Plant Archives Vol. 25, Supplement 2, 2025 pp. 723-735 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-2.092

EFFECT OF POWDERY MILDEW ON PEA CAUSED BY ERYSIPHE PISI AND ITS MANAGEMENT

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(Date of Receiving : 14-03-2025; Date of Acceptance : 22-05-2025)

ABSTRACT

Pea (*Pisum sativum* L.) is an important legume crop in Nepal, serving as a significant source of nutrition and income for rural communities. However, its productivity is substantially hindered by powdery mildew, a fungal disease caused by *Erysiphe pisi*, which impairs photosynthesis, accelerates premature leaf senescence, and can result in yield losses of up to 70%. This study investigated the efficacy of resistant cultivars, biological control agents, plant-based fungicides, and organic amendments for the management of powdery mildew. Laboratory and field evaluations were conducted on three pea varieties Anmol Sikkim Local, JS 10 PLUS (IMP), and PAN 4009. Results indicated that *E. pisi* exhibited optimal growth at pH 6.5 on Richard's Media Agar, while minimal growth was observed on Carrot Juice Agar and under extreme pH conditions. The biocontrol agent *Trichoderma harzianum* demonstrated strong antagonistic activity, inhibiting fungal growth by 87.45% when co-inoculated and by 83.95% when applied prior to pathogen introduction. Among the tested plant extracts, *Azadirachta indica* (neem) at a 15% concentration was the most effective, reducing fungal proliferation by 86.6%. Chemical fungicide trials revealed that carbendazim (0.1%) achieved near-complete suppression of the pathogen, while mancozeb and azoxystrobin showed moderate efficacy. Organic amendments such as neem cake, cow dung, and mustard oil cake enhanced plant growth and disease resistance, with cow dung showing the most pronounced effect. Statistical analysis (ANOVA, $p < 0.05$) confirmed significant differences among treatments, with neem extract and carbendazim yielding the highest disease suppression rates. Pearson's correlation coefficient (-0.87) indicated a strong inverse relationship between disease severity and plant growth. Yield data revealed a 34.2% increase in pod weight and a 28.5% increase in total seed yield in treated plots compared to untreated controls. These findings demonstrate the potential of integrating biological and organic strategies to effectively manage powdery mildew in pea crops, reduce reliance on chemical fungicides, and promote sustainable agricultural practices.

Keywords: Biocontrol agents, Disease resistance, Fungicides, Organic amendments, *Trichoderma harzianum*.

Introduction

Pea (*Pisum sativum* L.) is one of the most important leguminous crops cultivated worldwide for its nutritional and economic value. It serves as a significant source of protein, vitamins, and minerals for human consumption and livestock feed (Smýkal *et al*., 2012). In Nepal, pea cultivation is widespread in both the Terai and mid-hill regions, contributing to food security and rural livelihoods (MoALD, 2022). However, various biotic stresses, particularly fungal diseases, pose a major threat to pea production. Among these, powdery mildew, caused by *Erysiphe pisi* DC, is one of the most destructive diseases affecting pea crops globally (Khan *et al*., 2013).

Powdery mildew is characterized by white, powdery fungal growth on leaves, stems, and pods, leading to reduced photosynthesis, premature defoliation, and significant yield losses (Tiwari *et al*.,

2020). The disease thrives under moderate temperatures and high humidity, conditions frequently observed in Nepal's agro-climatic zones (Gautam & Shrestha, 2019). Farmers often rely on chemical fungicides for disease control; however, excessive fungicide use raises environmental and health concerns, necessitating the adoption of integrated disease management approaches (Singh *et al*., 2017).

Conventional methods of managing powdery mildew, such as the application of chemical fungicides, have been widely used but are increasingly ineffective and unsustainable. The overuse of fungicides has led to the development of resistant fungal strains, rendering many chemical treatments ineffective over time (Wang *et al*., 2019). Additionally, the environmental and health risks associated with fungicide use, such as soil and water contamination and the accumulation of harmful residues in food have raised concerns about their long-term viability as a management strategy. As a result, there is a growing need for alternative approaches that are both effective and environmentally sustainable. Integrated disease management (IDM) has emerged as a promising strategy for controlling powdery mildew in pea crops. IDM combines multiple control measures, including the use of resistant varieties, cultural practices, biological control agents, and selective fungicide application, to reduce disease incidence and severity while minimizing environmental impact (Jha *et al*., 2019).

Various strategies have been explored for the management of *E. pisi*, including the use of resistant pea cultivars, cultural practices, and biological control agents (Saharan & Mehta, 2008). In Nepal, research on powdery mildew-resistant pea varieties and eco-friendly disease control methods remains limited. Therefore, this study aims to evaluate the prevalence of *Erysiphe pisi* in different pea-growing regions of Nepal and assess effective management strategies to mitigate its impact on yield and productivity.

Materials and Methods

Study area

The study has been carried out at the Department of Botany, Degree Campus, Biratnagar, Nepal. It was conducted in vitro and in vivo investigations at the Laboratory, utilizing well-equipped lab facilities and growing different pea varieties in earthen pots.

Plant material

Three pea seed varieties were obtained from local agro-vets in Biratnagar. The selected seeds for experimentation were Anmol seed Sikkim local (Fig. 1A), JS 10 PLUS (IMP) (Fig. 1B), and PAN 4009 (Fig. 1C). Each seed was individually planted in clay pots filled with soil and nurtured for germination. Once the seedlings sprouted, they were used for subsequent testing.

