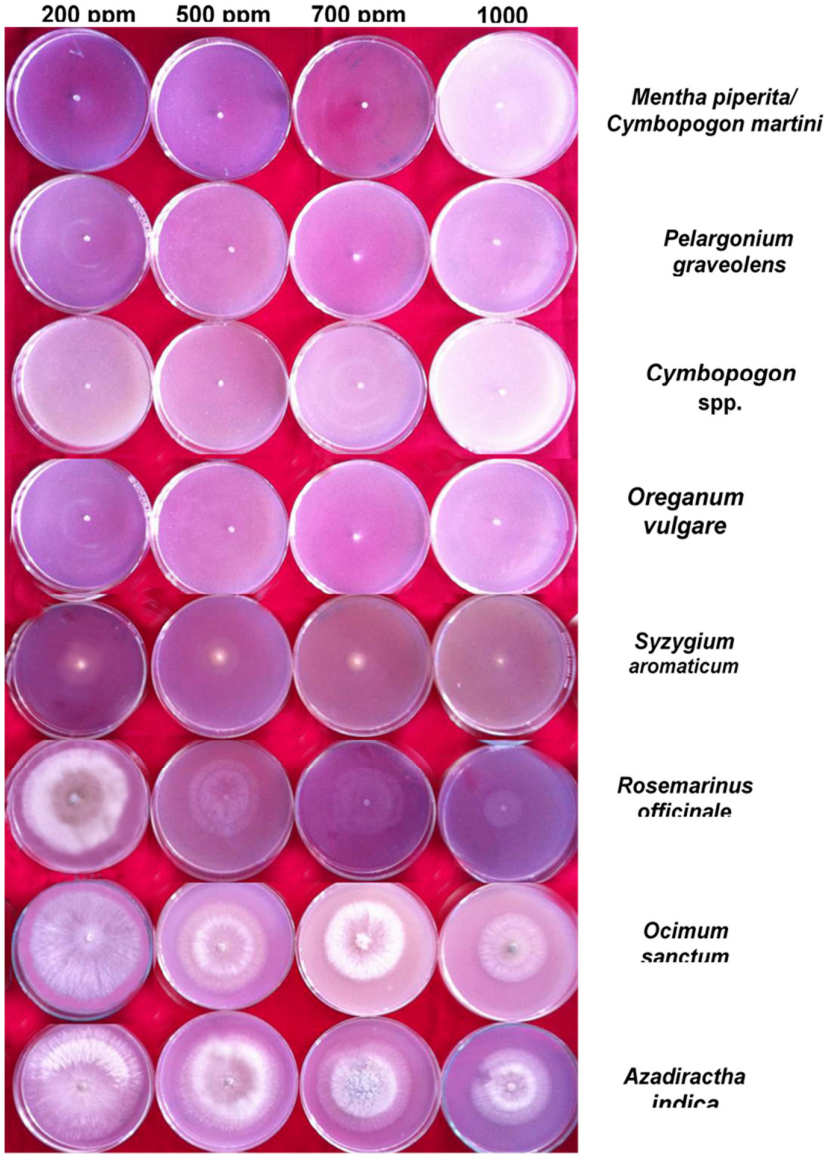

Figure 2. Mycelial growth of *B. cinerea* at different concentrations of chemicals

These results are in consonance with the findings of several scientist, including Camili *et al.* (2010) and Adebayo *et al*. (2013) who reported that the essential oil of *Thymus vulgaris* controlled fruit rot caused by *Botrytis cinerea*, *Phytophthora citrophthora,* and *Rhizopus stolonifer*. Abdolahi *et al.* (2010) reported that thyme essential oils exhibited strong antifungal activity against *Botrytis cinerea* and *Mucor piriformis.* According to Klaric *et al.* (2007) the thyme essential oil, which contains p-cymene (36.5%), thymol (33.0%), and 1,8-cineole (11.3%) as main components, and pure thymol exhibited antifungal activities. Oregano and lemon essential oils showed significant activity against *B. cinerea*. Di Vaio *et al.* (2010) reported that the main component in the lemon essential oil was limonene, accounting for a 72.5-76.4 percent reduction in mycelial growth of *Botrytis cinerea*, followed by β-pinene (11.6-18.7%).

