Short Research Article

"Evaluation of the most effective plant extracts, bioagents and chemical fungicides *in vitro* against powdery mildew of pea."

ABSTRACT:

The present investigation was carried during 2022-23 at Plant Pathology Section, College of Agriculture, Pune-05with the objectives of evaluation of the most effective plant extracts, bioagents and chemical fungicides *in vitro.* Effect of six plant extracts, bioagents and chemical fungicides were studied *in vitro* by hanging drop method where the study revealed that garlic (*Allium sativum*),followed by neem (*Azadiracta indica*) showed highest inhibition of spore germination for powdery mildew. After 48 hours of incubation, the spore germination rate ranged from 3.15 % to 34.45 %, where garlic showed the highest inhibition at 95.30 %, followed by neem at 91.03 %. The efficacy of bioagents was evaluated by preparing the bacterial and fungal culture filtrateswhere *Trichoderma harzianum* and *Pseudomonas fluorescens* were found to be the most effective with tune of 56.86% and 50.72% of efficacy respectively against *Erysiphepisi*. Among all chemical fungicides, hexaconazole (0.05 %) resulted as the most effective chemical fungicide against powdery mildew of pea.These findings can be useful in developing safer and more eco-friendly methods to control powdery mildew and protect crops.

Key words*: Pea, powdery mildew, conidia, spore inhibition,bioagents, plant extracts,fungicides*.

1. INTRODUCTION

Pea (*Pisum sativum* L.) is a valuable vegetable as well as pulse crop all over the world, is also known as 'Matar' or Garden pea. It belongs to the family Fabaceae and a self-pollinated crop. Green peas are the number-one processed vegetable in modern world. The United States, Canada, China, Russia, and India come under some top pea-producing nations. Peas, especially green peas, are a widespread staple in many parts of India. *Pisum sativum* is a versatile and valuable plant that is esteemed for its culinary and nutritional attributes. Peas are highly nutritious and offer a range of essential nutrients[1]. Pea farming is impacted by a variety of biotic and abiotic stressors. Rust, powdery mildew, fusarium wilt, and other fungi-related illnesses fall under the most prevalent biotic stressors. Garden peas are frequently afflicted with fungi-related diseases including powdery mildew and rust. The amount of damage caused by powdery mildew varies significantly depending on the stage of plant growth at which the illness manifests itself and is proportional to the disease's severity. The pathogen functions as a parasitic biotroph. Fungicide-based plant disease management can occasionally produce positive outcomes. However, inappropriate fungicide use mostly results in global disasters, pollution of the environment, and the emergence of pathogen resistance [2].The antimicrobial properties of plant extracts against fungi make them a promising source of antifungal agents[3].The efficacy of bioagents has been gaining significant attention as a potential solution to

plant disease management. These agents offer practicality, affordability, and environmental safety. Compared to conventional non-sustainable control methods, bioagents exhibit remarkable field persistence[4]. However, this research is done to understand their potential and ensure their effectiveness to control the powdery mildew of pea.

2. MATERIAL AND METHODS

2.1 EXPERIMENTAL SITE

The current research was conducted at the section laboratory in the Department of Plant Pathology at the College of Agriculture, Pune.

2.2 FUNGAL PATHOGEN (DISEASE SAMPLE)

Pea leaves bearing the conidia of *E. pisi* were frequently collected from the experimental field for *in vitro* study.

2.3 CHEMICAL FUNGICIDES USED

Total six fungicides, bioagents and plant extracts were used to test efficacy against sporegermination of powdery mildew of pea. Their details are as follows:

Table No.1: List of fungicidestested against powdery mildew of pea.

Sr. No.	Common name	Trade name	Concentrations used
1.	Hexaconazole 5 EC	Contaf	0.05 %
2.	Dinocap 48 EC	Karathane	0.1 %
3.	Tebuconazole 250 EC	Folicur	0.1 %
4.	Propiconazole 25 EC	Tilt	0.1 %
5.	Wettable sulphur 80% WP	Sulfil	0.2 %
6.	Triadimefon	Calixin	0.1 %

BIOAGENTS USED: -

Table No.2: List of bioagentstested against pea powdery mildew.

