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BIOLOGICAL MANAGEMENT OF DOWNY MILDEW DISEASE IN CUCUMBER

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ABSTRACT

Downy mildew caused by *Pseudoperonospora cubensis* is a major foliar disease in cucumber which causes more damage and devasting loses to cucumber production. In the present study revealed the biological management of downy mildew disease in cucumber. The *in vitro* studies presented that, garlic bulb extract at a concentration of 15% revealed greater percentage inhibition (71.42%), followed by clove oil (64.51%), between all the botanicals studied *Pseudomonas fluorescence* inhibited sporangia germination powerfully (58.137%), followed by *Trichoderma harzianum* (46.67%). *in vivo* results also confirmed that, garlic extract @10% found to be best in decreasing the downy mildew incidence (42.28 %) with lowest AUDPC value (1278.51) compared with control plants and the next best was clove oil @ 4% (47.77%). However, The minimum disease incidence was observed in POP (UHS, Bagalkot) recommended fungicide Ridomil gold @ 0.2% sprayed plants (41.19%) with lowest AUDPC value (1143.94).The bio control agent *Pseudomonas fluorescens* was found to be less operational under field conditions (55.39%), when compared with other treatments and control (86.37%).

Key words : Downy mildew, Cucumber, Botanicals and Bio control agents.

Introduction

Cucumber crop is widely grown in temperate and tropical regions of world and occupies the fourth most important vegetable crop after tomato (*Lycopersicon esculentum* Mill.), cabbage (*Brassica oleracea* var. *capitata* L.), and onion (*Allium cepa* L.). Cucumber originated from Asia and present cultivated cucumber is closely related to wild cucumber *Cucumis sativus* var. *hardwickii* (Royle) Alef., found in the foothills of Nepal and northern India (Whitaker and Davis, 1962; Harlan, 1975).

The fruit of cucumber own a number of medicinal properties namely, cooling effect, prevent constipation, checks jaundice and indigestion (Nandkarni, 1927). Along with these, consumption of cucumbers also provides good nutritional benefits to human beings. 100 g of cucumber fruit affords 5 g carbohydrates, 0.4 g protein, 0.1 g fat, 0.3 g minerals, 10 mg calcium, 0.4 g fiber and traces of vitamin C and iron (Choudhary *et al*., 2009). Cucumbers are boon to cosmetic industry. Many cosmetic products such as soaps, lotions, creams and perfumes contain cucumber extracts. In addition, the seed of cucumbers are used in Ayurvedic preparations (Goker, 2017). At the global level, about 397 million tons of cucumber were produced from 22-million-hectare land with average productivity of approximately 19.58 t/ha (Anonymous, 2021). In India, 105 metric tons of cucumber was produced from an area of 1673 hectare and in Karnataka

cucumber is cultivated under an area of 8.27 thousand ha with production of 131.96 MT.

Cucumbers are prone to several diseases like downy mildew, powdery mildew, fungal and bacterial wilts and viral diseases (Cucumber Mosaic Virus, Watermelon Bud Necrosis Virus). It causes more economic losses in the sense of production and export. Among this downy mildew is a major foliar disease which causes more damage and devasting loses to cucumber production (Palti and Cohen, 1980).

The yield losses from downy mildew remained minimal compared to other diseases until 2004, later the pathogen developed more virulent races, which causes yield losses up to 40-80% in cucumber (Colucci *et al*., 2006). So, this disease currently continues to be a big threat to cucumber production in all cucumber growing areas of India (Haveri *et al.*, 2019).

Downy mildew is caused by the fungal pathogen *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov. It is an obligate parasite which requires live cucumber host tissue to grow and reproduce (Waterhouse, 1973; Palti, 1975). The pathogen affects more than 60 vegetable species belonging to 20 genera in Cucurbitaceae family (Lebeda, 1992a; Lebeda and Cohen, 2011). Among the different genera of cucurbits, species from *Cucumis* genera are most affected by *P. cubensis* (Kirkbride, 1993; Lebeda *et al*., 2007).