Antifungal activity of bioresources

The efficacy of twelve plant origin biopesticide were evaluated, and the data on % growth inhibition of mycelium of *Botrytis cinerea* by various extracts of plants exhibited that 57.39% was shown by the aqueous extract of cloves of *Allium sativum* L. followed by the leaves of *Azadirachta indica* L. with 45.47% growth inhibition at all tested concentrations (Table 4 and Figure 3). The *Euphorbia halicoscopia*, sour butter milk and vermiwash showed no inhibition of growth at all tested concentrations. The popularity of botanical pesticides is once again increasing and some plant products are being used globally as green pesticides (Gurjer *et al.,* 2012).

Table 4. *In vitro* evaluation of bioresources against *B. cinerea*

Bioresources	Mycelial growth (mm)				M Mean	Mycelial growth inhibition (%)				Mean
	20	50	75	100		20	50	75	100	
A. Botanicals										
Allium sativum L.	62.00	48.00	33.33	15.33	**39.67**	31.03 (37.47)	46.66 (43.07)	62.92 (52.47)	82.96 (65.60)	**57.39 (49.65)**
Azadirachta indica L.	56.67	49.33	46.67	39.00	47.92	31.11 (33.88)	45.18 (42.22)	48.14 (43.92)	56.66 (48.81)	45.47 (42.20)
Murraya koengii L.	66.00	62.33	55.67	43.33	56.83	26.66 (31.07)	30.73 (33.65)	38.14 (38.12)	52.00 (46.13)	36.88 (37.24)
Melia azedarach L.	80.00	71.00	56.33	46.33	63.42	11.11 (19.45)	21.11 (27.34)	37.40 (37.68)	48.51 (44.13)	29.53 (32.14)
Spindus laurifolia L.	90.00	85.33	65.00	56.33	74.17	0.00 (0.00)	5.19 (13.13)	27.78 (31.79)	37.40 (37.69)	17.59 (20.65)
Vitex nigundo Linn.	90.00	5.00	3.00	63.33	7.83	0.00 (0.00)	5.55 (13.58)	18.88 (25.74)	29.66 (32.96)	13.52 (18.06)
Adathoda vesica L.	90.00	0.00	2.00	77.00	4.75	0.00 (0.00)	0.00 (0.00)	4.67 (12.12)	14.44 (22.32)	4.78 (8.60)
Eucalyptus globules L.	90.00	0.00	8.00	80.67	7.17	0.00 (0.00)	0.00 (0.00)	2.00 (7.95)	10.07 (18.44)	3.02 (6.59)
Euphorbia halioscopia L.	90.00	0.00	0.00	90.00	0.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
B. Bioproducts										
Cow urine	90.00	87.33	76.33	65.00	79.67	0.00 (0.00)	2.97 (9.86)	15.18 (22.92)	27.77 (31.78)	11.48 (16.14)
Sour butter	90.00	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Vermiwash	90.00	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Control	90.00	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
$CD_{0.05}$ Bioresources(B) = 0.73 Concentration (C) = 0.40 B × C = 1.44										

