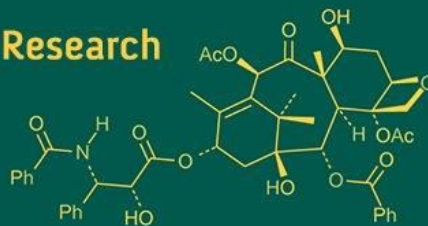
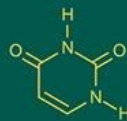
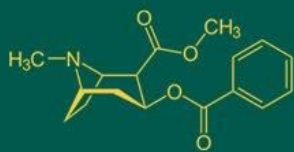


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Assessment of fungicides, phyto extracts and bioagents against grey mold of strawberry incited by *Botrytis cinerea* Pers

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Abstract

Strawberry is one of the most important crop of temperate and sub tropical regions. The grey mold disease caused by fungus *Botrytis cinerea* is a serious strawberry disease that causes significant yield loss. In the present study fungicides, phyto-extracts and bioagents were tested against grey mold of strawberry caused by *Botrytis cinerea* under *in vitro* conditions. The observations revealed that among the fungicides the maximum growth inhibition was observed in Hexaconazole (100%) and Carbendazim (100%) at all the concentrations of 50, 100, 150, and 200 ppm. Among the phyto- extracts at the concentration of 5% and 10% the maximum growth inhibition was observed in garlic phyto extract *i.e.*, 68.05% and 71.66% respectively. At the higher concentration of 15% and 20% neem extract showed maximum growth inhibition *i.e.*, 78.61% and 82.27% respectively. Among the bioagents the maximum growth inhibition was recorded in *Trichoderma harzianum* which was 64.16%.

Keywords: Bioagents, *Botrytis cinerea*, fungicides, grey mold, phyto extract

Introduction

The strawberry, (*Fragaria × ananassa*) Duch., is a member of the Rosaceae family and is considered as one of the world's most beautiful and refreshing fruits. The fruits of this plant are abundant in bioactive phytochemicals, particularly phenolic compounds with a high antioxidant capacity. These fruits also contain vitamins and minerals, contributing to a regular diet's overall health benefits. Strawberries are the rich source of vitamin C, they also contain a high amount of potassium. Several diseases caused by bacteria, viruses, and fungi harm strawberry cultivation, which results in yield loss. Some important diseases of strawberries are powdery mildew caused by *Sphaerotheca fragariae*, leaf spot caused by *Mycosphaella fragariae*, Colletotrichum rot caused by *Colletotrichum* spp., angular leaf spot caused by *Xanthomonas fragariae*, grey mold caused by *Botrytis cinerea*, etc.

Due to its broad host range, the fungus *Botrytis cinerea* is highly prevalent as a pre and post-harvest disease in various crops. Based on its scientific and economic significance, the fungus *B. cinerea* is ranked second in the world's top 10 pathogens, (Dean *et al.*, 2012) [3]. Approximately 200 or more dicotyledonous crop species worldwide are estimated to be hosts for *B. cinerea* (Williamson *et al.*, 2007) [14]. Fruit gets attacked by *Botrytis cinerea* at any point. The infected fruit has a light brown patch that initially gets tender and then gets dry. In the early stage of the infection, a darker circular area is visible where the fruit tissues are softer than the other fruit parts, subsequently abundant sporification, whose colour ranges from white to grey, depending on the quantity of light, can develop from the site of infection. Conidia have a smooth, hyaline or grey appearance, and are shaped like eggs. (Romanazzi and Feliziani, 2014) [10]. Fruits with high moisture content exhibit the fungus's white growth, eventually turning grey. Overripe fruits are more susceptible to the pathogenic infection.

The present study was conducted to evaluate the effectiveness of fungicides, phyto-extracts, and bioagents against *Botrytis cinerea*, the causative agent of grey mold in strawberries.

Materials and Methods

Isolation of pathogen: The diseased sample was collected from the Fruit Block, College of Horticulture, Bharsar, Pauri Garhwal. For the isolation of pathogen, the first step was to cut the infected strawberry sample into 5mm pieces with the help of a sterilized scalpel.