The symptoms are small, pale green to greasy looking angular or rectangular spots on upper surfaces initially. Later these patches turn into chlorotic to bright yellow colour with powdery downy growth on undersurface of leaves. Lower leaves of the plant are infected first later infection spreads to upward causing defoliation, stunted growth, poor fruit development and the entire plant shows burnt appearance. Finally, the lesions expand, shrivel and cause death of the leaf surface (Haveri *et al*., 2019).

Management of *P. cubensis* is a challenging task, because it has the ability to overcome the control measures (resistance and fungicide application) very quickly and it has long distance dispersal capacity. More usage of fungicides creates pollution of the environment and will be health hazardous. Thus, many researchers and scientists are now focusing on alternative methods such as biological management of disease. The use of bio control agents and botanicals against downy mildew provides ecofriendly management of disease and identification of active compounds in botanicals will help in development of commercial botanical formulations. So, we have conducted this study to identify effective bio agents and biological control agents against downy mildew in cucumber.

Materials and Methods

In- vitro evaluation of botanicals and bio control agents

Six different botanicals were assessed in this study to test its efficacy against *P. cubensis* under *in vitro* conditions through sporangia germination technique. The botanicals were tested at three different concentrations 5, 10 and 15% by v/v. The required concentration of botanicals was extracted by two different extraction methods. The list of botanicals used to give below

List of botanicals used

Name of the botanical	Plant part used/ type of product
Ginger	Rhizomes
Garlic	Cloves
Neem	Leaves
Pudina	Leaves
Castor	Oil
Clove	Oil

Water extraction method

Leaf samples from neem, tulsi, pudina collected from the fields, College of Horticulture, Bengaluru, India and cloves of garlic, castor and clove oil were purchased from retail shop. One hundred grams of each botanical sample were cleaned with tap water and shade dried at room temperature until complete evaporation of moisture. The samples were then made into powder by using an electric blender. Three concentrations of 5, 10 and 15% were prepared by suspending 5 g., 10 g and 15 g. of each botanical powder in 100 ml of sterile distilled water followed by filtration through cheesecloth to remove unwanted coarse particles. The filtered extract was centrifuged at 5000 rpm for 5 min to obtain a clear extract (Petrikovszki *et al.*, 2019; Bommesh *et al*., 2018; Yehia, 2016; Vincent, 1947).

Methanol extraction method

The procedure for the methanolic extraction of the botanicals was followed according to (Vincent, 1947). Hundred grams of each botanical sample were cleaned and made into powder. Thirty grams of each powdered botanical were extracted with 90 ml of methanol and kept on a rotary shaker for three days with periodic shaking. Then, the extract was filtered with muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected in tubes and kept in a hot air oven until complete evaporation of the solvent. Then the leftover material in the tubes was utilized for the experimentation.

Bio control agents

The bio control agents (*Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus megatherium*) at 3, 6 and 9% concentration were tested against *Pseudoperonospora cubensis* under *in vitro* conditions. The bio control agent commercial formulations were collected from Department of Microbiology, University of Agricultural Sciences, GKVK, Bengaluru. The required concentration of was prepared by dissolving known quantity of bio control agent formulations in sterile distilled water under aseptic conditions.

The fresh sporangia of *Pseudoperonospora cubensis* were collected from the naturally infected cucumber research plot maintained at the College of Horticulture, Bengaluru, India. The procedure for sporangia collection was followed as per Bommesh *et al*. (2018). Five-day-infected cucumber leaves were picked and cut into small pieces before being soaked in sterile distilled water to make a sporangial suspension. Using a hemocytometer, the sporangia concentration was adjusted to 100 sporangia/ml. Then, a drop of sporangia suspension was mixed with a drop of botanical extract of 5%, 10% and 15%, respectively, and kept in a BOD incubator at 20°C and 100 per cent relative humidity for 2 h. After 2 h. of incubation, the sporangial germination was recorded under a microscope. A cavity slide with sterile distilled water was maintained as the control. The percentage of sporangia germination was calculated by the formula.