Sr. No.	Name of bioagent	Concentration used
1.	*Trichoderma viride*	5%
2.	*Trichoderma harzianum*	5%
3.	*Trichoderma koningii*	5%
4.	*Trichoderma hamatum*	5%
5.	*Bacillus subtilis*	1×10^6 cfu/ml
6.	*Pseudomonas fluorescens*	1×10^6 cfu/ml

PLANT EXTRACTS USED: -

Table No. 3: List of Plant extractstested against pea powdery mildew.

Sr. No.	Common Name	Botanical Name	Plant Part used	Concentration
1.	Onion	*Alliumcepa*	Bulb	10%

2.	Tulsi	*Ocimum sanctum*	Leaves	10%
3.	Nilgiri	*Eucalyptusglobulus*	Leaves	10%
4.	Periwinkle	*Catharanthusroseus*	Leaves	10%
5.	Neem	*Azadiracta indica*	Leaves	10%
6.	Garlic	*Alliumsativum*	Clove	10%

2.4 *IN-VITRO* EXPERIMENTAL DETAILS

The six chemical fungicides, six crude plant extracts and six bio-agents evaluated under *in vitro* against pea powdery mildew (*Erysiphe pisi* DC) pathogen by spore germinationusing hanging drop technique [5]. One control maintained to calculate the % inhibition of spore germination.

1. Design : CRD (Completely Randomized Design)
2. No. of treatments : 07
3. No. of replication : 03

2.4.1 Collection of disease samples and preparation of spore suspension

A composite representative sample of fresh pea leaves diagnosed as infected on the basis of typical symptoms of powdery mildew (*Erysiphe pisi* DC) were collected from experimental farm. Samples were placed in separate plastic bags, appropriately labelled, brought to the laboratory and stored at 4 ºC for further examination. For powdery mildew conidia, they are incorporated into distilled water containing host tissue exudates[5].

2.4.2 Preparation of culture filtrates of bioagents

The pure cultures of *Trichoderma viride, T. hamatum, T. harzianum, T. koningii, Pseudomonas fluorescens, Bacillus subtilis* were procured from the Agricultural Bacteriologist, Biological Nitrogen Fixation Scheme, College of Agriculture, Pune.Bioagents were grown individually on 100 ml of sterilized liquid media (nutrient broth for bacteria and potato dextrose broth for fungi) for preparation of culture filtrates in 250 ml conical flasks. The culture filtrates were obtained after 72 hr of incubation by filtering through Whitman filter paper No.42. The bacterial filtrates were adjusted to contain $1x10^6$ cfu /ml and then evaluated for spore germination studies [6].

2.4.3 Hanging drop method

One drop of pathogen spore suspension was placed at the centre of clean glass slide and mixed with one drop of bioagent suspension using micropipette on different glass slides. The glass slides containing drop of mixed suspension was placed in an inverted position supported over two pieces of glass rods kept in a sterilized Petri plate lined with double layered moist filter paper at 25±1°C for 24 h in moisture chamber. These experiments were laid out in completely randomized design with three replications for each treatment in case of fungicides, plant extracts and bioagents, respectively. A control set was also run concurrently in which spores were mixed in sterilized distilled water. After 48 hr, spore germination was recorded under the microscope [6]. The germinated conidia were counted and mean of percentages of germination was calculated and recorded for each treatment.

2.4.4 Effect of plant extracts on spore germination

The crude plant extracts were used to evaluate their spore inhibition potential against powdery mildew conidia. *E. pisi* conidia from infected pea plants were tapped on the drops of the plant extracts and mixed thoroughly with the help of a needle. The glass slides were placed in moist chambers prepared by placing moist filter paper on the lower surface of the lid and on inner surface of the base of Petri plates and incubated at 25^{o} C for 24 h. Conidia tapped only on sterile distilled water containing pea exudates for germination served as control[7].

