INVESTIGATION OF PEA (*Pisum sativum* L.) SEED MYCOFLORA AND ITS CONTROL

A Dissertation Submitted for the Partial Fulfillment of
M. Sc. Degree in Botany

BY
PRUSHA VAIDYA (JOSHI)
Reg. No. – 5-1-33-582-97
Roll No: 274

Central Department of Botany
Tribhuvan University,
Kathmandu, Nepal

RECOMMENDATION

This is to certify that the dissertation work entitled **'INVESTIGATION OF PEA (*Pisum sativum* L.) SEED MYCOFLORA AND ITS CONTROL'** submitted by Ms. Prusha Vaidya (Joshi) for the partial fulfillment of M.Sc. degree in Botany, has been performed under my supervision. The entire work is based on the results of her own work and has not been submitted for any other degree to the best of my knowledge.

Date: - 2069-5-3

Dr. Usha Budathoki

Professor, Central Department of Botany, T.U., Kirtipur

TRIBHUVAN UNIVERSITY

INSTITUTE OF SCIENCE AND TECHNOLOGY

CENTRAL DEPARTMENT OF BOTANY

OFFICE OF THE HEAD OF DEPARTMENT

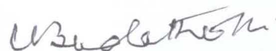
KIRTIPUR, KATHMANDU
NEPAL

Ref No:

LETTER OF APPROVAL

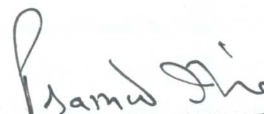
This dissertation paper submitted by Ms. Prusha Vaidya (Joshi) entitled "INVESTIGATION OF PEA (*Pisum sativum* L.) SEED MYCOFLORA AND ITS CONTROL" has been accepted as a partial fulfillment for Master's of Science in Botany.

Expert Committee



....

Dr. Usha Budathoki
(Research Supervisor)
Professor
Central Department of Botany
T.U., Kirtipur



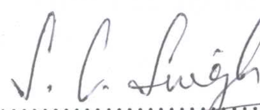
....

Dr. Pramod Kumar Jha
(Head of Department)
Professor
Central Department of Botany
T.U., Kirtipur

Head
Central Department of Botany

....

Dr. Ram Dev Tiwari
(Internal Examiner)
Professor
Central Department of Botany
T.U., Kirtipur



....

Sailesh Chandra Singh
(External Examiner)
Professor

Date of Examination: September 20, 2012

Phone: 4331322, 4332636, Fax: ++977-1-4333722, Post Box: 26429
E-mail: info@cdbtu.edu.np, Webpage: www.cdbtu.edu.np

ACKNOWLEDGEMENT

First of all I would like to acknowledge Central Department of Botany, Tribhuvan University for providing the grant to conduct this study.

My sincere thanks goes to my supervisor Professor Dr. Usha Budathoki, who has effortlessly guided, encouraged and supported me ever since the beginning of this research.

My special thanks goes to our Department Chief Professor Dr. Pramod Kumar Jha for providing all the necessary facilities during my laboratory work.

I am also indebted to my teacher Mr. Shiva Devkota, Mr. Pramesh Lakhey for their valuable support. Similarly my deep acknowledgement goes to my friends. I am very grateful to all who helped me directly or indirectly to complete my work and my heartiest thanks goes to all teaching and non teaching staff of the Central Department of Botany. Lastly, I have no words to describe the support of my family members, who, always have shown patience and support in all I have done.

ABSTRACT

The study aims to isolate the seed mycoflora of Pea (*Pisum sativum* L.) and control the pathogens. Seed sample of pea were collected from local markets of Kathmandu valley and randomly mixed. Three standard methods recommended by the International Seed Testing Association, 1950 were performed, *viz.* the standard blotter technique, the agar plate method and the moist sand method.

Total 11 fungal pathogens were isolated by all the three methods. The agar plate method was comparatively the most suitable method for the isolation purpose because large number of fungal species was isolated adopting this method. *Aspergillus clavatus, Penicillium sp., Rhizopus sp., Cladosporium sp.* etc. were isolated only in unsterilized seed so they are labelled as surface contaminants. Rest of the species like *Aspergillus niger*, *Aspergillus flavus, Aspergillus fumigatus, Fusarium sp.* were proved as truly seed-borne species because they are found in both sterilized and unsterilized seeds.

Effect of some fungicides like Bavistin, Derosal and Dithane M-45 against the control of test pathogens, *Aspergillus niger* and *Aspergillus flavus* were studied by the sand and the thread method. The sand method showed Derosal was effective in controlling *Aspergillus niger* while Bavistin was effective in controlling *Aspergillus flavus*. In thread method, Bavistin and Derosal prevented the growth of pathogens. In conclusion, Derosal was found most effective against both test pathogens.

CONTENTS

	Page No.
Acknowledgements	
Abstract	
List of Tables and Figures	
1. INTRODUCTION	1–9
2. LITERATURE REVIEW	10–13
3. MATERIALS AND METHODS	14–17
4. RESULT	18–25
5. MORPHOLOGICAL CHARACTER OF TEST PATHOGENS	26-29
6. DISCUSSION	30–33
7. SUMMARY	34
8. REFERENCES	35–40
9. PHOTOGRAPHS	41–46

LIST OF TABLES AND FIGURES

Tables

1. Isolation of fungi by the Standard Blotter Technique 18
2. Isolation of fungi by the Agar Plate Method 19
3. Isolation of fungi by the Moist sand Method 20
4. Pathogens in any of three methods employed for isolation 21
5. Effect of different fungicides on pre and post- emergence rot of pea seeds 23
6. Growth of two pathogens on sterilized inoculated threads treated with three fungicides 24

Figures

1. Effect of fungicides on *Aspergillus niger* 25
2. Effect of fungicides on *Aspergillus flavus* 25

1. INTRODUCTION

Many of the fungi are so prevalent and abundant that they must be considered one of the more successful forms of life. They are among the chief causes of diseases in plants. They cause heavy loss in seeds of all kinds, especially when they are stored. Roughly these fungus parasites can be divided into “facultative” (dead material) parasites and “obligate” parasites. The majority of fungi that cause diseases in plants are “facultative” parasites and some of our most destructive plant diseases are caused by them. They cause heavy losses not only in all our crop plants but also in all the goods and materials processed from these plants and spoil many kinds of manufactured products derived from things other than plant or plant products.