Percent Germination of sporangia (PG) = (A/B) × 100

Where,

A = Number of sporangia germinated

B = Number of sporangia observed

The experiment was laid out with a Completely Randomized Design (CRD) with three replications. The cavity slides of each botanical concentration (5%, 10%, and 15%) were maintained with three replications along with the control under similar conditions. All slides were kept in a BOD incubator at 20°C and 100 percent relative humidity for 2 h. The percentage of sporangia was calculated from all three replications along with the control.

Experimental design and statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) and each treatment was replicated thrice. The analysis of variance was performed based on CRD. The data was changed into arc-sine transformation for statistical analysis using OPSAT (Sheoran *et al*., 1998).

In-vivo evaluation of botanicals and bio control agents

A field trail was conducted during *Rabi* 2020-21 under field conditions at College of Horticulture, Bengaluru to determine the effective botanicals and bio control agents against *Pseudoperonospora cubensis.*

Experimental details

Crop	Cucumber (Swarna Ageti)
Location	College of Horticulture, Bengaluru
Statistical design	RBD
Treatments	5
Replications	5
Spacing	60×60 cm

Planting materials

The seeds of downy mildew susceptible variety (Swarna Ageti) were sown on pro trays filled with coco peat. Then covered completely for 3 days to enhance germination and watering given regularly. The seedlings at 2 true leaf stage transplanted at the experimental field. The effective botanicals and bio control agent found under *in vitro* studies along with POP (UHS Bagalkot) recommended fungicide were evaluated against downy mildew under field conditions.

Treatment details

S. no.	Treatment	Description
1.	T_1	Garlic @ 15%
2.	T_2	Clove oil @ 15%
3.	T_3	Ridomil gold @ 0.2%
4.	T_4	*Pseudomonas fluorescence* @ 9%
5.	T_5	Control

Spraying was started on observation of disease on lower leaves of cucumber. Second, third and fourth spray was given at 14 days interval. The disease severity was recorded one day before first spray and seven days after every spray by using the Percent Disease Index (PDI) formula given by Wheeler (1969).

$$PDI = \frac{\text{Sum of all disease rating}}{\text{Number of observations} \times \text{Maximum disease grade}} \times 100$$

Results and Discussion

In-vitro evaluation of botanicals and bio control agents

In the present investigation, five botanicals, *i.e.*, neem, tulsi, pudina, clove and garlic and three bio control agents *i.e. Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus megaterium* were tested as

Table 1 : Evaluation of botanicals against sporangia germination of *P. cubensis* under *in vitro* conditions.

Treatment	% Inhibition of Sporangia Germination		
	Concentration		
	5 %	10 %	15 %
Clove	47.41(43.49) **	57.14(49.08)	**64.51**(53.41)
Garlic	57.14(49.08)	61.9(51.86)	**71.42**(57.66)
Tulsi	38.09(38.09)	47.61(43.61)	52.38(46.34)
Pudina	38.09(38.09)	47.41(43.49)	57.14(49.08)
Neem	33.33(35.24)	42.85(40.87)	57.14(49.08)
Control	0.00(0.00)	0.00(0.00)	0.00(0.00)

	Treatment	Concentration	Treatment × Concentration
CD@ 1% (p < 0.05)	0.776	0.508	1.344
S.Em ±	0.271	0.177	0.469

Note: **- Values in parenthesis are arc sine transformed values.