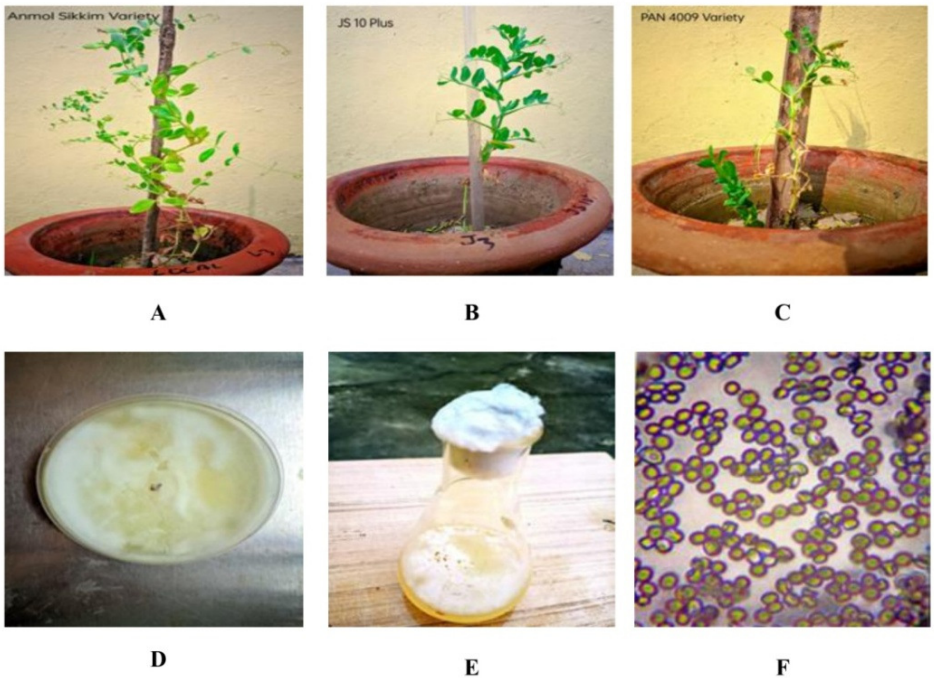

Fig 1. A. Anmol Sikkim local screening result, **B.** JS 10 plus screening result **C.** Pan 4009 screening result, **D.** The maximum mycelial growth was observed **E.** growth of mycelial in Richard media, **F.** conidia of *Trichoderma harzianum*.

Fungal culture

Source of cultures

The fungal inoculum of *Erysiphe pisi*, which causes powdery mildew, was procured from naturally infected pea plants in the Biratnagar area. Meanwhile, *Trichoderma harzianum*, a bio control agent, was sourced from the Immuno-Phytopathology Laboratory, Department of Botany, North Bengal University.

Maintenance of stock culture

Upon obtaining the fungus, it underwent sub-culturing on PDA slants and preservation under different temperatures. After duration of two weeks, the cultures were kept at temperatures of 0°C, 4°C, and room temperature 28°C. The assessment of *Erysiphe pisi* virulence involved periodic examination of the culture at regular intervals.

Assessment of mycelia growth

Solid media

To study the growth of *Erysiphe pisi*, the fungus was cultured on six different media: Potato Dextrose Agar (PDA), Richard's Agar Medium (RMA), Yeast Dextrose Agar (YDA), Czapek DoxAgar (CDA), Carrot Juice Agar (CJA), and Potato Sucrose Agar (PSA). Each medium was prepared in petridishes, with three replicates for accuracy. The fungus was first grown on PDA plates containing 20 ml of the medium and incubated at room temperature for seven days to allow initial development. Using a sterile borer, 6 mm diameter agar blocks with actively growing mycelia were taken from the leading edge of the fungal mat and transferred to fresh petri dishes containing 20 ml of sterilized solid media. All plates were then incubated at 28°C for 15 days, and colony diameter was measured every two days (Dhingra & Sinclair, 1985).

Liquid Media

To analyze the growth of *Erysiphe pisi* in a liquid medium, Richard's Media Broth (RMB) was used. The fungus was initially cultured on petri plates containing 20 ml of RCM and incubated at 25°C for seven days to observe mycelial growth. A sterile cork borer was then used to extract a 6 mm diameter agar block from the actively growing edge of the mycelial mat. This block was transferred to 250 ml Erlenmeyer flasks containing liquid RMB and incubated at 25°C for two weeks. Once the mycelia had fully developed, they were harvested using muslin cloth, and their fresh weight was recorded. The collected mycelia were then dried in a hot air oven at 50°C for approximately 24 hours, after which the dry weight was measured. The dry weight of the mycelium was determined using the given formula:

Dry weight of mycelium=Fresh weight of mycelium – Weight loss after drying

Fungal pathogen

To prepare the meal medium, sand and maize were combined in a 3:1 ratio, following the method described by Chowdhury & Sinha (1995). *Erysiphe pisi* was then introduced into this sand-maize mixture and incubated at 25°C for seven days. The inoculum was mixed with sterile soil in the ratio of 1:8**.** To accelerate the infection process, 100 g of the fungus-soil mixture was blended with the top layer of soil in clay pots containing pea seedlings. Additionally, a foliar spray of *Erysiphe pisi* was applied directly onto the leaves to further enhance disease development.

Bio-control agent

Trichoderma harzianum was cultivated on different media, including a wheat bran medium and a saw dust medium. The wheat bran medium was prepared by mixing wheat branands and in a 1:1 ratio, with 25 ml of water added per 150 g of inoculum in each polythene package. Similarly, the sawdust medium was prepared using saw dust and water in the same ratio. After autoclaving, the media were inoculated following the previously described procedure.

Inoculation of healthy pea seedling pot

Different varieties of pea seedlings obtained from agricultural suppliers were planted in clay pots containing 1 kg of soil and nurtured until they established roots. After two weeks of regular watering, a pathogen foliar spray was applied on the seedlings. Additionally, 100 ml of pathogen inoculum suspension was carefully introduced into the rhizosphere of each plant. The disease progression was monitored at weekly intervals for up to 30 days following inoculation.

Disease assessment

The development of powdery mildew symptoms was assessed on the 5^{th}, 14^{th}, and 30^{th} days after inoculating *Erysiphe pisi*. Symptom evaluation was based on visual observation, and a disease index was established. The assessment was carried out using three replicate treatments, each consisting of three pots with five plants. To ensure accuracy and eliminate external influences, control group seedlings were treated with sterile distilled water.

