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# Allelopathic effect of *Penicillium* sp. and *Fusarium* sp. on the seeds of Pisum sativum

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## ABSTRACT

Plant pathogenic fungi produce several toxic substances by their metabolic processes which include various types of toxins, organic acids, proteins or enzymes etc. These toxic metabolites are responsible for degradation of plant cell wall as well as alteration of several host physiological processes and also effective at chromosomal levels that create abnormalities in chromosome. *Pisum sativum* is used as a tester plant for evaluating the toxic effect of two pathogenic fungi viz., *Penicillum* sp. and *Fusarium* sp. on the seed germination. The seeds of *Pisum sativum* were treated with cell free supernatant (CFS) as aqueous extract of mycelium and plant growth medium of these pathogenic fungi and examined for percentages of seed germination, seedling length, mitotic and abnormality indices. Variation in the results for different concentrations of CFS suggested strong correlation between the concentration of toxic substances and the morphological or physiological abnormalities. During the comparative analysis between two fungal strains, *Fusarium* sp. showed more adverse effect on the seed germination process.

**Key-words:** *Penicillium* sp., *Fusarium* sp., *Pisum sativum*, liquid growth medium, seedling vigour index, mitotic index, abnormality.

## INTRODUCTION

Pulses are the second most important group of food plants of the Family Fabaceae after cereals. It is an important source of dietary carbohydrates, protein, essential amino acids and micronutrients such as calcium, phosphorus and iron. The various seed borne fungi like *Aspergillus flavus, A. fumigatus, A. niger, Drechslera tetramera* and *Rhizopus stolonifera* reported to effect the test pulses i.e., green gram, black gram, chickpea, pigeon pea. The mycotoxins of fungus found to be adversely affecting seed health of test pulses (Kandhare 2015). The metabolites are products of some amino acids, cyclic peptides, aromatic, phenols, terpenoids and plant growth regulators. These metabolites are diverse and they are known to cause diseases in plants, animals and humans who eat infected food. Fungi belonging to the genera *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizoctonia* are commonly known to produce toxic substances, such as aflatoxin B1 and B2, aspergellic acid, cyclopiczonic acid, kojic acid, naphthoquinones, fumonizin and fusaric acid that threaten the health of our plants and animals (Haikel 2008). The role of toxic metabolites of pathogenic fungi in plant disease development has been reported by several workers. Anaso et al. (1981) found out that toxic metabolites of *Drechslera rostrata* and *Fusarium equiseti* retarded root growth of wheat. Reddy et al. (1989) reported that the culture filtrate of seed borne strains of *Alternaria alternate* inhibited the germination and vigour of sunflower seeds and maize kernels. Reduction in seed germination and yield of exotic potato cultures by filtrates of *Pythium* sp., *Chaetomium*

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*globosum* and *Rhizopus* sp. was reported by Dwivedi (1988). Similarly, culture filtrates of *Fusaruim moniliforme*, *F. semitectum* and *F. oxysporum* gave very high percentage reduction in seed germination and also inhibited root and shoot growth of sorghum (Haikel 2008).

Allelopathy is the direct or indirect effect of plants with one another through producing chemical compounds (Rizvi & Rizvi 1992). Allelopathic compounds generally occur in natural plant communities and are suggested to be one mechanism by which they interfere with crop growth (Bell & Koeppe 1972). Primary growth of soyabean seedlings was reduced significantly by the allelopathic effect of DDW fraction of *Echinochloa colona* L. and *Cyperus iria* L. (Chopra et al. 2017). The present study was undertaken to evaluate the allelopathic effect of filtrates of seed-borne fungi (*Fusarium* sp. and *Penicillium* sp.) on the seeds of *Pisum sativum*. *Penicillium* is a genus belonging to Ascomycetes of major importance in the natural environment as well as food and drug production. Some members of this genus produce Penicillin, a molecule that is used as an antibiotic, which kills certain types of bacteria inside the body. Its thallus typically consists of highly branched network of multinucleate, septate usually colour less hyphae. Species of *Penicillium* are ubiquitous soil fungi, preferring cool and moderate climates commonly present.The *Penicillium* have capacity to infect the seeds and other stored foods (Singh & Prasad 2016). *Fusarium* is a large genus of filamentous fungi widely distributed in soil and associated with plants. Some species produce mycotoxins in cereal crops that can affect human and animal if enter into the food chain. Main toxins produced by the different species of *Fusarium* are fumonisins and trichothecenes. The peas are widely used because of their high seed protein content (~20-27%), their balanced amino acid composition, as well as good taste and digestibility (www.asiafarming.com). In the present contribution, the screening of allelopathic interaction of both the fungal genera i.e., *Fusarium* and *Penicillium* on the seeds of *Pisum sativum* has been done by the estimation of seedling vigour index, mitotic index, abnormality index and response coefficient.

## MATERIAL AND METHODS

The study has been carried out in Genetics and Medicinal Plant Biology laboratory, Department of Botany, Visva-Bharati during the month of February 2018 maintaining temperature of 20-25°C. The pure culture of the fungus i.e., *Penicillium* sp. and *Fusarium* sp. were provided by the Mycology Pathology laboratory, Department of Botany, Visva-Bharati and seeds were purchased from the local agricultural shop.