Then the sample was washed with distilled water and HgCl₂ solution. Then, under the sterilized conditions in laminar air flow cabinet the small pieces of diseased samples were placed on PDA media in Petri Plates and incubated at 25±2° C temperature in BOD incubator. The incubated plates were observed periodically for mycelial growth of fungus. Developed colonies from the culture were observed for identification. The microscopic observations were also done to analyse the morphological characteristics of the pathogen for identification. The sub-culturing of the pathogen was done for purification of the pathogen and slant cultures were also prepared and maintained at 4° C in the refrigerator for further studies.

Evaluation of different fungicides: Evaluation of six fungicides was done against *Botrytis cinerea* under *in vitro* conditions. The fungicides that were used were Mancozeb, Difenconazole, Hexaconazole, Carbendazim, Propiconazole and Thiophanate Methyl. The Poisoned Food technique was used to study the effect of fungicides against the pathogen. The desired concentration of fungicides was added to the 100 mL of molten PDA separately under aseptic conditions in the sterilized conical flask. To ensure proper mixing of fungicides and PDA the flasks were shaken gently. 20 mL of PDA was then poured into the sterilized petri plates. After the solidification of PDA media, the mycelial disc of 5 mm from the edges of seven days old culture was taken with the help of a cork borer and placed in the middle of petri plates and incubated at temperature of 25±2° C. The culture discs which were grown under same conditions without fungicides, were kept as control. The mycelial growth was observed after seven days of incubation. Per cent mycelium growth inhibition was calculated by using the formula suggested by Vincent (1947)^[13].

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

Where, PGI= Per cent growth inhibition

C= Mycelial growth in control

T= Mycelial growth in treatment

Evaluation of different phyto-extracts: Six different phyto-extracts *i.e.* Aloe vera, Tulsi, Neem, Lantana, Garlic and Datura were used against the pathogen. For the preparation of phyto-extracts plant material was crushed with distilled water in a 1:1 (W/V) ratio with the help of mortar and pestle. Then the mixture was filtered with the muslin cloth and then by Whatman no. 1 filter paper. The efficacy of phyto extracts was tested by Poisoned Food technique. The Petri plates were incubated at temperature of 25±2° C. The mycelial growth was recorded after seven days of incubation. The per cent mycelium growth inhibition was calculated by formula suggested by Vincent (1947)^[13].

Evaluation of different bioagents: Six bioagents *i.e.* *Trichoderma harzianum*, *T. viride*, *T. hamatum*, *Bacillus subtilis*, *Pseudomonas fluorescens-2* and *P. fluorescens-4* were tested against the *Botrytis cinerea* by using dual culture technique. In this technique, the bioagent and test fungus was placed at opposite ends of each other in petri plate and incubated at temperature of 25±2° C. The mycelial growth was recorded after seven days of incubation.

Statistical analysis

The data obtained was analysed by using simple Completely Randomized Design (CRD) with the help of OPSTAT.

Results and Discussion

Efficacy of fungicides: Six fungicides were used against *Botrytis cinerea* at concentrations of 50, 100, 150 and 200 ppm.

Table 1: Effect of Fungicides on per cent mycelial growth inhibition of *B. cinerea* at different concentrations

Treatments	Average per cent growth inhibition			
	Concentration (ppm) ± S.E. (m)			
	50	100	150	200
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mancozeb	43.05 (40.98)	49.72 (44.82)	55.83 (48.33)	64.16 (53.20)
Difenconazole	64.16 (53.21)	69.44 (56.42)	77.22 (61.47)	85.27 (67.42)
Hexaconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Carbendazim	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Propiconazole	81.66 (64.63)	89.44 (71.03)	92.49 (74.12)	100.00 (90.00)
Thiophanate Methyl	49.72 (44.82)	60.83 (51.23)	69.44 (56.42)	79.72 (63.22)
S.E(d)	0.82 (0.51)	0.75 (0.49)	0.79 (0.60)	0.64 (0.46)
CD (0.05)	1.73 (1.07)	1.58 (1.04)	1.66 (1.26)	1.35 (0.97)

() = Values in parentheses are angular transformed

At 50 ppm concentration, maximum per cent mycelial growth inhibition was observed in Hexaconazole (100%) and Carbendazim (100%) followed by Propiconazole (81.66%), Difenconazole (64.16%) and Thiophanate Methyl (49.72%). Minimum per cent mycelial growth inhibition was recorded in Mancozeb *i.e.* 43.05%.