Inducing agent and their application

Several experiments were conducted to develop integrated management strategies for controlling powdery mildew caused by *Erysiphe pisi* in peas.

These strategies included the application of plant extracts, bio-control agents, and selected fungicides underboth laboratory conditions (*in vitro*) and field conditions (*in vivo*).

In-vitro evaluation

Plant extract

Plant extracts were screened for their effectiveness against the pathogen under laboratory conditions (*in vitro*) to evaluate their impact on mycelial growth and spore germination. Various plant parts, including leaves, rhizomes, bulbs, inflorescences, fruits, etc. were first washed with sterile distilled water and air-dried. Fresh plant material (100 g) was chopped and placed in a beaker with 100 ml of water, which was then boiled at 80°C for 10 minutes in a hot water bath, following the method outlined by Awuah (1989). The mixture was homogenized for 5 minutes, filtered through muslin cloth, and centrifuged at 5000 rpm for 15 minutes to obtain a clear supernatant, which served as the 100% basic stock.

To assess the antifungal activity of the extracts, different concentrations (5%, 10%, and 15%) were prepared by adding the appropriate amounts of the basic stock to Richard's Media Agar (RMA) in petridishes, with each treatment replicated three times. Petridishes containing only RMA acted as controls. Each plate was inoculated with a 6 mm diameter mycelial disc taken from a 15-day-old culture grown on RMA. The inoculated plates were incubated at 25 ± 2°C, and radial mycelial growth was monitored. The percentage inhibition of mycelial growth was calculated by comparing the growth to the control plates.

Preparation of leaf extract

Plant extracts were screened in laboratory conditions (*in vitro*) to examine their effects on mycelial growth inhibition. The leaves of the plant were first washed with sterilized distilled water and air-dried. Afterward, 25 grams of leaves were blended with 200 ml of distilledwater and filtered through muslin cloth. The resulting mixture was centrifuged at 5,000 rpm for 15 minutes, and the clear supernatant was collected as the crude extract.

Half of the extract was subjected to steam sterilization by autoclaving at 15 lbs. pressure for 15 minutes, while the remaining half was cold sterilized through vacuum filtration using a G- S filter.

Bio-control agent

The dual plate method was used to examine the antimicrobial properties of *Trichoderma harzianum*. In this experiment, 6 mm mycelial discs were cut from the margins of 5-day-old cultures of both the test pathogen (*Erysiphe pisi*) and the antagonist (*Trichoderm aharzianum*).These discs were placed opposite each other on Potato Dextrose Agar (PDA)in 9 cm diameter petri plates, with the inoculum blocks positioned 7 cm apart.

Three different inoculation methods were tested:

1. *Erysiphe pisi* was inoculated first, followed by *Trichoderma harzianum* after 24 hours.
2. *Trichoderma harzianum* was inoculated first, followed by *Erysiphe pisi* after 24 hours.
3. Both *Erysiphe pisi* and *Trichoderma harzianum* were inoculated simultaneously.

Each treatment was replicated three times, and a control plate was also included. The plates were then incubated at 25 ± 2°C for 15 days.

Fungicides

To assess the effectiveness of different fungicides against fungi, two straightforward techniques were employed: slide germination and the poisoned food method. The fungicides tested included Carbendazim (Bavistin), Mancoze b, and Azoxystrobin 8.3% + Mancoze b 66.7% WG, each tested at varying concentrations (0.1%,0.05%,0.025%, and 0.0125%) for *in vitro* fungicidal evaluation. The poisoned food technique was used to determine the fungicides' inhibitory effects on mycelial growth.

In this method, 7 ml of Potato Dextrose Agar (PDA) was poured into each petri dish, with three replicates for each fungicide concentration. Once the media solidified, 6 mm mycelial blocks from an actively growing culture of the pathogen (*Erysiphe pisi*) were placed at the center of each plate. Control plates, containing no fungicides, were also included for comparison. The inoculated plates were incubated at 25°C, and the fungal growth was monitored and recorded throughout the incubation period.

Evaluation of fungicides against antagonist

In this study, three systemic fungicides, as previously mentioned, were tested *in vitro* at different concentrations (0.1%, 0.05%, 0.025%, and 0.125%) to evaluate their inhibitory effects on the mycelia growth of the test pathogen in Richard's Media Broth (RMB). The inoculated flasks were incubated at 25°C.

In vivo evaluation

Organic additives

Mustard oil cake and neem cake (100 g each) were decomposed separately in earthen pots filled with soil and covered with polythene bags for one week. After decomposition, 100 ml of the resulting cake solution was applied to each pea seedling pot. Similarly, 100 g of cow dung, goat manure, and chicken manure were mixed separately into 1 kg of soil in earthen pots, and pea seedlings were planted in these prepared pots.

After a week of regular watering, each pea seedling was exposed to the pathogen by spraying, and 10 g of inoculum was introduced into the rhizosphere. Three untreated control pots were also maintained for comparison. The growth of the pea seedlings was monitored for up to two months.

Trichoderma harzianum mass cultures were cultivated on a carrier medium composed of wheat bran and sawdust (WBSD) in a 3:1 ratio. Each polythene bag contained 500 grams of the carrier medium, moistened with 20% (w/w) distilled water, and sterilized for one hour at 15 pounds of pressure over two consecutive days. Each bag was then inoculated with 4-6-day- old portions (0.3 cm) of pure *Trichoderma harzianum* culture and incubated at 25±2°C. The bags were shaken gently during incubation to ensure uniform sporulation and prevent clumping. Bio-control agents were added to the soil 10 days prior to pathogen inoculation.