The quality of seed plays an important role in the production of healthy crop. Generally the quality of seed is effected by fungi, bacteria, virus and nematodes as seeds are attacked by devastating seed borne diseases. A seed is a small embryonic plant enclosed in a covering called the seed coat, usually with some stored food. The term seed also generally means anything that can be sown. Some seeds are edible; others are harmful, poisonous or deadly. Many seeds are edible and the majority of human calories come from seeds, especially from cereals, legumes and nuts. Seeds also provide most cooking oils, many beverages and spices and some important food additives. In different seeds, the seed embryo or the endosperm dominates and provides most of the nutrients. The seeds of many legumes, including the common bean (*Phaseolus vulgaris*), contain proteins called lectins which can cause gastric distress if the beans are eaten without cooking. The common bean and many others, including the soybean, also contain trypsin inhibitors which interfere with the action of the digestive enzyme trypsin. Normal cooking processes degrade lectins and trypsin inhibitors to harmless forms. Particularly in developing countries, a major constraint is faced in the inadequacy of the marketing channels to get the seed to poor farmers. Thus, the use of farmer-retained seed remains quite common. Seeds are also eaten by animals, and are fed to livestock. Many seeds are used as birdfeed.

Seed is the primary and essential starting point of a wide range of horticultural crops. Seed germination and seedling emergence are affected by many factors such as seed genotype, seed quality (seed viability and seed vigor) and the environmental conditions (moisture and temperature). Seeds of cereal grains and of many other crop plants may be invaded by a variety of fungi before the seeds mature. Approximately, 50 genera of fungi have been isolated from seeds of barley. When ground up and cultured, tens of thousands of individual colonies of fungi, hundreds of thousands of colonies of yeasts, millions of colonies of bacteria, which indicate that seed, is not just seed.
All seeds contain vitamins. The presence of vitamin is especially important in those seeds which are used for food or fodder. The figures shown below are for the Air- dry Seeds (From Food Composition Tables, FAO, 1954).

mg/100g					I.U.
Peas	Thiamin	Riboflavin	Nicotinic Acid	Ascorbic Acid	Vitamin A
	0.72	0.15	2.4	4	100

As seeds are carrier of some important disease inciting micro-organisms, there are often considerable losses in the yield due to diseases arising by their attack. Disease interferes with normal function of the seeds and may be physiogenic caused by the direct effect of unfavorable environmental factors. Among these different factors, many fungi cause prominent or severe loss in seed due to association as spores and spore bearing structures like sclerotia, pycnidia, perithecia, acervuli simply mycelia fragments inside or outside the seed. However all these fungi are not always able to produce diseases. Skoric (1927) showed that *Pseudomonas pisi* on peas penetrates through the micropyle into the seed coat. Plant pathogenic fungi are commonly associated with seed of agricultural, horticultural and forestry crops. This intimate association of seed and pathogen was reported over two hundred years ago by 'Tillet' who in 1755 proved in France that the seed of wheat could transmit bunt (*Tilletia sp*) through contamination by spores invisible to naked eye. Apparently it was Hellwig who first demonstrated a pathogen (*Claviceps purpurea*) accompanying seed of rye. Later Michelle reported seed transmission of a

pathogen (*Orobinche miones*) on beans. While Needham reported the internal seed transport on wheat and in 1833 Frank reported the internal seed transmission of *Colletotrichum lindemuthianum* on bean. Scarmeas et al., 2006; Scarafoni et al., 2007; Zhang,2007; Tang et al., 2008; Villegas et al., 2008 showed that legume seeds help to prevent cancer.

Seed-borne mycoflora is a generalized term indicating the association of fungi with seeds, which may or may not have the potential of causing diseases of the seed or plant. The term externally or internally seed-borne refer to the location of pathogen in relation to the seed. If a pathogen is located on the outer covering of the seed, it is externally seed-borne, if inside the seed, it is known as internally seed-borne. Moreover seed-borne pathogens may have higher possibility to establish and spread all over the country when they are introduced. It is very important therefore to inspect the imported seeds thoroughly, whether from outside the country or not they are infested by the harmful seed-borne pathogens.

Treatment of seed is aimed to prevent infection of the seedling and the subsequent crop, but should be appropriate to the type of seed to be treated. Thus, for disinfection or protection of seed against pathogenic organisms a considerable range of physical and chemical procedures have been use. For this, fungicides used are mercuric compounds such as Ceresan, Mercuran, Germisan etc. and non-mercuric fungicides like Captan,Thiram, Dithane M-45, Maneb, Zineb, Bavistin etc, were developed. In Nepal, seed health tests of various economically important crops have been carried out in the section of seed pathology under the Ministry of Agriculture, Khumaltar.

The National Plant Quarantine Service of Korea carried out the project on the detection of seed-borne pathogens from 2001-2003.All seed-borne pathogens are potentially harmful to a crop. It was common and severe in leguminosae and that they are also prominent in almost any other plant family. Almost all the seedlings and plants raised from the diseased seeds will most likely be diseased and the seeds produced from these plants also become diseased. Seed also transfer diseases from diseased area to disease

free area. Transmission of pathogens by seed is very important. A large number of fungal pathogen is transmitted through the seeds. The direct transmission of fungi on the seed is considerable. Many fungi are serious parasites of seed primordial and maturing seeds and reduced yields of seed both quantitatively and qualitatively. Other fungi including saprophytes and very weak parasites may lower the quality of seeds by causing discoloration which may seriously depreciate the commercial value of seeds, particularly of grain when graded for consumption. The following types of disease and disorder are encountered.