### Preparation of fungal inoculums:

The fungus *Penicillium* and *Fusarium* were cultured aseptically in Potato dextrose liquid medium in conical flasks (of 250 ml capacity) for adequate growth of fungal mycelium. These flasks were incubated at 24°C for 5 days maintaining darkness. After that fungal mycelium were grinded in the mortar and pestle with distilled water and centrifuged at 4000 rpm for 10 minutes. The supernatant were taken for carrying out the experiment. This supernatant was taken as 100% and further diluted to 50% and 25%. The growth medium on which fungus were grown, further diluted to 50% and 25%.

### Standard Blotter Paper Test:

The seeds were surface sterilized with 1% sodium hypochlorite solution and soaked in treatment solutions (aqueous mycelium extract and growth medium of fungus) along with control for 24 hours in the dark. The treated seeds were placed on three layers of moistened blotting papers in the petri plates of 9 cm diameter size. The petri plates were then incubated at ±25°C in alternating cycles of 12 hours light and darkness for 6 days. The two replicates were maintained for each concentration. The seeds were examined after 2 days for percentage seed germination, seedling length, mitotic and abnormality index of root tips (Figure 2). The cytological study was done following the method of Sharma et al. (1994).

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total no of cells}} \times 100$$

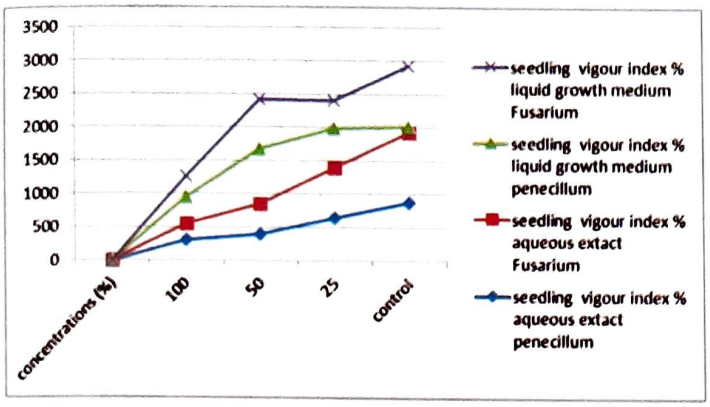


**Figure 1**. Seedling vigour index of *Pisum sativum* treated with aqueous mycelium extract and liquid growth medium of *Penecillium* sp. and *Fusarium* sp.

$$\text{Abnormality index} = \frac{\text{Total no of abnormal sells in treated seeds}}{\text{Total no of cells in division}} \times 100$$

The Seedling vigour index was calculated according Randhawa et al. (1985)

V = L×FG, where L = mean seedling length, FG = final germination percentage (%)

The response co-efficient of aqueous mycelium extract and growth medium on which fungus was grown for both the fungus was also calculated (Figure 3).

$$\text{Response coefficient} = \frac{\text{Value of treated set- value of control set}}{\text{Value of control set}}$$

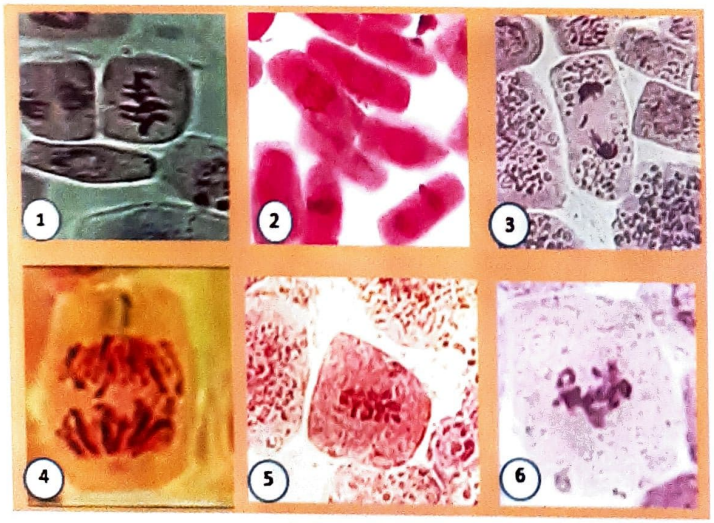


**Figure 2.** Mitotic cell division of root tips of *Pisum sativum* treated with aqueous mycelium extract and liquid growth medium of *Penecillium* sp. and *Fusarium* sp. 1. Normal metaphase, 2. Normal anaphase and telophase, 3. Laggard, 4. Disturbed anaphase, 5. C-mitosis, 6. Multipolarity.