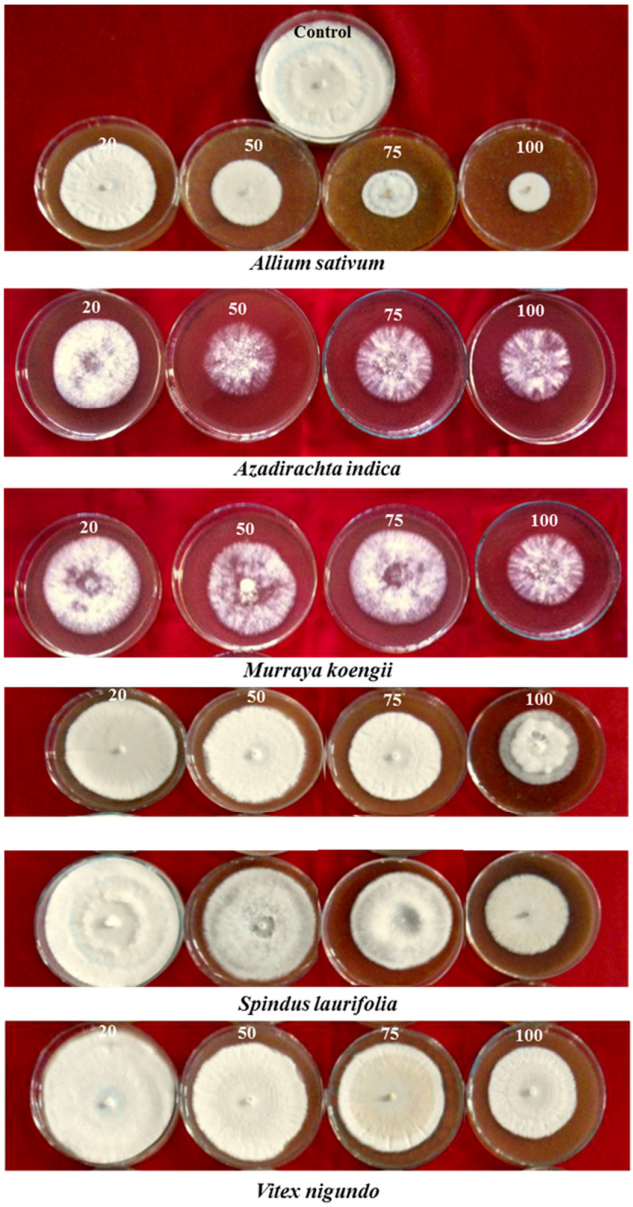

Figure 3. Mycelial growth of *B. cinerea* at different concentrations of chemicals

Plants have the ability to synthesize aromatic secondary metabolites like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins, and coumarins (Cowan, 1999). The components with phenolic structures, like carvacrol, eugenol, and thymol were highly active against the pathogen. These groups of compounds show antimicrobial effects and serve as plant defense mechanisms against an array of pathogenic microorganisms (Das *et al.,* 2010). Allicin has antibacterial, antiviral, antitumor, anticoagulation, antihypertensive, antiparasitic, and hepatoprotective effects. It is also efficient against many fungal species, such as *Aspergillus flavus, A. niger, Candida albicans, Fusarium laceratum, Microsporum canis, Mucor racemosus, Penicillium* spp.*, Rhizopus nigricans, Saccharomyces* spp.*, Trichophyton granulosum* and *Botrytis cinerea* (Josling, 2003; Parvu, 2010). The volatile antimicrobial substance allicin (diallyl thio sulfinate) is synthesized in garlic when the tissues are damaged and the substrate alliin (S-allyl-L-cysteine sulfoxide) mixes with the enzyme alliin-

lyase. Allicin is readily membrane-permeable and undergoes thiol-disulfide exchange reactions with free thiol groups in proteins.

Neem (*Azadirachta indica* Juss), a large tree of India, has been used for centuries in Asia as insecticides, fungicides and anticonceptional in popular medicine. Almost every part of this tree like seeds, leaves, roots, bark, trunk, and branches has multiple uses and has been recommended for planting in Africa and Asia by many international organizations. The bioactivity of neem extract has been attributed to various compounds found in the seed and leaf such as nimbin, nimidin and salannin, but most of the compound is azadiractin. Some extracts from neem plant have been shown to be toxic to fungal pathogens (Dhyani *et al.,* 2004), *Aspergillus flavus* from the soybean seeds (Krishnamurthy *et al.,* 2008), *Pyricularia oryzae* infecting rice plants in the field and the harvested rice (Amadioha, 2000). Natural pesticides represent a sound alternative to the use of synthetic fungicides, as they generally have fewer damaging effects on the environment and human health (Wilson, 1997).