1. Seed Necroses:

Many seed-rotting fungi produce superficial necroses on the seed; other fungi never penetrate deeply into the tissues. Most seed-borne fungi usually not found beyond the protective layers, the seed coat or pericarp. In Leguminosae seeds anthracnose fungi, *Colletotrichum spp.* as well as *Ascochyta spp.* often penetrates into the fleshy cotyledons, producing conspicuous necrotic lesions in seeds of bean, soybean, pea, chickpea, cowpea and other hosts. (Neergaard 1977)

2. Seed discoloration, toxic production by physiological alternation and reduction of size:

Discoloration of seeds is a very important degrading factor. There are at least three categories of seed discoloration. When causes and effects are considered: (1) superficial necrotic lesions, (2) fungous coatings and (3) pigmentation. Many seed-borne parasitic fungi infect the seed coat causing conspicuous necrotic black, brown to grey discoloration. Well known examples are the effects of *Ascochyta pisi* in pea; *Colletotrichum lindemuthianum* in bean. Seed-borne fungi cause death of the ovule shriveling of the seeds that reduces the size of the seed. It may discolor or damage the whole seed which may reduce the grade and price of the grain, by bringing about biochemical changes, make grains unfit or unattractive for food. They also produce toxins that causes disease in human and animals when consumed. They also cause

heating which usually is accompanied by drastic reduction in quality by complete spoilage of seed. (C.M.Christenson and H. H. Kaufman, 1965).Pathogenic fungi may be so profusely present in seeds that these appear discolored; heavy sporulation of *Fusarium graminearum*, scab or head blight in wheat makes the kernels pink or orange.

3. Seedling blight and rot:

Many seed–borne fungi produce seed rot either in the crop or during germination. Seedlings coming out of infected seeds get often blighted. *Alternaria spp*. causes blight of radish seed. *Fusarium spp.*, including *F. moniliforme*, *F. graminearum*, *F. nivale*, F. *semitectum*. Of which *F. semitectum* may cause dry rot in rice grains.

4. Disease symptoms on adult plants:

Certain seed-borne fungi produce symptoms in mature plants as they are particularly pathogenic to flowers and young seed structure of the host where they are replaced by the fructification of the parasites. Many smut diseases, e.g. loose smuts of wheat and barley, loose smuts of oats and loose smuts of jowar, which loose their infectivity as seeds approach maturity. In these cases, controlling of seed infection will completely control the disease. On the other hand, there are many more pathogens that may be found in plant debris mixed with seed or as contaminants on the seed itself. E.g. *Sclerotina sclerotiorum, Alternaria sp., Tilletia sp., Fusarium oxysporium, Claviceps purpurea* etc. Here, eliminating seed infection will only give partial control of these diseases depending upon the amount of disease brought about by seed infection and that through inoculums surviving in soil and plant debris (Neergaard, 1977)

5. Reduction or elimination of germination capacity:

The most important damage caused by seed–borne fungi is reduction in germination capacity and thus decrease the germination percentage. As in paddy, maize etc., several storage species like *Aspergillus flavus, A. glaucus*, *A. candidus, Curvularia lunata,*

C.pallescens, *Fusarium moniliforme*, infect outer seed coat, endosperm or embryo of seeds and damaged by producing non specific toxins which reduce the germination capacity of seed.

Vegetables are important crops of economic value in Nepal. They are important components in the daily human diet all over the world. Value of vegetable production is 45 percent greater than that of fruit production (APP, 1995).Nepal is not self-reliant in vegetable production. Major factors for insufficient vegetable production include poor technical knowledge, weak marketing system, ineffective management of farmer's cooperative, post harvest loss, instability of vegetable price and significant pest damage. The basic of the high yield and the good quality of the vegetable is the good propagation material, the top quality of germinative seed. The demand for high seed quality that exhibit early, uniform, vigorous seedlings, early and high fruiting of good quality from each seed sown at optimum or adverse conditions has been increased greatly in recent years.

People took most of their protein from the crops. There are more than 500 varieties of pulses that play a useful role in increasing soil fertility by an association with nitrogen-fixing bacteria. The Leguminosae or Fabaceae is the third largest flowering plant family, containing 19,400 species, and accounts for over 8% of the world's flowering plants (www.kew.org/science-research-data). The legume or bean family includes lentils, peas, beans, peanuts and soybean and are hugely important as a source of food due to its high protein content.

Pea is among the fourth important cultivated legumes next to soybean, groundnut, and beans (Hulse, 1994). It is a cool season crop. It is an important vegetable crop of the tropics. It is the predominant export crop in world trade and represents about 40% of the total trade in pulses (Oram and Agcaoili, 1994). It is originally called pisos by the Greeks and pisum by the Romans. It is grown all over the world for its fresh use, preservation and high level of digestibility which is more than most of the legumes. The green, smooth pods are straight or slightly bent, rounded or flat, and hold seeds of variable size,

round in shape or slightly square. Usually green, these seeds can be grayish, whitish or brownish. It is the seed-pod of the pod fruit *Pisum sativum*. Each pod contains several peas. Owning to great nutritional importance, cultivation of peas in the world is increasing. It provides much-needed protein to complement the starches of the cereals. Wild peas generally have a rough or granular seed surface, while domesticated peas are characterized by a smooth seed coat. The appearance of a smooth seed coat is thought to be the most reliable indicator of domestication. In many regions, especially where meat is scarce or expensive, legumes like peas, beans, lentils, peanuts, and soybeans fulfill the diet.