2.4.5 Effect of chemical fungicides on spore germination

Different concentrations of fungicide solutions were prepared as per requirement for the experiment. *E. pisi* conidia were trapped individually on the drop of the different fungicide solution in cavity slide and mixed thoroughly with the help of needle.

Observations: Percent spore germination was calculated by the following formula:

$$\text{Percent spore germination} = \frac{\text{Total number of spores germinated}}{\text{Total number of spores observed}} \times 100$$

The percent inhibition of spore germination calculated by using the following formula given by Vincent (1947):

$$\text{Percent inhibition of spore germination (I)} = \frac{C - T}{C} \times 100.$$

Where,

I = Percent inhibition of spore germination.

C = Number of spores germinated in control.

T = Number of spores germinated in treatment.

3. RESULTS

3.1 Effect of plant extracts on spore germination of *Erysiphe pisi* DC:

The observation of spore germination inhibition was taken by using light microscope at 40x objective lens. The mean spore germination rate after 24 hours was ranged from 3.12 % to 36.59%, with the control having the highest rate at 62.07 %. Treatment T_6, which is Garlicclove extract exhibited the highest level of inhibition of spore germination, i.e., 94.98 %. This was followed by T_5, which is Neem leafextract, at 87.63 %. T_4 i.e., Periwinkle leaf extract, showed the least inhibition of spore germination, at 41.06 %.After 48 hours, the mean spore germination rates were ranged from 3.15 % to 34.45 %, with highest rate in control i.e., 63.33%. The maximum inhibition of spore germination was recorded in Garlicat 95.30 %, followed by T_5, which is Neem, at 91.03%. Treatment T_4 i.e., Periwinkleat 45.60 % showed the least inhibition of spore germination among all.

Table No.4: Effect of plant extracts on spore germination of *Erysiphe pisi* DC after 24 hrs & 48 hrs.

Tr. No.	Treatments	Conc.used	24 Hours		48 Hours	
			% spore germination	% Inhibition	% spore germination	% Inhibition
T_1	Onion	10%	23.47	62.19	22.93	63.79

	(*Alliumcepa*)		(28.96)		(28.6)	
T_2	Tulsi (*Ocimum sanctum*)	10%	24.56 (29.69)	60.43	22.42 (28.25)	64.60
T_3	Nilgiri (*Eucalyptus globulus*)	10%	31.02 (33.83)	50.03	33.33 (35.24)	47.37
T_4	Periwinkle (*Catharanthus roseus*)	10%	36.59 (37.20)	41.06	34.45 (35.24)	45.60
T_5	Neem *(Azadiracta indica)*	10%	7.68 (16.05)	87.63	5.68 (13.75)	91.03
T_6	Garlic (*Allium sativum*)	10%	3.12 (10.05)	94.98	3.15 (10.14)	95.03
T_7	Absolute control		62.07 (51.97)	**-**	63.33 (52.71)	-
	S.E.(m) ±		**0.605**	**-**	**0.539**	**-**
	CD (1%)		**1.854**	**-**	**1.652**	**-**

Note: Valuesin parentheses are arcsine transformed values.

3.2 Effect of bioagents on spore germination of *Erysiphe pisi* DC:

All tested bioagents decreased the germinated conidia of *E. pisi* compared with the control. After 24 hours, treatment T_2 i.e., *T. harzianum*was most efficient with tune of 56.86 % efficacy, followed by T_6 i.e., *P. fluorescens* with 50.72% efficacy over the control. The lowest inhibition of conidia germination was observed in case of T_3 i.e., *T. koningii*at 25.26 %.

Table No. 5: Effect of bioagents on spore germination of *Erysiphe pisi* DC after 24hrs.

Tr. No.	Treatments	Concentration	% Spore germination	(%) Efficacy
T1	*Trichoderma viride*	5%	63.53 (52.85)	31.02
T2	*Trichoderma harzianum*	5%	39.73 (39.07)	56.86
T3	*Trichoderma koningii*	5%	68.84 (56.07)	25.26
T4	*Trichoderma hamatum*	5%	57.61 (49.38)	37.45
T5	*Bacillus subtilis*	1×10^6 cfu/ml	47.20 (43.39)	48.75
T6	*Pseudomonas fluorescens*	1×10^6 cfu/ml	45.39 (42.35)	50.72
T7	Absolute control	-	92.10 (73.67)	-
	S.E.(m)±	-	**0.854**	-
	CD (1%)	-	**2.600**	-

Note: Values in parentheses are arcsine transformed values.