Integrated management of grey mould with fungicides, bioresources and plant oils (in vivo)

The testing of most potent fungicides and botanicals viz., Avtar, Contaf, and Quintal together with neem plant oil were evaluated at various concentrations against *Botrytis cinerea* in the field, and the data on severity of disease and disease reduction are represented in the Table 5. The cloves of *Allium sativum* L. showed 57.39% followed by the leaves of *Azadirachta indica* L. showed 45.47% growth inhibition at all tested concentrations. Although higher concentrations of 75 and 100% resulted in maximum growth inhibition of the fungal mycelium. At all concentrations, vermiwash, sour butter milk, and *Euphorbia halicoscopia* showed no growth reduction. Against *Botrytis cinerea,* the *Euphorbia halioscopia* L. was found to be the least effective among all the botanicals.

In Table 5, the presented data clearly depicted that in the study of two years, the severity of disease vary between 1.33-20.67% and 1.99-22.07%, respectively, in all the combination treatments as compared to the control in which the severity of disease was 28.00 and 29.33% respectively. All documented combinations of fungicides, neem oil, and botanicals were highly efficient in field conditions against grey disease. However, the combination of Quintal + *Allium sativum* + neem oil exhibited a maximum reduction in disease 94.40% followed by the combination of Contaf + *Allium sativum* + neem oil that showed 93.19% disease reduction. The treatment combinations viz., Quintal+ *Azadirachta indica* + neem oil showed 84.19% and Contaf + *Azadirachta indica* + neem oil 81.36% reduction in disease followed by the combination treatment of Quintal + *Murraya koengii* + neem oil (79.62%) and Contaf + *Murraya koengii* + neem oil (77.67%) against the grey mould disease of gladiolus. However, the use of botanicals along with neem oil were found to be the least efficient against *Botrytis cinerea* under field conditions.

Table 5. Integrated management of grey mould with fungicides, botanicals and plant oil *(in vivo)*

Fungicides + Botanicals + oil	Conc. (%)	Disease Severity (%)			
		2018	2019	Pooled	Disease Reduction (%)
Quintal + *Allium sativum* + Neem oil	0.25+0.10+0.05	1.33 (1.52)	1.99 (1.73)	1.66 (1.62)	94.40
Quintal + *Azadirachta indica* +Neem oil	0.25+0.10+0.05	4.33 (2.30)	4.74 (2.39)	4.53 (2.35)	84.19
Quintal +*Murraya koengii*+ Neem oil	0.25+0.10+0.05	5.67 (2.58)	6.00 (2.64)	5.84 (2.61)	79.62
Contaf + *Allium sativum* + Neem oil	0.03+0.10+0.05	1.83 (1.68)	2.07 (1.74)	1.95 (1.71)	93.19
Contaf + *Azadirachta indica* + Neem oil	0.03+0.10+0.05	5.00 (2.45)	5.66 (2.58)	5.34 (2.51)	81.36
Contaf + *Murraya koengii* + Neem oil	0.03+0.10+0.05	6.11 (2.67)	6.70 (2.78)	6.40 (2.70)	77.67
Avtar+ *Allium sativum* + Neem oil	0.25+0.10+0.05	10.00 (3.31)	11.33 (3.51)	10.66 (3.41)	62.80
Avtar + *Azadirachta indica* + Neem oil	0.25+0.10+0.05	12.18 (3.62)	12.67 (3.69)	12.42 (3.66)	56.67
Avtar + *Murraya koengii* + Neem oil	0.25+0.10+0.05	13.22 (3.78)	13.92 (3.86)	13.57 (3.81)	52.65
Allium sativum + *Murraya koengii* + *Azadirachta indica* + Neem oil	0.10+0.10+0.10++0.05	20.67 (4.65)	22.07 (4.95)	21.37 (4.75)	26.87
Bavistin (standard check)	0.05	17.33 (4.28)	18.00 (4.35)	17.67 (4.31)	38.34
Control		28.00 (5.38)	29.33 (5.50)	28.66 (5.45)	
CD $_{0.05}$	Fungicides = 0.15 Year = 0.07 Treatments × Year = 0.24				