In the U.S.A. average annual losses of the national production of green peas for the period 1951-1960 (USDA, 1965) were estimated at 2 percent for 'Ascochyta blight' which, however, may not include *Mycosphaerella* foot rot, a part of the Ascochyta complex a further 2 percent for *Fusarium* wilt 1 percent for bacterial blight, totaling 5 percent. Total world dry pea production rose from 8.127 million metric tons in 1979-81 to 14.529 million metric tons in 1994 while acreage varied from 7.488 to 8.060 million hectares for the same years (FAO, 1994). The highest productivity for pea was reported in France at 5088 kg per hectare in 1994, about eight times more than the African average yield. In 1994, USA total acreage was 54, 000 hectares with an average yield of 2587 kg per hectare (FAO, 1994). France, Russia, Ukraine, Denmark and United Kingdom in Europe; China and India in Asia; Canada and USA in North America; Chile in South America; Ethiopia in Africa, and Australia (FAO, 1994) were the important pea production areas of the world. It was grown for home use or for fresh market, are picked by hand before the seeds are fully matured and still in the pod and are used for immediate consumption.

Seed germination can occur over a wide range of soil temperatures. The optimum temperature for seed germination is 20°C. The germination rate increases with increasing temperature. At temperature greater than 25°C, germination percentage decreases. Seeds germinate slowly at 16°C, for good healthy seedlings, the soil temperature should be at least 18°C. The optimum mean temperatures for pea growth are between 13°C and 18°Cs

and growth stops above 29°C. However, peas are considered to be a vegetable in cooking. It is an annual plant, with a life cycle of one year. Planting can take place from winter to early summer depending on location. The average pea weighs between 0.1 and 0.36 grams. At higher temperatures germination is rapid, but seedlings may die from various pathogens in the soil. As temperature rises during growing season, yield drops off rapidly.

It was cultivated for the fresh green seeds, tender green pods, dried seeds and foliage (Duke, 1981). Green peas are eaten cooked as a vegetable, and are marketed fresh, canned, or frozen while ripe dried peas are used whole, split, or made into flour (Davies et al., 1985). In some parts of the world, dried peas are consumed split as dahl, roasted, parched or boiled. Green peas are the number one processed vegetable specifically in UK and USA. Green foliage of garden pea is also used as vegetable in parts of Asia and Africa. Some cultivars are grown for their tender green pods, which are eaten cooked or raw. It is being used in a growing snack market. Fresh peas are used in various dishes such as aloo matar or matar paneer. Dried peas are often made into a soup or simply eaten on their own. It is also eaten raw, as they are sweet when fresh off the bush. In modern times, however, peas are usually boiled or steamed, which breaks down the cell walls and makes the taste sweeter and the nutrients more bio-available.

There are many varieties of garden pea. Some of the most common include the following:

- *Pisum sativum* var. *macrocarpon* is commonly known as the snow pea.
- *Pisum sativum* var. *macrocarpon* ser. cv. is known as the sugar or snap pea.

Peas are high in fiber, protein, vitamins, minerals, and lutein. The protein concentration of peas range from 15.5-39.7% (Davies et al., 1985; Bressani and Elias, 1988). Fresh green peas contain per 100 g: 44 calories, 75.6% water, 6.2 g protein, 0.4 g fat, 16.9 g carbohydrate, 2.4 g crude fiber, 0.9 g ash, 32 mg Ca, 102 mg P, 1.2 mg Fe, 6 mg Na, 350 mg K, 405 mg carotene equivalent, 0.28 mg thiamine, 0.11 mg riboflavin, 2.8 mg niacin,

and 27 mg ascorbic acid, while dried peas contain: 10.9% water, 22.9% protein, 1.4% fat, 60.7% carbohydrate, 1.4% crude fiber, and 2.7% ash (Duke, 1981; Hulse, 1994). Flour contains: 343 calories, 10.9% moisture, 22.8 g protein, 1.2 g fat, 62.3 g total carbohydrate, 4.2 g fiber, 2.8 g ash, 72 mg Ca, 338 mg P, 11.3 mg Fe, 0.86 mg thiamine, 0.18 mg riboflavin, and 2.8 mg niacin (Duke, 1981). Seeds are thought to cause dysentery when eaten raw. In Spain, flour is considered emollient and resolvent, applied as a cataplasm. It has been reported that seeds contain trypsin and chymotrypsin which could be used for contraceptive, ecbolic, Fungistatic and spermicide (Duke, 1981).

Peas are affected by fungal diseases like, *Ascochyta pisi*, *Cladosporium pisicola* (leaf spot or scab), *Erysiphe polygoni* (powdery mildew), *Fusarium oxysporium* (wilt), *Peronospora pisi* (downy mildew), *Pythium sp*. (pre- emergence damping-off), *Botrytis cinerea* (grey mold), *Aphanomyces euteiches* (common root rot), *Thielaviopsis basicola* (black root rot), and *Sclerotina sclerotiorum* (Sclerotina white mold). Pea Early Browning Virus (PEBV), Pea Enation Mosaic virus (PEMV), Pea Mosaic Virus (PMV), Pea top yellows (PTY), Pea seed-borne Mosaic Virus (PSbMV) and Pea Streak Virus (PSV) constitute diseases caused by viruses, while the most important bacterial disease is caused by *Pseudomonas pisi* (bacterial blight).Important seed-borne disease of *Pisum sativum* L. are Leaf, Stem and Pod Spot - *Ascochyta pisi,* Powdery Mildew- *Erysiphe polygoni,* Wilt- *Fusarium oxysporium f. pisi. ,* Alternaria Blight- *Alternaria spp*., Bacterial Blight- *Pseudomonas pisi* and Anthracnose- *Colletotrichum pisi*.

2. LITERATURE REVIEW

All available literature, which seems to meet the requirement of present work, had been reviewed through related papers and were cited.

Tillet in 1755(Cited from G. Rangaswami 1979) proved the transmission of bunt in wheat.

Storage fungi of the genera *Aspergillus* and *Penicillium* may also appear with high moisture. These fungi produce aflatoxins which damage the liver and induce carcinogenic, mutagenic and teratogenesis (Pereyra *et al*., 2008).

Skoric (1927) showed that *Pseudomonas pisi* in peas penetrates through the micropyle into the seed coat.