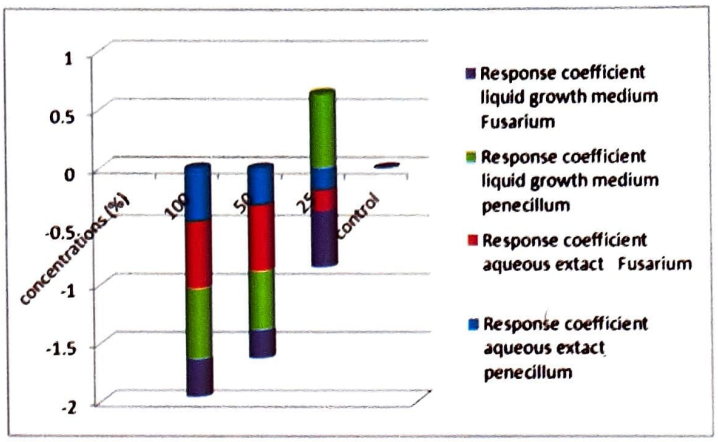


**Figure 3.** Response coefficient of *Pisum sativum* treated with aqueous mycelium extract and liquid growth medium of *Penecillium* sp. and *Fusarium* sp.

## RESULTS AND DISCUSSION

The saturated concentrations of aqueous mycelium extract and filtered liquid growth medium of *Penecillium* sp. and *Fusarium* sp. inhibited seed germination as well as reduced seedling length. The 50% concentration of filtered liquid growth medium recorded more seed germination and seedling length in comparison to 100% saturated and 25% dilute concentrations (Table 1 & 2). The 50% Potato dextrose liquid medium can act as a supplement and support more conducive environment for seed germination and seedling elongation. The Seedling vigour index (Figure 1) of aqueous extract of *Penecillium* sp. and *Fusarium* sp. are of less value than control, but liquid growth medium of *Fusarium* sp. had more or less near value of vigourness in 50% and 25% concentrations respectively. The radicle and plumule length decreased with increasing concentrations resulted to the decrease of seedling length in comparison to the control except 50% liquid growth medium. The toxins produced by fungus may hinder the metabolic pathway or nutrient uptake needed for seed germination or cell division resulted in retarded growth compare to the control. Similar work on effect of fungal metabolites on seed viability and seedling vigour of *Trigonella foenum-graecum* has been reported by Khokhar et al. (2011). The least reduction in seed germination, radicle and plumule length was observed in culture filtrate of *Alternaria alternata* followed by *Penicillium* sp. (Khokhar et al. 2011)

**Table 1. Seed germination percentage of *Pisumsativum* treated with aqueous mycelium extract and liquid growth medium of *Penecillium* sp. and *Fusarium* sp.**

| Concentrations | Aqueous Extract *Penecillium fusarium* | | Liquid growth medium *Penecillium fusarium* | |
|---|---|---|---|---|
| 100 | 60.1±0.05 | 48.7±0.04 | 66.2±0.06 | 56.4±0.03 |
| 50 | 64.2±0.08 | 68.4±0.03 | 89.1±0.04 | 84.2±0.01 |
| 25 | 80.1±0.17 | 88.4±0.13 | 74.9±0.02 | 74.7±0.06 |
| Control | 97.1±0.99 | 100±0.0 | 99.7±0.001 | 99.2±0.02 |

The differences in the values of mitotic index and abnormality index of the root tips in the treated and control seeds of *P. sativum* were recorded. The mycelium extract treated seeds had lower value of mitotic index than in liquid growth medium and control. The 50% concentration of liquid growth medium had recorded good mitotic index due to inducing and ameliorating effect of basic nutrients like carbohydrate, suger etc. present in the the medium which help in enhancement of the seed germination, mitotic cell division and seedling vigourness. The chromosome abnormality like laggard, disturbed anaphase, c-mitosis and multipolarity were observed. The cytological observations showed that mitotic index decreased with increased concentrations, whereas, abnormality increases with increasing concentrations. Moreover, it was noticed that mycelium fungal extract and growth medium did not relatively impose much damage on the seed germination, seedling length and mitotic cell division. The mitotic abnormality may be due to toxins produced by the particular fungus under study which can bind to tublin and either inhibits tublin assembly or cause depolymerisation of assembled microtubules (Kiso 2004). The response coefficient is a criterion for determining the effectiveness of treatment which indicate that all saturated concentrations have negative effect on seed germination, seedling length and mitotic index. The treatment of seeds with aqueous mycelium extract of *Penecillium* sp. and *Fusarium* sp. were much toxic than liquid growth medium which can act as a nutrient source for better seedling growth. Our observations in the experiment suggested that *Fusarium* sp. had much adverse effect on seed germination, seedling length and mitotic index than *Penecillium* sp.

**Table 2. Seedling length (cm) of *Pisumsativum* treated with aqueous mycelium extract and liquid growth medium of *Penecilliumsp.* and *Fusarium sp.***

| Concentrations | Aqueous Extract *Penecillium fusarium* | | Liquid growth medium *Penecillium fusarium* | |
|---|---|---|---|---|
| 100 | 5.1±0.05 | 4.7±0.04 | 6.2±0.06 | 5.4±0.03 |
| 50 | 6.2±0.08 | 6.4±0.03 | 8.1±0.04 | 7..2±0.01 |
| 25 | 8.1±0.17 | 8.4±0.13 | 7.9 ±0.02 | 6.7±0.06 |
| Control | 9.1±0.99 | 10.1±0.0 | 9.7±0.001 | 9.2±0.02 |

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