*Figures in parentheses are square root transformed value

Integrated disease management with fungicides, botanicals and plant oil

Integrated disease management (IDM) can simply be defined as a strategy or system that combines all available methods to ensure the healthy growth of crop plants so that they produce high yields (Youdeowei, 2004). The combination of fungicides along with botanicals and the neem oil have the capability to boost the growth factors like plant height (cm), yield of corms, spike length (cm), number of florets and number of flowers per plot in gladiolus during the two succeeding years (2018 and 2019), respectively (Table 6 and Figure 4). Among the tested treatment combinations, Quintal + *Allium sativum* + neem oil considerably improved the plant height (70.34 cm), yield of corms (38.00), spike length (53.17 cm), number of florets (15.65) and number of flowers (32.67) followed by Contaf + *Allium sativum* + neem oil up to the extent of 69.67 cm as plant height, yield of corms (36.00), spike length (52.50 cm), number of florets (15.19) and number of flower (31.84) as compared to control treatment where 40.50 cm was recorded plant height, and other factors like yield of corms (10.33), spike length (29.00 cm), number of florets (5.84) and number of flower (9.00) remained considerably less improved.

Plants are attacked by various phytopathogenic fungi. For many years, synthetic fungicides have been used to control plant diseases. Although synthetic fungicides are highly effective, their repeated use has led to problems such as environmental pollution, development of resistance, and residual toxicity. This has prompted intensive research on the development of biopesticides, including botanical fungicides (Yoon *et al.,* 2013). Laminarin (also known as laminaran) is a storage glucan (a polysaccharide of glucose) found in the blue green algae *Laminaria digitata* its application reduced infection by *B. cinerea* and *Peronospora viticola* by approximately 55 and 75 percent, respectively, as reported by Copping (2004) and Liangbin *et al*. (2010).

Table 6. Integration of fungicides with botanicals and plant oil on the plant growth parameters of gladiolus under field condition