Table 2 : *In vitro* evaluation of bio control agents against sporangial germination of *P. cubensis.*

Treatment	Inhibition of sporangial germination (%)*		
	Concentration		
	3 %	6 %	9 %
Pseudomonas fluorescens	34.52(35.96)	46.34(42.88)	**58.13(49.65)**
Trichoderma harzianum	30.26(33.36)	39.25(38.77)	46.66(43.06)
Bacillus megaterium	20.34(26.79)	28.17(32.04)	34.71(36.08)
Control	0.00(0.00)	0.00(0.00)	0.00(0.00)
CD @ 1%		21.07	
SE± m		0.79	

*Figures in the parenthesis are arc sine transformed values.

these are good sources of anti-microbial compounds and are used against many fungal pathogens, especially the oomycetes fungi (Feng and Zheng, 2006; Fawzi *et al*., 2009; Hayat *et al*., 2016; Doshi *et al*., 2020; Najdabbasi *et al*., 2020). The botanicals and bio control agents were tested based on per cent inhibition of sporangial germination of *P. cubensis* (Table 1).

Evaluation of botanicals against sporangial germination of *P. cubensis in vitro* was carried out with five botanicals at three different percent concentrations. The data revealed that all the botanicals significantly inhibited the sporangial germination of *P. cubensis*. Amidst all, garlic bulb extract at 15 percent showed significantly higher percentage inhibition (71.42%) followed by clove oil @15% (64.51%) (Fig. 1). The slightest inhibition of sporangial germination (33.33%) was observed at 5 percent concentration of neem (Table 1).

Evaluation of bio control agents revealed that, *Pseudomonas fluorescens* effectively inhibited sporangia germination (58.13%) followed by *Trichoderma harzianum* (46.67%) (Fig. 2). However, the bio control agent *Bacillus. megatherium* did not show much inhibition on sporangia germination (34.71%) and the percent reduction was similar to control (34.66%) (Table 2).

In vivo evaluation of botanicals and bio control agents

Field experiment conducted during *Rabi* season, to evaluate the best botanicals and bio control agents obtained from *in vitro* studies. Among the botanicals tested, Garlic extract (15%) and clove oil (15%) were found effective under *in vitro* studies whereas, the bio control agent *P. fluorescens* @ 9% with 10^8 CFU/ml was also found effective under *in vitro* studies. These botanicals and bio control agent were selected for field experiment along with the recommended fungicide Ridomil Gold @ 0.2%. The control plants were maintained without spraying of any botanical/bio control agent/ fungicide (Table 3).

The disease index of downy mildew was observed 40 days after sowing and was recorded before spraying at 14 days intervals. The disease severity was recorded one day before first spray and seven days after every spray.

Among the botanicals and bio control agents, minimum disease index was recorded in garlic extract @ 15% (47.77%) with lowest AUDPC value (1277.49) and shown significantly best in minimizing the disease index compared to control. It was followed by clove oil @15% (42.28%) with an AUDPC of (1277.49). The lowest disease index was observed in POP (UHS, Bagalkot) recommended fungicide Ridomil Gold sprayed plants (41.19%) with lowest AUDPC value of (1143.94). *P. fluorescens* sprayed plants showed more disease index (55.39%) compared with other treatments. However, the maximum disease index was recorded in control plants (86.37%) with highest AUDPC value (2001.54) at 14 days after 3rd spray (Table 3).

Biological management is another sustainable ecofriendly way to manage downy mildew disease. Bio control agents control the disease by competition, antagonism, or by the production of secondary

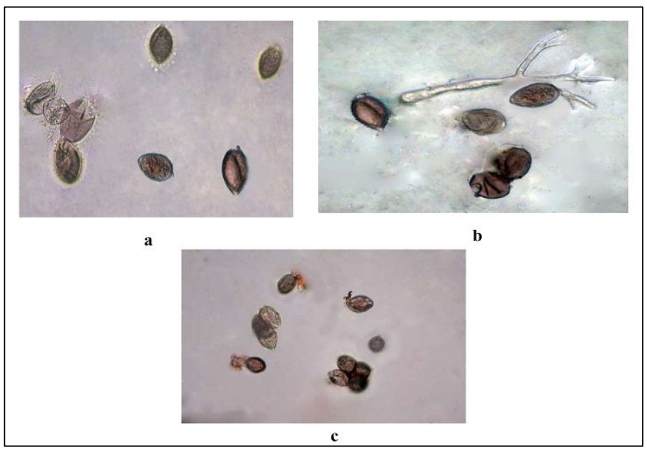

Fig. 1 : Inhibition of sporangia germination of *P. cubensis* (A) clove oil @ 5% (B) Garlic @ 15% (C) Control.