At 100 ppm concentration, maximum per cent mycelial growth inhibition was observed in Hexaconazole (100%) and Carbendazim (100%) followed by Propiconazole (89.44%), Difenconazole (69.44%) and Thiophanate Methyl (60.83%). Minimum per cent mycelial growth inhibition was recorded in Mancozeb *i.e.* 49.72%.

At 150 ppm concentration, maximum per cent mycelial growth inhibition was observed in Hexaconazole (100%) and Carbendazim (100%) followed by Propiconazole (92.49%), Difenconazole (77.22%) and Thiophanate Methyl (69.44%). Minimum per cent mycelial growth inhibition was recorded in Mancozeb *i.e.* 55.83%.

At 200 ppm concentration, maximum per cent mycelial growth inhibition was observed in Hexaconazole (100%), Carbendazim (100%) and Propiconazole (100%) followed by Difenconazole (85.27%) and Thiophanate Methyl (79.72%). Minimum per cent mycelial growth inhibition was recorded in Mancozeb *i.e.* 64.16%.

The experimental results revealed that the Hexaconazole and Carbendazim completely inhibit the growth of *Botrytis cinerea* *i.e.* 100% at all the concentrations (50, 100, 150 and 200 ppm). Meanwhile, minimum inhibition was recorded in Mancozeb at all concentrations.

Kaur *et al.*, 2023^[7] in their experimental results found that Iprodione + Carbendazim and Hexaconazole at the concentrations of 50, 100, 150 and 200 ppm completely

inhibit the growth of *Botrytis cinerea* (100%) under *in vitro* conditions. The 100% inhibition by Carbendazim was recorded by Manjunath *et al.*, 2023 against the pathogen *Ascochyta rabiei* and *Botrytis cinerea* at 100, 200, 500 ppm concentrations.

Efficacy of Phyto-extracts: Six Phyto-extracts from different plants were used against the *Botrytis cinerea* to varying concentrations of 5, 10, 15 and 20%.

At 5% concentration, maximum per cent mycelial growth inhibition was observed in Garlic (68.05%) followed by Datura (67.50%), Neem (59.72%) and Aloe vera (58.61%). Minimum per cent mycelial growth inhibition was recorded in Tulsi (35.00%) followed by Lantana (36.94%).

At 10% concentration, maximum per cent mycelial growth inhibition was observed in Garlic (71.66%) followed by Datura (69.16%), Neem (63.88%) and Aloe vera (63.88%). Minimum per cent mycelial growth inhibition was recorded in Tulsi (37.22%) followed by Lantana (44.72%).

At 15% concentration, maximum per cent mycelial growth inhibition was observed in Neem (78.61%) followed by Garlic (74.16%), Datura (70.55%) and Aloe vera (65.27%). Minimum per cent mycelial growth inhibition was recorded

in Tulsi (40.83%) followed by Lantana (48.33%). At 20% concentration, maximum per cent mycelial growth inhibition was observed in Neem (82.27%) followed by Garlic (81.94%), Datura (71.38%) and Aloe vera (66.38%). Minimum per cent mycelial growth inhibition was recorded in Tulsi (47.78%) followed by Lantana (51.66%). The findings of Jatoi *et al.*, 2022 [6] revealed that neem at a concentration of 15% significantly reduces mycelial growth (23.33mm). Garlic has antimicrobial compounds like allicin. Allicin (diallyl-thiosulfinate) is produced when garlic cloves are crushed, and alliin-an organosulfur precursor is transformed into allicin (Gruhlke *et al.*, 2010 and Stoll *et al.*, 1951) [5, 12].

Neem contains at least thirty-five biologically active principals of which nimbin and azadirachtin are the significant compounds which are present predominantly in the seeds, leaves and other parts of the neem tree (Zakawa *et al.*, 2018) [15]. The bioactivity of neem extract has been due to various bioactive compounds found in the seed and leaf such as nimbin, nimidin and salannin, but most of the compound is azadirachtin. Extracts from neem plant have been proved to be toxic against fungal pathogens (Dhyani *et al.*, 2004) [4].