Treatments	Conc. (%)	Plant Height (cm)			No. of flowers/plot			No. of florets/ plant			Spike length (cm)			Yield of corms/plot		
		2018	2019	Pooled	2018	2019	Pooled	2018	2019	Pooled	2018	2019	Pooled	2018	2019	Pooled
Quintal + *Allium sativum* + Neem oil +	0.25 + 0.10 + 0.05	71.00	69.67	70.34	33.33	32.00	32.67	16.00	15.00	15.65	53.67	52.67	53.17	38.00	38.00	38.00
Quintal+ *Azadirachta indica* + Neem oil	0.25+ 0.10 + 0.05	69.33	68.67	69.00	31.00	30.33	30.67	15.00	14.00	14.82	52.00	50.67	51.34	37.00	37.00	37.00
Quintal + *Murraya koengii* + Neem oil	0.25 +0.10 + 0.05	67.67	67.67	67.67	29.67	28.00	28.84	14.00	13.00	13.82	51.33	49.67	50.50	35.00	35.00	35.00
Contaf + *Allium sativum* + Neem oil	0.03 +0.10 + 0.05	70.33	69.00	69.67	32.67	31.00	31.84	16.00	14.00	15.19	53.33	51.67	52.50	36.00	36.00	36.00
Contaf + *Azadirachta indica* + Neem oil.	0.03+ 0.10 + 0.05	68.00	67.33	67.67	30.67	29.00	29.84	14.00	13.00	13.84	51.67	49.33	50.50	35.00	35.00	35.00
Contaf + *Murraya koengii* + Neem oil	0.03+ 0.10 + 0.05	66.67	65.33	66.00	30.00	27.33	28.67	13.00	12.00	12.67	50.33	47.67	49.00	33.33	33.33	33.33
Avtar + *Allium sativum*+ Neem oil	0.2+ 0.10 + 0.055	63.67	62.67	63.17	27.00	25.00	26.00	12.00	11.00	11.67	48.00	45.67	46.84	31.33	31.33	31.33
Avtar + *Azadirachta indica* + Neem oil	0.25+ 0.10 + 0.05	60.67	61.67	61.17	26.00	23.00	24.50	11.00	11.00	11.00	46.67	44.67	45.67	29.00	26.00	27.50
Avtar + *Murraya koengii* + Neem oil	0.25 +0.10 + 0.05	58.33	57.33	57.83	25.67	22.00	23.84	10.00	9.00	9.82	46.33	41.67	44.00	17.67	17.67	17.67
Allium sativum + *Azadirachta indica* + *Murraya koengii* + Neem oil	0.10+0.10+ 0.10+0.05	50.00	48.00	49.00	17.33	14.00	15.67	8.00	7.00	7.85	39.00	36.33	37.67	16.00	16.00	16.00
Bavistin (Standard Check)	0.05	53.67	51.67	52.67	20.67	16.00	18.34	9.00	8.00	8.67	41.00	38.67	39.84	19.00	19.00	19.00
Control		45.67	35.33	40.50	9.33	8.67	9.00	6.00	5.00	5.84	30.00	28.00	29.00	10.33	10.33	10.33
Mean		62.08	60.66		26.11	23.86	24.99	12.13	11.33		46.94	44.72		28.14	27.89	28.01
$CD_{0.05}$		Treatments (T) =1.97 Plant Height (P.H.) = 0.80 T X P.H.= 2.78			Treatments (T) =0.74 No. of Flower = 0.32 TX No. of flowers =1.05			Treatments (T) = 0.76 No. of florets= 0.31 TX No. of florets = 0.92			Treatments (T) =1.46 Spike length = 0.59 TX Spike length = 0.70			Treatments (T)= 0.58 Yield = 0.23 TX Yield = 0.82		