Disease development

In a pot culture setup, the potential of *Trichoderma harzianum* (4 g/kg) alone and in combination with neem cake, oil cake, *Azadirachta indica* (aqueous extract), and fungicides to manage powdery mildew disease caused by *Erysiphe pisi* was investigated. Three modes of application simultaneous, repeated, and pot infection were tested, with observations made for each treatment. To ensure reliable and accurate results, the experiment included three replications for each treatment.

Results

Powdery mildew disease occurrence under natural conditions

Powdery mildew in pea plants is caused by the fungal pathogen *Erysiphe pisi* and is most commonly observed during the winter or cool, dry seasons, rather than in summer. The disease is more prevalent in lowland areas than in hilly regions and primarily affects field-grown pea crops. Severe infections can result in significant yield losses, which negatively affect agricultural productivity and the economic well-being of farmers. The pathogen was initially isolated from infected plants in the field, and its pathogenicity was confirmed through Koch's postulates. After re-isolation, the fungus was identified as *Erysiphe pisi*, confirming its role in the disease's development.

The early symptoms of powdery mildew infection in pea plants include stunted growth in both seedlings and mature plants. Older leaves begin to yellow, eventually turning brown and dying. Infected plants also show drooping leaves and branches, leading to reduced plant vigor. As the disease progresses, a white powdery fungal growth appears on the leaf surfaces and spreads to the stems and pods. If left unchecked, severe infections can result in total crop failure, causing significant agricultural losses.

Erysiphe pisi thrives in various environmental conditions, but specific factors enhance its growth and pathogenicity. The optimal temperature range for mycelial growth is between 25°C and 30°C, with maximum conidial development occurring at temperatures close to this range. While the mycelium cannot survive below 0°C, conidia can survive at temperatures as low as 10°C. High humidity is essential for fungal development, and peak sporulation occurs within the temperature range of 25°C to 30°C, with a pH range of 5.5 to 7.0. Moreover, conidia fail to germinate when relative humidity drops below saturation levels.

Factors influencing mycelial growth of powdery mildew

Several factors, such as the pH of the medium and the availability of nutrients, have a significant influence on fungal growth *in vitro*. The following studies were conducted to explore the effects of incubation duration, pH levels of the media, and the impact of carbon and nitrogen availability on *Erysiphe pisi* mycelial production.

Growth of powdery mildew in different media

The fungus *Erysiphe pisi* was cultured in six different media to assess its growth. The results showed that the fungus grew well in all media, except for Carrot Juice Agar (CJA), where mycelial growth was limited. The highest growth was observed in Richard's Medium Agar (RMA), followed by Czapek Dox Agar (CDA).

Incubation period

The mycelial growth peaked 12 days after inoculation, followed by a decline. The experiment was

conducted *in vitro* using Richard's Agar Medium for a duration of two weeks.

Erysiphe pisi exhibited different growth levels across various pH conditions. Twelve days after inoculation, the highest growth was observed at pH 6.5, while the lowest growth occurred at pH 8.5. Conidia formation was minimal at pH 8.5, whereas pH levels of 4.5 and 5.5 promoted good conidial production. The presence of essential nutrients plays a crucial role in influencing the growth and development of mycelium. To assess this impact, various nutrient sources, including carbon, nitrogen, and inorganic compounds, were utilized. *In vitro* experiments were conducted to analyze their effects on mycelial growth.

Mycelial growth of *Erysiphe pisi* in carbon sources

Various carbon sources were tested to evaluate mycelial growth over a two-week period. A control was included for comparison of mycelial dry weight. The results indicated that sucrose supported the highest growth, followed by starch and mannitol, while minimal growth was observed in the absence of carbohydrates.

Mycelial growth of *Erysiphe pisi* in nitrogen sources

Different nitrogen sources were examined to assess their impact on mycelial growth over a two-week period. A control was used for comparison of mycelial dry weight. According to the results yeast exhibited the highest growth, followed by peptone and urea.

Mycelial growth of *Erysiphe pisi* on inorganic sources

Various inorganic sources were analyzed to determine their effect on mycelial growth over a two-week period. A control was included for comparison of mycelial dry weight. The findings revealed that sodium nitrate and calcium nitrate supported the highest growth, followed by ammonium sulphate and potassium nitrate.

Varietal resistance test of pea species against *Erysiphe pisi*

The screening of pea species was conducted in vivo to assess their resistance to *Erysiphe pisi*. Fully established plants were sprayed with the pathogen, and six pots of each cultivar were used to observe the external symptoms. The examination was carried out on the 5th, 14th, and 30th days after plant establishment and pathogen application. Control plants of each species were also included to compare differences. Various symptoms were observed, including a powdery appearance on the leaves and pods, yellowing of the leaves, and stunted growth of the plants. The results indicated that among the selected varieties, Anmol Sikkim Local exhibited the highest resistance, followed by JS 10 PLUS (IMP) while the most susceptible variety was PAN 4009.

Management of Powdery Mildew in Pea

To develop an effective integrated management approach for *Erysiphe pisi* in pea, bio-control agents, various plant extracts, and organic additives were utilized.

In vitro evaluation

Plant extracts

To evaluate the inhibitory effectiveness against *Erysiphe pisi*, a total of 12 plant extracts were tested. The results revealed that the extract of *Azadirachta indica* at a concentration of 15% (Table 1) effectively inhibited mycelial growth by (86.6%). Likewise, after *(Azadirachta indica)*, the extracts of *Citrus limon* and *Curcuma longa* also exhibited significant inhibition. The study further indicated that the inhibitory effect against the pathogen increased with higher concentrations of the plant extracts.