Table 2: Effect of Phyto extracts on per cent mycelial growth inhibition of *B. cinerea* at different concentrations

Treatments	Average per cent growth inhibition			
	Concentration (per cent) $\pm$ S.E. (m)			
	5%	10%	15%	20%
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Aloe vera	58.61 (49.94)	63.88 (53.04)	65.27 (53.87)	66.38 (54.54)
Tulsi	35.00 (36.25)	37.22 (37.58)	40.83 (39.70)	47.78 (43.70)
Neem	59.72 (50.58)	63.88 (53.04)	78.61 (62.43)	82.27 (67.43)
Lantana	36.94 (37.41)	44.72 (41.95)	48.33 (44.02)	51.66 (45.93)
Garlic	68.05 (55.57)	71.66 (57.81)	74.16 (59.43)	81.94 (64.83)
Datura	67.50 (56.22)	69.16 (56.25)	70.55 (57.11)	71.38 (57.64)
S.E(d)	0.88 (0.53)	0.93 (0.55)	0.85 (0.53)	0.97 (0.65)
CD (0.05)	1.85 (1.11)	1.95 (1.16)	1.79 (1.11)	2.03 (1.36)

(=) Values in parentheses are angular transformed

Efficacy of Bioagents: Among the six bioagents *i.e.*, *Trichoderma harzianum*, *T.viride*, *T. hamatum*, *Bacillus subtilis*, *Pseudomonas fluorescens*-4, *P. fluorescens*-2 which were evaluated against the pathogen three were fungal bioagents and three were bacterial bioagents. The maximum per cent mycelial inhibition was recorded in *Trichoderma harzianum* (64.16%) followed by *Trichoderma viride* (60.55%), *Trichoderma hamatum* (55.27%) and *Bacillus subtilis* (54.16%). The minimum per cent mycelial growth was observed in *Pseudomonas fluorescens*-4 (34.16%) followed by *Pseudomonas fluorescens*-2 (36.39%). Khan *et al.*, 2011 under *in vitro* conditions isolated bioagents from seven commercial formulations out of which three formulations were of *T. harzianum* viz., PBAT-1, Biowilt- X and Tricodex which have shown the per cent mycelial inhibition of 72.2%, 70.5% and 67.5% respectively. *Trichoderma* spp. has a broad host range. *Trichoderma* spp. produces hydrolytic enzymes like cellulase and chitinase (Chaube and Pundir, 2009) [2] which help in inhibiting the growth of pathogen. A fungistatic metabolic compound gliotoxin is produced by the *T. viride* which has antimicrobial properties whose efficacy was tested by Brian and Hemming (1945) [1] against different pathogens. Among the bacterial bioagents the maximum inhibition was observed in *Bacillus subtilis* (54.16%). The *Bacillus* spp. contains metabolites like bacteriocins, lantibiotics, lipopeptides and

polyketides (Samsienenea and Otriz, 2011) which helps in controlling the growth of pathogen.

Table 3: Effect of bioagents on per cent mycelial growth inhibition of *B. cinerea*

Treatments	Average per cent growth inhibition(mm) $\pm$ S.E(m)
Control	0.00 (0.00)
<i>Trichoderma harzianum</i>	64.16 (53.21)
<i>Trichoderma viride</i>	60.55 (51.07)
<i>Trichoderma hamatum</i>	55.27 (48.01)
<i>Bacillus subtilis</i>	54.16 (47.37)
<i>Pseudomonas fluorescens</i> 4	34.16 (35.74)
<i>Pseudomonas fluorescens</i> 2	36.39 (37.08)
S.E(d)	0.98 (0.58)
CD (0.05)	2.05 (1.21)

(=) Values in parentheses are angular transformed

Conclusion

Among the fungicides, the maximum per cent growth inhibition was found in Hexaconazole and Carbendazim at concentrations of 50, 100, 150, 200 ppm. Meanwhile, minimum per cent growth inhibition was recorded in Mancozeb at all the concentrations. Among the phyto-extracts, the maximum per cent growth inhibition was recorded in Garlic extract at 5% and 10% concentrations. At

the higher concentrations of 15% and 20% the maximum per cent growth inhibition was recorded in the Neem phyto-extract. Minimum per cent growth inhibition was recorded in the Tulsi extract. In bioagents, maximum per cent growth inhibition was recorded in *Trichoderma harzianum* and minimum was observed in *Pseudomonas fluorescens* 4.

Declarations

Conflict of interests: Authors declare no conflict of interest.

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