Sharma *et.al*. in 1989 found that Vitavax (carboxin), a fungicide seed treatment has been utilized by several investigators not only to control fungal pathogens but also to improve percentage and rate of seed germination, to increase seedling growth and yielding, in rough lemon seeds.

Flentje (1964a) demonstrated that the attack of pea seeds by *Pythium* is usually occurred from 48–96 hours after planting and if no attack by *Pythium* that occurred within the first 96 hours then no attack took place at all. He also reported that the attack by the fungi may preceded by the leakage of materials from the germinated pea seed into the soil in the vicinity of the seed, leading to stimulation and prolific growth of *Pythium* around the seed, causing rotting. He found that the percentage of seeds attacked increased with increased of soil moisture level from wilting point to field capacity and soil moisture may affect the leakage of materials from the seed rather than have a direct effect on *Pythium* activity.

Extreme temperatures are not preferable to growth and development of the seeds. Halligan (1986) observed that at high temperature and seed moisture of 70-80 %, the incidence of hollow heart was the highest and it increased with the length of exposure to high temperature. At pod wrinkle stage (70-80% moisture content), exposure for 5 days resulted in 20 % of the seeds had hollow heart. Over 80 % of the seeds had hollow heart symptom after 5 days exposure to daily mean temperature of 32.5°C.

Treatment of seeds with very low concentration of alcohol, methanol, ethanol and isopropanal resulted in increased storability of pea (Bhattacharya and Basu, 1990).

Moussart *et. al*. (1998) stated that infected seeds caused significant losses due to poor and high disease transmission to parts of the plant under soil level. Losses were more serious by low temperatures during the early stages of the crop development.

Gibberellins and Naphthalene acetic acid (NAA) were utilized to improve seed germination, seedling emergence and seedling height in some ornamental plants (Renard and Cler, 1978; Abdulla and McKelvie, 1980; Grzesik and Chognowuski, 1992).

Fusarium spp. is the most common root rot pathogens isolated from peas in Alberta (Sumar & Howard 1979; Hwang & Chang 1989).

Seed rot and seedling damping-off caused by *Pythium* species are considered a major limiting factor in pea production in Alberta (Hwang et al. 1997) *Pythium* species are common in Alberta soils.

Thielaviopsis basicola Berk & Br.. is considered a serious root rot pathogen of peas in some pea growing areas of the United States. Although reported in Ontario, it is not yet considered a problem in Canada (Tu l987a).

Susuri *et*. *al*. in 1982 found that *Alternaria alternata* (Fr.:Fr.) Keissl. is the causal organism of Alternaria Blight. Although symptoms can be damaging to a pea plant, this

disease is not considered to be of economic importance because of its infrequent and isolated occurrence.

In Anonymous 1998a, the number of fungicides registered for use on peas in western Canada is limited. At the current time, there are only three fungicides for use as seed treatments and two as foliar sprays. Thiram (Thiram 75WP) and Captan (Captan FL) are registered as seed treatments for control of seed decay, seedling blight, damping off and root rot and Metalaxyl (Apron FL) is registered for control of seed rot and seedling blight caused by *Pythium* spp. Thiram and Metalaxyl can be mixed together to give a broader spectrum of protection.

Hwang *et al.* in 1997 found that seed treatment will prevent the introduction of seed-borne pathogens from diseases such as Mycosphaerella blight. It will also provide protection against soil pathogens such as *Pythium spp.*, *Fusarium spp.* and *Rhizoctonia solani* as the seed germinates and emerges from the ground.

Shah and Jain in 1993 and Klich *et al*. in 1994 found that seed infection can be effectively reduced if seeds are treated with suitable agents before sowing. Fungicidal seed treatments are known to reduce the seed- borne mycoflora and thereby improve the seed germination.

Crocker and Barton in 1957 mentioned that Plant disease organism associated with seed was reported over 200 years ago and long before that time, farmers had recognized.

According to seed health test carried out by Shrestha in 1986 on some legumes of Nepal, seed-borne organisms include such fungi as *Myrothecum verrucaria* on horse gram, *Colletotrichum lindemuthianum* on common bean and soybean, *Macrophomina phaseoli* in moth bean and soybean.

Suryanarayanan and Suryanarayanan in 1990 isolated seed-borne fungi in stored sunflower seeds eight spp. of fungi- *Aspergillus flavus* , *A. nidulas* , *A. glaucus*,

Chaetomium abunse, *Fusarium solani*, *Mucor sp*., *Rhizopus nigricans* and *Penicillium sp*. were found of associated with stored k1 variety of sunflower seeds.

Nair, Neeta and Arora in 1994 isolated nine seed-borne fungal pathogens from legume crops, *Alternaria alternata, Verticillium sp. Aspergillus niger, A.flavus, Phoma sp., Rhizoctonia bataticola, Gliocladium viriens*, *Botrydiplodia theobromea* were obtained from diseased and percentage was very high in all the healthy seeds followed by wrinkled and diseased seeds.

Nayak B.K. *et. al.* in 1995 observed the seed-borne fungi and their effects on germination and seedling mortality in black gram. They have used agar plate and blotter method for the isolation of fungi. A total of 37 fungi taxa including with *Aspergillus spp.* were isolated which were responsible for seedling rot during germination.

According to the test for seed borne fungi carried out by Verma in 2004, total of 22 fungi were associated with pea seeds, among which *Gliocladium virens*, *Cladosporium cladosporioides*, *C. herbarum*, *Rhizopus stolonifer*, *Pythium ultimum* and *Absidia cylindrospora* were new to India.

According to the test for seed-borne fungi conducted by standard International Seed Testing methods carried out by Haware in 1967, seed-borne organisms include fungi like *Alternaria*, *Aspergillus*, *Rhizopus*, *Mucor* and *Fusarium*. *Fusarium* and *Rhizopus* were dominant in all the varieties. They were also responsible for reduction in germination percentage of seeds.

Professor Noble established a seed testing station at Thrandt, near Dersden, in East Germany in 1969.