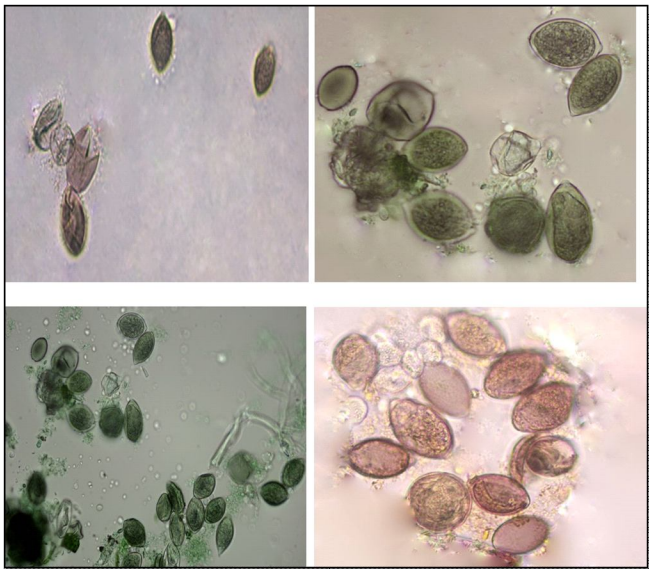

Fig. 2 : Inhibition of sporangia germination through bio control agents a) *P. fluorescens* b) *T. harzianum* c) *B. megaterium* d) Control.

metabolites. Now a days, it has been found that higher plant products can be used as innovative plant protection methods (Amar and El-shennway, 2023). Botanical pesticides are becoming more popular due to the widespread use of several plant products as green pesticides (Malkhan *et al*., 2012). In the present study, we have utilized five botanical extracts of different plant materials and three bio control agents against downy mildew disease under both *in vitro* and *in vivo* conditions.

Among the five botanicals tested, garlic bulb extract showed maximum inhibition (71.42%) followed by clove oil (64.51%). Garlic bulb extract at a 15 percent concentration showed maximum inhibition of sporangial germination (71.42%), followed by clove oil at a 15 percent concentration (71.76%) under *in vitro* conditions. Spaying or application of essential oils and plant extracts reduce the intensity of downy mildew (Adirano-Anaya *et al*., 2018; Islam *et al*., 2019; Deweer *et al*., 2017; Fialho *et al*., 2017). Results from earlier reports found that the concentrations of 50–1000 µg ml/L allicin in garlic juice reduced the severity of cucumber downy mildew caused by *P. cubensis* by approximately 50-100 per cent under controlled conditions (Rani *et al*., 2017). The volatile antimicrobial substance allicin (diallyl thiosulphinate) from garlic (*Allium sativum*) at concentrations 50-100 ìg/mL reduced the severity of *P. cubensis* on cucumber by approximately 50-100% (Chen *et al*., 2016). In addition, clove oil at 4 per cent effectively reduced the downy mildew incidence in cucumber (Portz *et al*., 2008). This might be because of the strong effect of fatty acids and their derivatives on *P. cubensis*. Studies have shown that fatty acids, as well as their salts, have an antimicrobial effect (Skøivanova *et al*., 2005).

The low incidence of the disease was recorded by the spray Ridomil Gold (41.19%) compared with control (86.37%). The similar results were quoted by Gabriel-Ortega *et al*. (2020); Chaudhry *et al*. (2009) and Sharma *et al*. (2003). In supportive to the result, earlier Cohen (1979) advised that systemic fungicides of phenyl amides group were effective in controlling the downy mildew disease.