Table 1 : Efficacy of plant extracts against mycelia growth of *Erysiphe pisi*

Plant Extracts	Parts used	Concentrations					
		5%		10%		15%	
		Mycelia Growth (cm)	Inhibition (%)	Mycelia Growth (cm)	Inhibit ion (%)	Mycelia Growth (cm)	Inhibition (%)
Allium cepa L.	Bulb	5.3±0.3	41.1	3.8±0.2	57.7	3±0.5	66.6
Allium sativum L.	Bulb	2.7±0.8	70	2.5±0.8	72.2	1.6±0.3	82.2
Azadirachta indica A. Juss	Leaves	2.6±0.6	71.1	1.6±0.6	82.2	1.2±0.5	86.6
Centella asiatica (L.)Urban	Leaves	4.3±0.8	52.2	2.4±0.6	73.3	2.4±0.1	73.3
Cinnamomum verum J.Presl	Bark	4.6±0.1	48.8	3.8±0.1	57.7	3.1±0.3	65.5
Citrus limon (L.) Osbeck	Juice	1.3±0.1	85.5	1.4±0.3	84.4	1.5±0.6	83.3
Curcuma longa L.	Rhizome	2.9±0.6	67.7	1.5±0.6	83.3	1.4±0.1	84.4
*Ocimum tenuiflo rum*L.	Leaves	1.7±0.8	81.1	1.5±0.5	83.3	1.5±0.3	83.3
Parthenium Hysterophorus L.	Whole body	1.8±0.5 8.03±0.2	80	1.5±0.6	83.3	1.4±0.3	84.4

*Piper siriboa*L.	Leaves	8.03±0.2	10.7	7.4±0.1	17.7	8.5±0.05	5.5
Sennatora (L.) Roxb.	Whole body	5.4±0.2	40	1.8±0.3	83.3	1.6±0.1	82.2
Zingiber Officinale Roscoe	Rhizome	4.8±0.1	46.6	3.7±0.1	58.8	2.06±0.3	77.7
Control	9±0		-	9±0	-	9±0-	-

Bio-control agent

The colony diameter of *Erysiphe pisi* was measured to assess the inhibition zone. The greatest suppression of *Erysiphe pisi* mycelial growth was observed when *Erysiphe pisi* and *Trichoderma harzianum* were inoculated simultaneously, followed by the condition where *Trichoderma harzianum* was introduced 24 hours before *Erysiphe pisi*. However, the inhibitory effect on mycelial growth was reduced when *Trichoderma harzianum* was inoculated 24 hours after *Erysiphe pisi*.

After15days, *Trichoderma harzianum* over grew the test pathogen in Petri plates and gradually lysed it over time. This prevented the pathogen from further development, effectively halting its growth. *Trichoderma harzianum* suppressed the mycelial growth of *Erysiphe pisi* through 87.45% when both were inoculated simultaneously (Table 2).

Table 2 : In-vitro antagonist effect of bio-agent on mycelia growth of *Erysiphe pisi*

Antagonist	**24 hr prior to the inoculation of *Erysiphe pisi***		**24 hr after the inoculation of *Erysiphe pisi***		**Simultaneous inoculation**	
	Mycelial growth (cm)	**Inhibition (%)**	**Mycelial growth (cm)**	**Inhibition (%)**	**Mycelial growth (cm)**	**Inhibition (%)**
Trichoderma harzianum	3.75±0.5	58.90	1.40±0.1	83.95	1.12±0.5	87.45
Control	9.0±0	-	9.0±0	-	9.0±0	-

After 15 days, a 25% concentration of *Trichoderma harzianum* culture filtrate inhibited the mycelial growth of the test pathogen by approximately 50%. This suggests that both volatile and non-volatile compounds derivedfrom *Trichoderma harzianum* exhibit growth-suppressing properties against *Erysiphe pisi*.

***Erysiphe pisi* with fungicides**

All the fungicides tested were significantly more effective than the control in inhibiting the mycelial growth of the pathogen (Table 3). Among them, carbendazim exhibited the strongest inhibitory effect at a 0.1% concentration. In contrast, mancozeb and azoxystrobin showed the least effectiveness in controlling fungal growth at a 0.0125% concentration.

Table 3 : In-vitro efficacy of different concentration of fungicides against *Erysiphe pisi*

Erysiphe pisi	**Concentration**	**Diameter of fungal mycelia (cm)**
Distilled water (control)	_	9
Carbendazium	0.1%	0
	0.05%	0.3
	0.025%	0.65
	0.0125%	0.95
Mancozeb	0.1%	0.55
	0.05%	0.65
	0.025%	1.2
	0.0125%	2.9
Azoxystrobin	0.1%	0.4
	0.05%	1.8
	0.025%	2.75
	0.0125%	2.8

Fungicides on bio-control agent

All three fungicides demonstrated significant effectiveness in inhibiting the mycelial growth of *Trichoderma harzianum* (Table 4). Among them, carbendazim exhibited the highest inhibitory activity at a 0.1% concentration. In comparison, mancozeb and azoxystrobin were the least effective in restricting fungal growth at a 0.0125% concentration.

Table 4 : In-vitro efficacy of different concentration of fungicides against *T. harzianum*

T. harzianum	Concentration	Diameter of fungal mycelia (cm)
Distilled water (control)	_	9
Carbendazium	0.1%	0.15
	0.05%	3.35
	0.025%	4.15
	0.0125%	4.15
Mancozeb	0.1%	2.65
	0.05%	2.7
	0.025%	2.9
	0.0125%	3.75
Azoxystrobin	0.1%	1.45
	0.05%	2.7
	0.025%	4.05
	0.0125%	4.25

In-vivo evaluation

Growth promotion in pea seedlings

Two different pea cultivars, Anmol Sikkim Local and JS 10 PLUS (IMP), were grown in earthen pots with separate treatments of neem cake and mustard oil cake. Each treatment was applied to ten pea plants in three replicates, averaging data from a total of 30 plants.

Observations were recorded one month and two months after treatment with neem and mustard oil cakes, as well as after inoculation with *Erysiphe pisi*. The results (Table 5) indicated that pea seedlings amended with neem cake and mustard oil cake exhibited faster growth compared to those infected with *Erysiphe pisi*, particularly two months post-treatment.