MacDonald *et. al* in 1996have found the incidence of *Fusarium spp*. On maize from Central America, Africa and Asia during 1992-1995 *F. monoliforme* was isolated as the mast frequently found sp. among all fungi species.

3. MATERIALS AND METHODS

Pea seeds were collected from the local markets of Kathmandu valley for the purpose of observation. All the seed samples collected from different places were mixed together to make composite samples from which “working sample” were made for the actual test. During the investigation period, Standard rules as recommended by the International Seed Testing Association, 1950 were performed.

3.1 Isolation of fungi:-

Three methods were used for the isolation of fungi as follows:

3.1.1 Standard Blotter Technique (De Tempe, 1953)

3.1.2 Agar Plate Method (Muskett, 1948)

3.1.3 Moist Sand Method (Surya Narayan and Bhombe, 1961)

3.1.1 Standard Blotter Technique:-

The blotter method is one of the isolation methods where seeds are incubated in petridish containing moistened blotter paper. First of all, clean petridishes were sterilized in hot air oven at 160 °c for 2 hours and blotter paper in autoclave at 121° c at 15lb/mg pressure for about 30 minutes. Then these sterilized petridishes were provided with three layers of well water soaked sterilized blotter paper of appropriate size, moistened by sterile water so as to retain the moisture during the periods of experiments. Then seeds were taken from the working sample, half of the sample was surface sterilized with 90% ethyl alcohol for 1-3 minutes and washed with sterilized water for three times by ringing process (Kemetz, Ellet, Schmithener 1978). Half of the sample was unsterilized. Then, sterilized as well as unsterilized seeds were placed asceptically in petridishes. Each plate

contains six seeds at equal distance on moist sheet. All the plates were moistened with sterilized water throughout the experiment.

3.1.2 Agar Plate Method:-

In the agar plate method, seed-borne inoculums were detected and identified based on characters of colonies on agar developing directly from seed. In this method, seeds were placed in sterilized petridishes containing potato-dextrose agar media. First of all, Potato-Dextrose Agar (PDA) media was prepared. The medium was sterilized in autoclave at 121°c and 15lb/mg pressure for about 30 minutes. Then, about 10ml sterilized medium was poured in each sterilized petridishes. Sterilized as well as unsterilized seeds were placed asceptically in petridishes, six seeds at equal distance in each plate after the agar media was solidified. Then petridishes were incubated in incubator at maintained temperature 25±2ºc upto 28 days. After incubation upto 28 days, observation of seed mycoflora was done and the isolation of fungal colonies were carefully performed.

3.1.3 Moist Sand Method:-

In this method, the sand was sterilized in autoclave at 121°c and 15lb/mg pressure at 30 minutes. Then sterilized as well as unsterilized seeds were placed asceptically in sterilized petridishes containing well soaked sterile sand, six seeds at equal distance per plate. The plates were moistened with sterilized water throughout the experiment.

All the plates were labelled as ‘SS’ for the surface sterilized seeds and ‘USS’ for the unsterilized seeds, incubated at maintained temperature, 25±2°c. After incubation upto 28 days, observation of seed mycoflora was done .

The isolation of fungal colonies was carefully performed in PDA culture slants by using single hyphal tip method. Then the fungi were identified with preparing slides with the help of following books:

a. “ A manual of soil fungi” by Joseph Gilman (1975)

b. “Illustrated genera of Imperfect Fungi” by Barnett and Hunter (1972)

c. “The Genera of Hyphomycetes from soil” by George L. Baron(1977)

3.2 Control

To control the seed-borne diseases, fungicides were used to kill or inhibit fungi or fungal spores. Fungicides are chemical compounds. It was used in agriculture and to fight fungal infections in animals. To test the efficiency of different fungicides against the test pathogen *Aspergillus niger* and *Aspergillus flavus* three fungicides were selected.

(a) Bavistin

(b) Derosal

(c) Dithane M-45

Efficiency test of the above fungicides against the test pathogen were evaluated by following methods:-

3.3.1 Sand Method (Neergaard, 1977)

3.3.2 Thread Method (Forsenberg, 1949)

3.3.1 Sand Method:-

In this method, well soaked and autoclaved sand was taken in sterilized earthen pot. Surface sterilized seed were rolled on the 7 days old culture of active test pathogens for 2 hours. Then inoculated seeds were treated with 2gm/kg of fungicides separately for 5 minutes (Neergaard, 1977) and sown in sterilized earthen pot filled with sand and 5 seeds on each pot asceptically at equal distance. Throughout the observation period along with the study of emergence, the sand was kept moistened by sterilized water after 6 days upto 28 days. Besides this a set of control without treatment with fungicides was also maintained. Replications of 3 pots were maintained for each test.

3.3.2 Thread method:-

In this method, cotton threads about 4-5 cm long were sterilized in autoclave at 121°c.Then each piece of thread was infested with test pathogen in slants for few days. Then each infested thread was separately treated with 3-fungicides for about five minutes. The treated threads were then inoculated in the nutrient PDA slants. The growth of the pathogen was recorded throughout the period of observation.

4. RESULT

4.1 Isolation of fungi

Mycoflora of pea seeds were isolated from three different methods were as follows:

4.1.1 Standard Blotter method

The mycoflora isolated by Standard Blotter method is shown below in Table No. 1

Table No. 1

S.no.	Organism	Colony color		SS	USS
		Surface	Reverse		
1	*Aspergillus niger*	Black	White to yellow	+	+
2	*Aspergillus flavus*	Yellow- green	Goldish to red brown	+	+
3	*Aspergillus clavatus*	Blue-green	White, brownish with age	-	+
4	*Aspergillus fumigatus*	Blue-green to gray	White to tan	+	+

Index:

+ = presence of pathogen

- = absence of pathogen

SS = sterilized seeds

USS = unsterilized seeds

4.1.2 Agar Plate Method

The mycoflora isolated by Agar Plate Method is shown below in Table No. 2.

