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 *rabi* 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 (*Allium sativum*) showed the highest inhibition at 95.30%, followed by neem~~(*Azadiracta indica*)~~ at 91.03%. The efficacy of bioagents was evaluated by preparing the bacterial and fungal culture filtrates ~~(Hassan and Eman.,2017)~~, where *Trichoderma harzianum* and *Pseudomonas fluorescens* were found to be the most effective with tune of 56.86% and 50.72% of efficacy respectively against *E. pisi.* Among all chemical fungicides, hexaconazole (0.05 %) resulted as the most effective chemical fungicide againstpowdery mildew of pea.

***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. *P~~isum~~ 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.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

Comment [D1]: It's advisable to avoid using local terms and provide context for readers unfamiliar with agricultural practices in specific regions

Comment [D2]: Suggest: Do not to repeatedly write the same Latin names of plants

Comment [D3]: Do not include the author's name in the abstract, Pls deleted it

Comment [D4]: What did you mean of this term?

Comment [D5]: References from each section discussed need to be included. Avoid writing overly long sentences without accompanying references.

with fungi-related diseases including powdery mildew andrust.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 (Brewer and Larkin, 2005).Therefore, it is imperative to use alternate safe, effective ways against such diseases or at the very least, to rationalise their application, in order to overcome these challenges.Therefore, disease resistant cultivars must be employed to overcome these limitations through research practices with holistic approach.

Comment [D6]: Pls write an explanation regarding information related to the use of extracts or compounds from plants that have the potential as biofungicides.

2. Material and methods:

Experimental site: -

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

Fungal pathogen (Disease sample):

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

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 againstpowdery 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%

Comment [D7]: How to make this concentration?Explain it in the method section, why different concentrations are used? Please provide an explanation.

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

Comment [D8]: How to make this concentration?Explain it in the method section, why different concentrations are used?

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%

Comment [D9]: Similar to the comment above, Explain it in method section

***In-vitro* experimental details**: -

The six chemical fungicides, six crude plant extracts and six bio-agents evaluated under *in vitro* againstpeapowdery mildew (*Erysiphe pisi* DC) pathogen by spore germinationusing hanging drop technique (Kahate R & Kahate N., 2022). 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

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 (Rao *et al*., 2020).

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 (Rao *et al.*, 2020). The germinated conidia were counted and mean of percentages of germination was calculated and recorded for each treatment.

Effect of plant extracts on spore germination:

In case of plant extracts, *E. pisi* conidia from infected pea plants were tapped on the drops of the plant extract 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 (Bahadur *et al*.,2009).

Comment [D10]: Explain in the Methods section how to perform the extraction, what solvent is used? How to make the bioagent concentration, what is the solvent?

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:- Per cent spore germination was calculated by the following formula:

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

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

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

Where,

I = Per cent inhibition of spore germination.

C = Number of spores germinated in control.

T = Number of spores germinated in treatment.

3. Results and discussion:

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 Garlic ~~(*Allium sativum*)~~ clove extract exhibited the highest level of inhibition of spore germination, i.,e., 94.98 %. This was followed by T_5, which is Neem leafextract~~(*Azadiracta indica)*~~, at 87.63 %. T_4 i.e., Periwinkle leaf extract ~~(*Catharanthus roseus*)~~, 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 Garlic~~(*Allium sativum*)~~at 95.30 %, followed by T_5, which is Neem *(Azadiracta indica)*, at 91.03%. Treatment T_4 i.e., Periwinkle (*Catharanthus roseus*) at 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 (*Alliumcepa*)	10%	23.47 (28.96)	62.19	22.93 (28.6)	63.79
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: Figures in parentheses are arcsine transformed values.

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

All tested bioagents decreased the germinated conidia of *Erysiphe pisi*compared with the control. After 24 hours, treatment T_2 i.e., *Trichoderma harzianum* was most efficient with tune of 56.86 % efficacy, followed by T_6 i.e., *Pseudomonas fluorescens* with 50.72% efficacy over the control. The lowest inhibition of conidia germination was observed in case of T_3 i.e., *Trichoderma koningii*at 25.26 %. Present findings are in line with the results of earlier studies done by Surwase *et al.* (2009), Mishra *et al.* (2017) and Patil *et al*. (2017).

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*	1×10^6cfu/ml	47.20 (43.39)	48.75
T6	*Pseudomonas fluorescens*	1×10^6cfu/ml	45.39 (42.35)	50.72
T7	Absolute control	-	92.10 (73.67)	-

	S.E.(m)±	-	**0.854**	-
	CD (1%)	-	**2.600**	-

Note: Figures?in parentheses are arcsine transformed values.

Comment [D11]: What did you mean?

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: Figures in parentheses are arcsine transformed values.

4. Conclusion:

In case of plant extracts, after 24 and 48 hours, Garlic (*Allium sativum*) showed the highest level of inhibition at 94.98% and 95.30 %, followed by Neem (*Azadiracta indica*) at 87.63% and 91.03 % respectively.In case of bioagents, after 24 hours, treatment T2 i.e., *Trichoderma harzianum* was most efficient with tune of 56.86% efficacy, followed by T6 i.e., *Pseudomonas fluorescens* with 50.72% efficacy over the control.In case of chemical fungicides; the laboratory study displayed that, after 24 and 48 hours, Hexaconazole (0.05%) at 94.12% and 95.05 % and Propiconazole (0.1%) at 90.55% and 92.09 %, respectively significantly inhibited spore germination. Thus hexaconazole (0.05%) and propiconazole (0.1%) were the most effective fungicides against powdery mildew after 24 and 48 hours.

Comment [D12]: In the Conclusion section, it is better not to write/include numbers/quantitative values; just write the categories, such as very high, low, and so on.

References:

Comment [D13]: Re-check the citation rules, pls see the journal writing guidelines

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