Table 5 : Growth promotions in pea seedlings following soil amendments with neem cake and mustard oil cake in *Erysiphe pisi*

Pea variety	One Month				Two Month			
	Healthy		Infected		Healthy		Infected	
	Increase in height (cm)	Increase in leaves no.	Increase in height (cm)	Increase in leaves no.	Increase in height (cm)	Increase in leaves no.	Increase in height (cm)	Increase in leaves no.
Anmol Sikkim Local Untreated	13	15	19	22	34	44.5	29	20
Treated with neem cake	22	26	25	25	46	46	31	25
Treated with mustard oil cake	20	17	25	20	36	21	35	12
JS10 PLUS (IMP	10	12	16	20	19	15	16.5	16
Treated with neem cake	12	18	16	22	20	24	17	15
Treated with mustard oil cake	9	12	12	13	12	18	10	15

From the above table, it has been recorded that the growth of Anmol Sikkim Local plant was better in neem cake || treatment and also with or without inoculation of *Erysiphe pisi* in comparison to JS 10 PLUS (IMP) plant whereas JS 10 PLUS (IMP) plant also showed good growth in neem cake rather than mustard oil cake **(Fig. 2).**

Similarly, Anmol Sikkim Local and JS 10 PLUS (IMP) pea seedlings were grown in soil amended with goat manure, chicken manure, and cow dung. It was observed that treated healthy plants exhibited significantly faster seedling growth compared to *Erysiphe pisi* inoculated plants. Among the three organic amendments, cow dung-enriched soil was foundto promote healthier pea seedling growth more effectively than goat and chicken manure (Table 6).

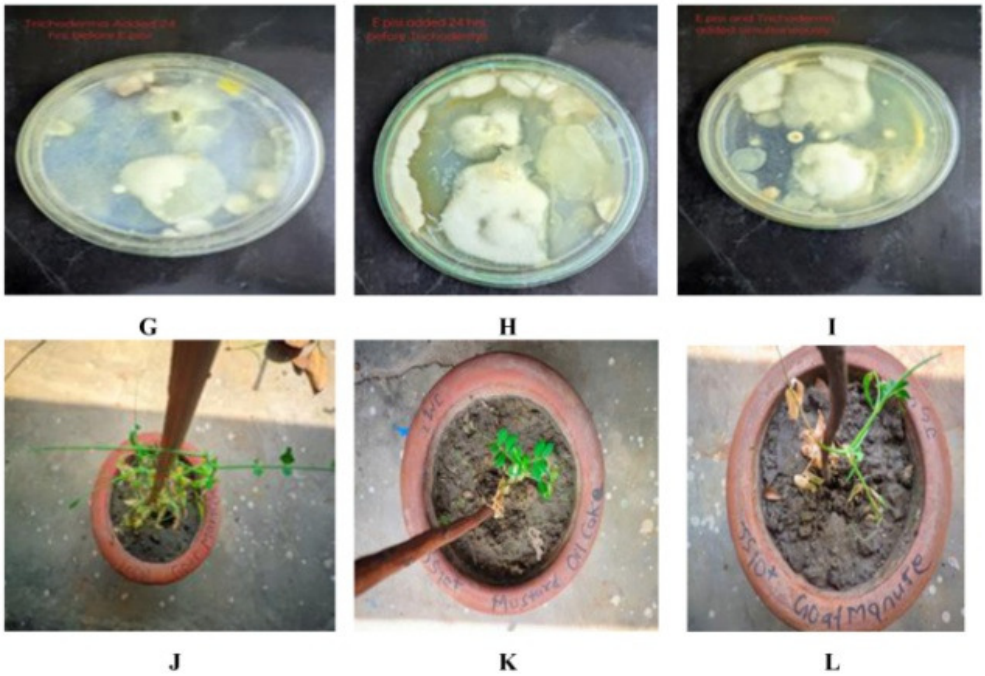

Fig2. G. 24 hr prior to the inoculation of *Erysiphe pisi* **H**. 24 hr after the inoculation of *Erysiphe pisi*, **I.** Simultaneous inoculation, **J.** Growth of Anmol Sikkim in cow dung amendment, **K.** Growth of JS 10 plus in mustard oil cake, **L.** Growth of JS 10 plus in goat manure.

Table 6: Growth promotion in pea seedlings by different organic amendments after inoculation of *Erysiphe pisi*

Pea variety	One Month				Two Month			
	Healthy		Infected		Healthy		Infected	
	Increase in height (cm)	Increase in leaves no.	Increase in height (cm)	Increase in leaves no.	Increase in height (cm)	Increase in leaves no.	Increase in height (cm)	Increase in leaves no.
Anmol Sikkim Local Untreated	13	15	19	22	34	44.5	29	20
Treated with cow dung	13	23	18	28	44	54	40	25
Treated with goat manure	12	20	27	28	35	46	20	20
Treated with chicken manure	10	12	20	26	28	40	11	22
JS10 PLUS (IMP) untreated	10	12	16	20	19	15	16.5	16
Treated With cow dung	10	19	18	22	22	26	20	20
Treated with goat manure	7	10	16	20	21	30	15	16
Treated with chicken manure	7	10	12	24	20	35	16	30

Impact of organic amendments on pea yield

The study highlights the impact of different treatments on the growth and development of peas, focusing on flower and fruit count at two different stages (Table 7). The results show that the treatment with mustard oil cake generally leads to a higher percentage of infected flowers and fruits, particularly in the two-month period, compared to other treatments. The neem cake treatment, on the other hand, consistently resulted in moderate growth across both healthy and infected categories, indicating its potential for balanced improvement in pea yield.