Among the three bio control agents tested, *Pseudomonas fluorescens* showed maximum inhibition of spore germination @ 9% (58.13%) followed by *Trichoderma harzianum* @ 9% (46.66%) under *in vitro* conditions. *Pseudomonas fluorescence* @ 9% with 10^8 CFU/ml was further evaluated under field conditions and recorded 55.39 % reduction of downy mildew disease incidence. Our results were in accordance with Abada *et al*. (2009); Umesha *et al*. (1999). They stated that foliar spray of *P. fluorescens* effectively reduced downy mildew disease in pearl millet. The downy mildew genera also controlled by *T. harzianum* (Abd-El-Moity *et al*., 2003; Nelson, 2004). Studies have shown that different *Trichoderma* species controls downy mildew (Al-Aswad and Al- Azzawi, 2021).

For further improvement in cucumber downy mildew control, understanding the biocontrol mechanism is an essential step. Controlling downy mildew through bio control agents involves production of hydrolytic enzymes or metabolites, which results in increase of resistance in plants against downy mildew disease. *T. harzianum* produce cell wall degrading enzymes like glucanase and *P. fluorescence* produces several secondary metabolites which are having antifungal nature against *P. cubensis*. It also activates defense enzymes such as peroxidase, and polyphenol oxidase in cucumber against downy

Table 3 : Field management of downy mildew using botanicals and bio control agents.

Treatment details	Disease severity (PDI %)						AUDPC
	Before spray 40 days	7 days after 1st spray (47 DAS)	14 days after 1st spray (54 DAS)	7 days after 2nd spray (61 DAS)	14 days after 2nd spray (68 DAS)	7 days after 3rd spray (75 DAS)	14 days after 3rd spray (82 DAS)	
T_1 - Garlic @ 15%	15.59*(23.24)	18.92(25.77)	23.92(29.26)	32.25(34.58)	36.92(37.39)	38.87(38.53	47.77(43.70)	1277.49
T_2 - Clove oil @ 15%	16.07(23.60)	20.74(27.07)	23.74(29.14)	33.74(35.49)	36.07(36.89)	39.18(38.72)	42.28(40.53)	1278.51
T_3 - Ridomil Gold @ 0.2 %	17.35(24.60)	19.68(26.30)	22.01(27.96)	28.35(32.15)	30.68(33.62)	33.43(35.30)	41.19(39.89)	1143.94
T_4 - *P. fluorescens* @ 9%	15.35(23.06)	23.69(29.10)	27.03(31.30)	35.69(36.66)	40.69(39.61)	50.37(45.19)	55.39(48.08)	1489.88
T_5 - Control	15.14(22.88)	27.49(31.61)	38.87(38.53)	42.28(39.89)	57.93(49.55)	68.61(55.93)	86.37(68.42)	2001.54
SEm (±)	1.31	1.34	1.43	1.53	1.27	1.29	1.32	
CD @ 5%	3.83	3.90	4.10	4.53	3.70	3.81	3.92	

Note: **DAS**: Days after sowing. *Figures in the parenthesis are arc sine transformed values.

mildew disease (Mohamed *et al*., 2015; Anand *et al*., 2007).

Conclusion

The *in vitro* studies showed that, garlic bulb extract at a concentration of 15% demonstrated much greater percentage inhibition (71.42%), followed by clove oil @ 15% concentration (64.51%), among all the botanicals examined *P. fluorescens* @ 9% reduced sporangia germination efficiently (58.137%), followed by *Trichoderma harzianum* (46.67%).

The results revealed that, garlic extract @ 15% found to be best in reducing the downy mildew disease incidence (47.77%) with lowest AUDPC value (1277.49) compared with control plants. It was followed by clove oil @ 15% (42.28%). However, the minimum disease incidence was observed in POP (UHS, Bagalkot) recommended fungicide Ridomil gold @ 0.2% sprayed plants (41.19%) with lowest AUDPC value (1143.94). The bio control agent *P. fluorescens* was found to be less effective under field conditions (55.39%) when compared with other treatments and control (86.37%).

Conflict of interest : The author's declared that no conflict of interest.

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