3.3 Effect of chemical fungicides on spore germination of *Erysiphe pisi* DC:

After 24 hours, as per the findings in the laboratory study, the mean spore germination rate ranged from 2.70% to 16.84%, with the control having the highest rate at 45.96%. Treatment T_1, Hexaconazole (0.05%) inhibited spore germination the most, at 94.12%. This was followed by T_4 (0.10% Propiconazole), at 90.55%. Treatment T_5, i.e., Triadimefon (0.10%), inhibited spore germination the least, at 63.36%.After 48 hours, Treatment T_1, Hexaconazole (0.05%) inhibited spore germination the most, at 95.05%. This was followed by T_4 (0.10% Propiconazole), at 92.09%. Treatment T_6, i.e., Triadimefon (0.10%), inhibited spore germination the least, at 59.06%. The mean spore germination rate ranged from 2.14% to 17.71%, with the control having the highest rate at 43.25%.

Table No. 6: Effect of chemical fungicides on spore germination of *Erysiphe pisi* DC after 24hrs & 48hrs.

Tr. No.	Treatments	Conc	24 Hours		48 Hours	
			% spore germination	% Inhibition	% spore germination	% Inhibition
T_1	Hexaconazole 5 EC	0.05%	2.70 (9.44)	94.12	2.14 (8.39)	95.05
T_2	Dinocap 48 EC	0.10%	11.14 (19.49)	75.77	9.83 (18.26)	77.27
T_3	Tebuconazole 25 EC	0.10%	10.03 (18.46)	78.17	8.56 (17.01)	80.20
T_4	Propiconazole 25 EC	0.10%	4.34 (11.98)	90.55	3.42 (10.63)	92.09
T_5	Wettable sulphur 80% WP	0.20%	9.67 (18.10)	78.97	7.72 (16.11)	82.16
T_6	Triadimefon	0.10%	16.84 (24.22)	63.36	17.71 (24.87)	59.06
T_7	Absolute control	-	45.96 (42.67)	-	43.25 (41.12)	-
	S.E.(m)±	**-**	**0.409**	**-**	**0.381**	**-**
	CD (1%)	**-**	**1.252**	**-**	**1.168**	**-**

Note: Valuesin parentheses are arcsine transformed values.

3.4 DISCUSSION

The finding of present investigation was in favour with the work done by Maurya *et al*. (2004) who reported more than 80 per cent spore germination inhibition of *E. pisi* causing pea powdery mildew with neem and motha. Choudhary *et al.* (2017)noted that the highest mycelial growth inhibition was recorded with garlic extract at 30% concentration (100%) followed by 91.80% and 80.52% at concentration 10%and 15% respectively.Mishra *et al.* (2017) through an experiment revealed that

P.flourescens (35.93%) and *T. harzianum* (42.02%) had minimum PDI compared to treated check (28.77%) and untreated check (51.33%).Patil *et al*. (2017) stated that *T. harzianum* (0.4%) were significantly superior over other treatments for managing powdery mildew.The significantly highest spore germination inhibition (81.82%) was recorded at 20 per cent concentration of garlic followed by 20 per cent concentration of neem (79.47%) on 72 hrs after the treatment [14].

4. CONCLUSION

The laboratory experiment aimed to find effective ways to combat powdery mildew. The study tested various plant extracts, bioagents, and chemical fungicides and found that garlic extract and neem showed great potential as plant extracts. Moreover, *T. harzianum* and *P. fluorescens* proved to be effective bioagents. Among the chemical fungicides, Hexaconazole (0.05%) and Propiconazole (0.1%) showed promising results in suppressing spore germination. These findings can be useful in developing safer and more eco-friendly methods to control powdery mildew and protect crops.

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