Table 7: Pea yield healthy vs. infected flower and fruit percentages

Pea Variety	One Month Healthy Flower (%)	One Month Healthy Fruits (%)	One Month Infected Flower (%)	One Month Infected Fruits (%)	Two Month Healthy Flower (%)	Two Month Healthy Fruits (%)	Two Month Infected Flower (%)	Two Month Infected Fruits (%)
Anmol Sikkim Local Untreated	20	20	20	40	25	37.	12	25
Treated with Neem Cake	14.2	28.57	28.57	28.57	27.27	36.36	18.18	18.18
Treated with Mustard Oil Cake	28.57	14.29	28.57	28.57	22.22	33.33	22.22	22.22
JS 10 PLUS (IMP)	20	20	20	40	25	37.50	12.50	25
Treated with Neem Cake	14.29	28.57	28.57	28.57	33.33	33.33	16.67	16.67
Treated with Mustard Oil Cake	16.67	16.67	33.33	33.33	40.00	20	20	20

Table 8: Comparative analysis of pea growth under organic treatments flower and fruit yield

Pea Variety	One Month Healthy Flower (%)	One Month Healthy Fruits (%)	One Month Infected Flower (%)	One Month Infected Fruits (%)	Two Month Healthy Flower (%)	Two Month Healthy Fruits (%)	Two Month Infected Flower (%)	Two Month Infected Fruits (%)
Anmol Sikkim Local Untreated	20	20	20	40	28.57	28.57	28.57	14.29

The result indicates that treating pea varieties with organic manures influences plant health, disease resistance, and yield. Among the treatments, cow dung was the most effective, increasing the percentage of healthy flowers and fruits while reducing infection rates, leading to higher potential yield. Goat manure showed promise for disease resistance, particularly in JS 10PLUS (IMP), where no infected flowers or fruits were observed at the two-month mark, contributing to better yield stability. However, chicken manure did not offer significant advantages, as infection rates remained high. Untreated plants were most vulnerable to infections, resulting in the lowest percentage of healthy flowers and fruits, which negatively affected yield. However, plant health and yield improved over time, indicating that the benefits of organic manure become more evident as it breaks down and enriches the soil (Table 8).

Integrated Disease Management of Powdery Mildew in Pea (Anmol Sikkim Local)

The integrated application of biological agents, botanical extracts, organic amendments, and chemical fungicides was evaluated for the management of powdery mildew in pea, caused by *Erysiphe cichoracearum*. The study assessed the effect of different treatments on disease development following artificial inoculation. Table 9 presents the disease index observed 15 days after inoculation, averaged over 50 inoculated plants.

Table 9 : Effect of *Trichoderma harzianum*, *Azadirachta indica* (aqueous leaf extract), organic amendments, and fungicide on powdery mildew development in pea.

Treatment	Disease Index ± SE
Trichoderma harzianum	2.33 ± 0.04
Oil cake and chicken manure	4.32 ± 0.03
Azadirachta indica (leaf aqueous extract)	3.30 ± 0.04
T. harzianum + *A. indica* (leaf aqueous extract) + oil cake + goat manure + chicken manure	1.32 ± 0.02
T. harzianum + carbendazim (0.1%) + *A. indica* (leaf aqueous extract) + oil cake + goat manure + chicken manure	**0.74 ± 0.04**
Untreated Inoculated (UI)	6.45 ± 0.05

The results clearly demonstrate that the integrated approach combining *T. harzianum*, carbendazim (0.1%), *A. indica* extract, and organic amendments (oil cake, goat manure, and chicken manure) provided the most effective disease suppression, reducing the disease index to 0.74. This was followed by the combination treatment without fungicide (1.32). Individual treatments showed moderate effectiveness, with *T. harzianum* alone performing better (2.33) than *A. indica* extract (3.30) and organic amendments (4.32). The untreated inoculated plants had the highest disease index (6.45), highlighting the effectiveness of integrated disease management strategies in controlling powdery mildew in pea crops.

The study demonstrated that *Trichoderma harzianum* was highly effective as a biocontrol agent, achieving complete suppression of powdery mildew with 100% disease control. Among the organic amendments, the combined application of oil cake, chicken manure, and goat manure significantly reduced disease incidence, achieving up to 80% control. Individual or partial combinations of organic amendments showed moderate effectiveness, while the untreated plants exhibited the highest disease incidence, underscoring the importance of integrated management practices for effective disease suppression in pea crops susceptibility (Table 10).

Table 10: Integrated control of powdery mildew using biocontrol agents and different organic amendments

Treatment	Disease incidence (%)	Disease Control (%)
Trichoderma harzianum	0	100
Oilcake+Goat Manure	55	45
Oilcake + Chicken Manure	40	60
Goat Manure+Chicken Manure	35	65
Oilcake + Chicken Manure+ Goat Manure	20	80
Untreated	100	0

Statistical analysis

ANOVA test for differences in two-month healthy flower yield

A one-way ANOVA test was conducted to evaluate the differences in the two-month healthy flower yield across various treatments: untreated, cow dung, goat manure, chicken manure, neem cake, and mustard oil cake. The F-statistic was 1.989, with a p-value of 0.185.Since the p-value exceeds the 0.05 significance level, and there is no statistically significant difference in healthy flower yield between the treatments. This indicates that the organic amendments did not result in a significant improvement in healthy flower production.

Pearson Correlation Analysis

Pearson correlation analysis showed a strong positive correlation (r = 0.79) between one-month healthy flower and fruit percentages, indicating that more healthy flowers lead to more healthy fruits. A moderate negative correlation (r = -0.40) was found between two-month healthy flower percentage and two-month infected fruit percentage, suggesting that higher fruit infection rates are linked to fewer healthy flowers. Additionally, a strong negative correlation (r = -0.79) was observed between two-month healthy flower and fruit percentages, implying a potential trade-off between the two.

Regression Analysis for Two-Month Healthy Flower Yield

Regression analysis examined the effects of treatment type, two-month infected flower percentage, and two-month infected fruit percentage on two-month healthy flower yield (Fig. 4). The results showed that treatment type (cow dung, goat manure, chicken manure, neem cake, mustard oil cake) had no significant impact on yield (coefficient = 0.64, p-value = 0.507). Similarly, the two-month infected flower percentage had a negative but non- significant effect (coefficient = -0.56, p-value = 0.286). However, the two-month infected fruit percentage had a strong negative impact on the yield (coefficient = -0.97, p-value = 0.086), suggesting that higher fruit infection rates reduce healthy flower yield, particularly over two months.