Table No. 2

S.no.	Organism	Colony color		SS	USS
		Surface	Reverse		
1	*Aspergillus niger*	Black	White to yellow	+	+
2	*Aspergillus flavus*	Yellow- green	Goldish to red brown	+	+
3	*Aspergillus clavatus*	Blue-green	White, brownish with age	-	+
4	*Aspergillus fumigatus*	Blue-green to gray	White to tan	+	+
5	*Penicillium sp.*	Blue green, gray green, or olive gray	Pale to yellowish	-	+
6	*Rhizopus sp.*	Initially white and turns grey to yellowish brown in time	White to pale	-	+
7	*Unidentified 1a*	-----	----	-	+
8	*Unidentified 2*	----	----	-	+
9	*Cladosporium*	Olivaceous green to black	Black	-	+
10	*Fusarium sp.*	Cottony White	White	+	+

Index:

+ = presence of pathogen

- = absence of pathogen

SS = sterilized seeds

USS = unsterilized seeds

4.1.3 Moist Sand Method

The mycoflora isolated by Moist Sand Method is shown below in Table No. 3.

Table No. 3

S.no.	Organism	Colony color		SS	USS
		Surface	Reverse		
1	*Aspergillus niger*	Black	White to yellow	+	+
2	*Aspergillus flavus*	Yellow- green	Goldish to red brown	+	+
3	*Fusarium*	Cottony White	White	+	-
4	*Unidentified 1b*	----	----	+	+
5.	*Penicillium sp.*	Blue green, gray green, or olive gray	Pale to yellowish	-	+

Index:

+ = presence of pathogen

- = absence of pathogen

SS = sterilized seeds

USS = unsterilized seeds

Pathogens in any of three methods employed for isolation

Commonly present fungal flora in all of three methods were *Aspergillus niger* and *Aspergillus flavus*. Similarly, *Aspergillus clavatus* and *Aspergillus fumigatus* were present in Blotter Method and Agar Plate Method, *Fusarium sp.* and *Penicillium sp.* were present in Agar Plate Method and Moist Sand Method.

Table No. 4

S. No.	Organism	Blotter	Agar plate	Moist sand
1	*Aspergillus niger*	+	+	+
2	*Aspergillus flavus*	+	+	+
3	*Aspergillus clavatus*	+	+	-
4	*Aspergillus fumigatus*	+	+	-
5	*Penicillium sp.*	-	+	+
6	*Rhizopus sp.*	-	+	-
7	*Unidentified 1a*	-	+	+
8	*Unidentified 2*	-	+	-
9	*Cladosporium*	-	+	-
10	*Fusarium sp.*	-	+	+
11	*Unidentified 1b*	-	-	+

Index:

+ = presence of pathogen

- = absence of pathogen

SS = sterilized seeds

USS = unsterilized seeds

4.3 Control

4.3.1 Sand method

Effect of different fungicides on pre and post-emergence rot of pea seeds

All seeds were observed daily and the pre and post-emergence rot was recorded after 28 days of sowing, then the survived plants were counted according to the following formula:

$$\text{Pre-emergence rot (\%)} = \frac{\text{Total number of ungerminated seeds}}{\text{Total number of planted seeds}} \times 100$$

$$\text{Post -emergence rot (\%)} = \frac{\text{Total number of rotted seedlings}}{\text{Total number of planted seeds}} \times 100$$

$$\text{Survived seedlings (\%)} = \frac{\text{Total number of survived seedlings}}{\text{Total number of planted seeds}} \times 100$$

Table No. 6

Pathogen inoculated	Fungicides used	Pre-emergence rot %	Post-emergence rot %	Survived seedling %
Aspergillus niger	Bavistin	22.85%	17.11%	60%
	Derosal	5.71%	0%	94.28%
	Dithane M-45	0%	94.28%	5.71%
Aspergillus flavus	Bavistin	11.42%	0%	88.57%
	Derosal	20%	0%	80%
	Dithane M-45	0%	97.14%	2.85%

From the above table, Derosal was effective in controlling *Aspergillus niger* while Bavistin was less effective and Dithane M-45 was found as not effective. Similarly Bavistin and Derosal were effective in controlling *Aspergillus flavus* while Dithane M-45 was found not effective. Derosal treatment for *Aspergillus niger* and Bavistin treatment for *Aspergillus flavus* showed the high percentage of survived seedling.

4.3.2 Thread method

<u>Growth of two pathogens on sterilized inoculated threads treated with three fungicides.</u>

Table No. 7

Pathogen inoculated	Fungicides used	Sign of pathogen on the ……………….day								
		3rd	6th	9th	12th	15th	18th	21st	24th	28th
Aspergillus niger	Bavistin	-	-	-	-	-	-	-	-	-
	Derosal	-	-	-	-	-	-	-	-	-
	Dithane M-45	+	+	+	+	+	+	+	+	+
Aspergillus flavus	Bavistin	-	-	-	-	-	-	-	-	-
	Derosal	-	-	-	-	-	-	-	-	-
	Dithane M-45	+	+	+	+	+	+	+	+	+

Index:-

+ = indicates growth

- = indicates no growth

Thus, from the above result it can be concluded that the most effective fungicides for all cases are Bavistin and Derosal while Dithane M-45 is least effective for all.