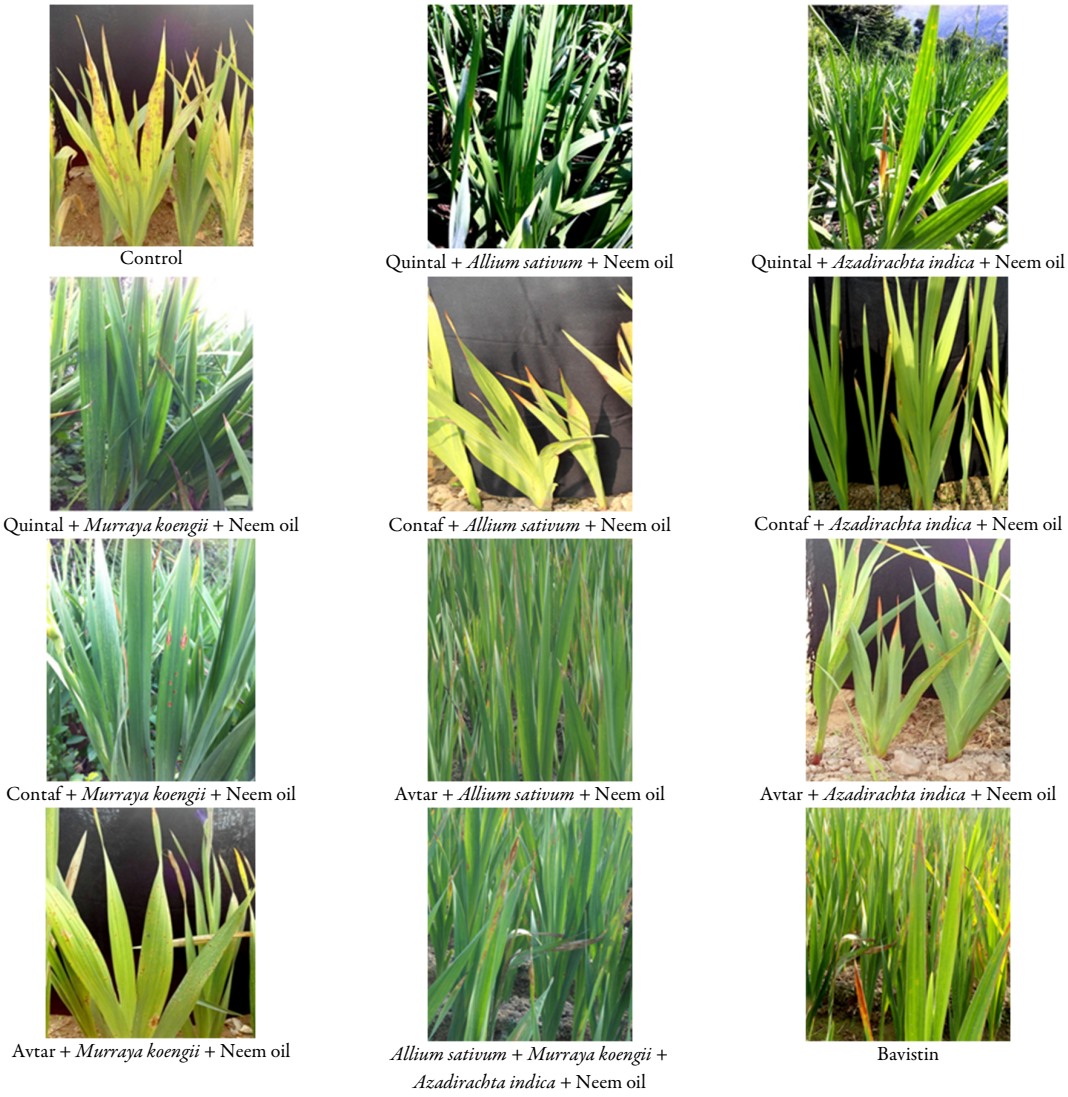

Figure 4. Integration of fungicides with botanicals and plant oil on the plant growth parameters of gladiolus under field condition

In the last few decades *Botrytis cinerea* has been embraced as a model organism in molecular plant pathology. Driven by the enormous economic importance of this fungi worldwide and its special infection strategy, academic and industrial research groups have joined together to resolve the biology of this necrotrophic pathogen, and find out its potential weaknesses for development of new management strategies. Natural plant-based protectants with antimicrobial activity have been because of their less impact on environment and higher public acceptance (Shao *et al.,* 2013). At the same time, the integrated use of fungicides and botanical based preparations can be useful alternative method against botrytis grey mold pathogen. On contrary, these strategies would enhance the chemical activity of modern preparations at very low concentrations and they would lessen the risk of the appearance against resistant pathogen strains (Ajmad *et al.,* 2023).

Conclusions

The *Botrytis* genus belongs to the Sclerotiniaceae family, which consists of different species of fungi that cause fatal damage to agricultural fields. In particular, the *Botrytis* contains approximately 30 recognized species with variable trophic lifestyles and is placed among the top ten most important fungal pathogens. *B. cinerea* species is the best-studied member of its genus, which shows a facultative secretive endophytic behavior ('hide and seek'). Here, we report the best fungicide, botanical and plant oils that inhibit the growth of *B. cinerea* at different concentrations in *in vitro* as well as at the field level.

Authors' Contributions

G.K. and H.N conducted the experiments. P.G., S.S., and P.O. designed the methodology, V.S and S.C. analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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