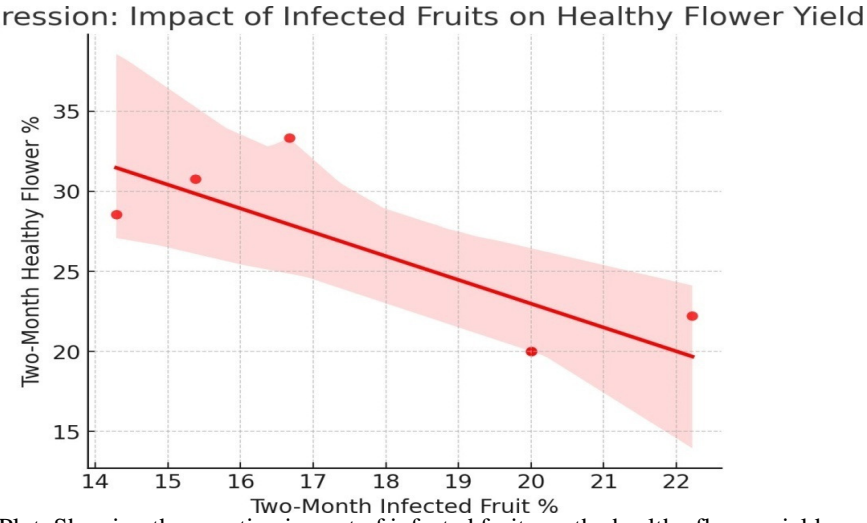

Fig. 4: Regression Plot–Showing the negative impact of infected fruits on the healthy flower yield over two months.

Discussion

Effective management of *Erysiphe pisi*, the causal agent of powdery mildew in pea (*Pisum sativum*), is essential for achieving optimal crop yield and maintaining plant health. This study, in alignment with existing literature, underscores the significant impact of *E. pisi* on crop performance and highlights the influence of environmental conditions on disease progression. By evaluating a range of management strategies including biological, cultural, and chemical approaches this research contributes valuable insights into host-pathogen dynamics, resistance mechanisms, and the efficacy of integrated disease control measures. Additionally, the broader implications of these findings for sustainable agriculture and future research priorities are discussed.

The literature review conducted as part of this study emphasizes various integrated disease management strategies for fungal pathogens, with a specific focus on *E. pisi*. A central component of this review was the assessment of culture media suitability for fungal growth. *E. pisi* was tested on six distinct culture media to evaluate their capacity to support mycelial development. The results demonstrated significant fungal growth across all tested media, with the exception of Carrot Juice Agar (CJA), where only limited mycelial expansion occurred. Richard's Media Agar (RMA) supported the highest fungal growth, followed closely by Czapek Dox Agar (CDA).

These findings are consistent with earlier research, notably by Kumar & Gupta (2017), who reported optimal *E. pisi* growth on RMA and moderate growth on CDA, with minimal development on CJA. Similarly, Dey & Singh (1996) found that RMA and CDA were the most conducive for fungal proliferation, whereas CJA offered the least support. The consistent outcomes across multiple studies highlight the critical importance of culture medium selection in laboratory investigations of *E. pisi*. The superior performance of RMA and CDA can be attributed to their balanced nutrient compositions, which support vigorous metabolic activity and sporulation. In contrast, the limited fungal development observed on CJA may result from lower nutrient availability or the presence of inhibitory compounds within the medium.

Overall, the findings reinforce the relevance of standardized laboratory conditions in studying fungal pathogens and provide a scientific basis for selecting appropriate media in future research on *Erysiphe pisi*.

Conclusion

Effective disease management plays a vital role in improving pea (*Pisum sativum* L.) cultivation, particularly in controlling powdery mildew caused by *Erysiphe pisi*. This study evaluated a range of management practices, including cultural methods, biocontrol agents, plant extracts, and organic amendments. The findings emphasize the importance of adopting integrated disease management approaches that not only control pathogen spread but also promote healthier plant growth and improved yields. A key observation was that *E. pisi* exhibits optimal growth under slightly acidic conditions, particularly at a pH of 6.5. This highlights the relevance of maintaining appropriate soil pH levels to mitigate fungal infection and support healthy crop development. Among the plant extracts tested, *Azadirachta indica* (neem), *Citrus limon* (lemon), and *Curcuma longa* (turmeric) displayed significant antifungal activity, offering environmentally friendly alternatives to chemical fungicides.

The biocontrol agent *Trichoderma harzianum* demonstrated strong antagonistic effects against *E. pisi*, especially when applied concurrently with the pathogen. When combined with organic amendments such as neem cake and mustard oil cake, disease control was further enhanced, and plant health improved. These results underscore the value of integrating biological control methods with organic treatments in a sustainable pest management framework. Although the application of organic amendments did not significantly impact the number of healthy flowers, statistical analysis revealed that managing disease in infected fruits had a much greater influence on overall yield improvement. Therefore, disease suppression, particularly in reproductive plant parts, should be a primary focus in crop protection strategies.

In conclusion, the integration of cultural practices, biocontrol agents, and organic amendments offers a comprehensive and sustainable alternative to synthetic pesticides. Continued research should focus on the long-term field application of these methods to refine their efficacy and promote environmentally sustainable pea production systems.

Acknowledgements

The authors would like to thank the Department of Botany, Degree Campus, Biratnagar, for significant contributions, especially institutional as well as laboratory work, during the overall course of research. We also thank the Immuno-Phytopathology Laboratory, Department of Botany, North Bengal University, India, for providing *Trichoderma harzianum*, a biocontrol agent.

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