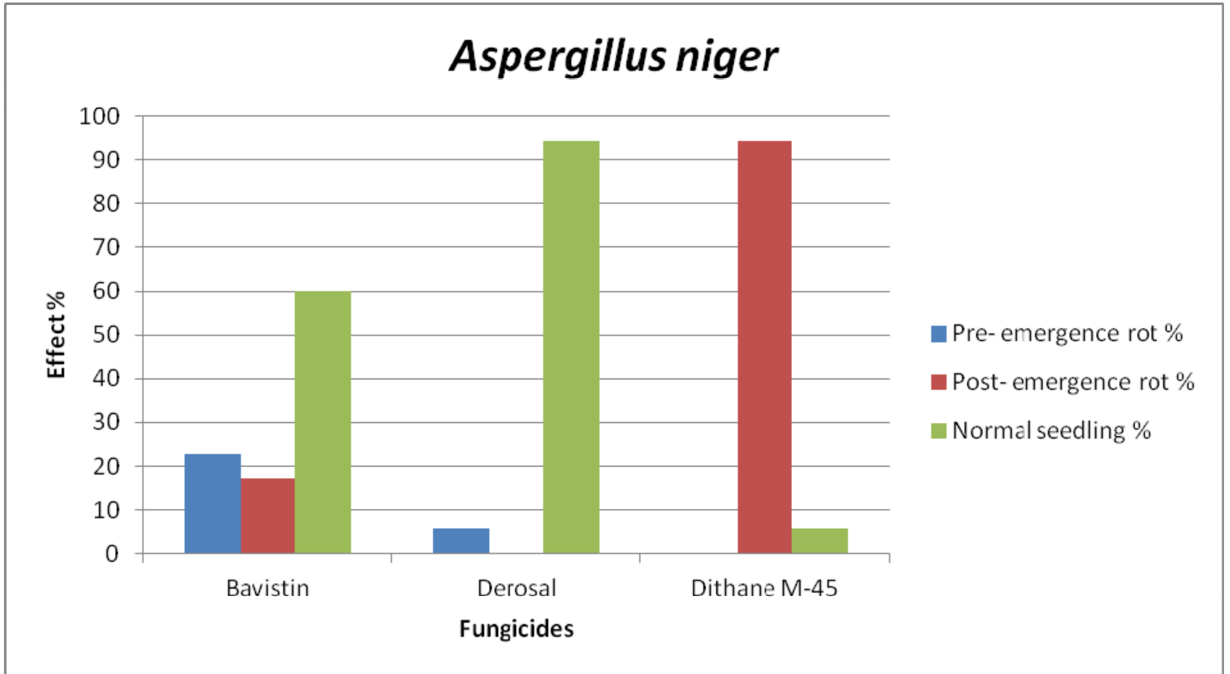


Fig no. 2 -<u>Effect of fungicides on *Aspergillus niger*.</u>

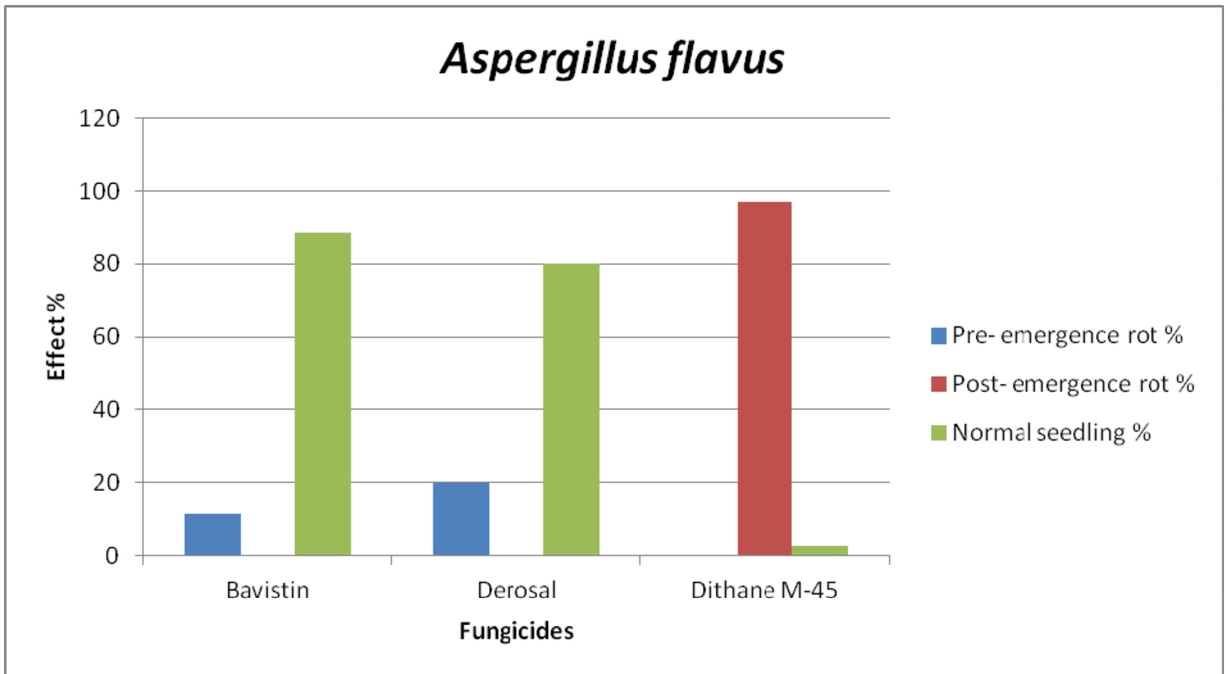


Fig no. 3 -<u>Effect of fungicides on *Aspergillus flavus*.</u>

5. MORPHOLOGICAL CHARACTER OF TEST PATHOGENS

Aspergillus clavatus **Desmazieres 1834**

Colonies are blue green with a white, brownish reverse. Conidiophores with walls smooth, colorless, up to one to several millimeters in length, commonly 15-20µm in diameter, gradually enlarged at the apex into a clavate vesicle which is fertile over an area up to 150µm long x 20-25µm in longest diameter. Phialides 7-10 x 2-3µm, in a single series, densely covering the fertile area, bearing long chains which frequently adhere more or less into masses. Conidia elliptical, green, 2.5-3 x 3.4-4.5µm, smooth and absence of Cleistothecia.

Aspergillus flavus **Johann Heinrich Friedrich Link 1809**

Colonies are olive to lime green with a cream reverse. Hyphae are septate and hyaline. Conidiophores arise separately from the substratum are coarsely roughened, uncolored, up to 400–700µm long x 5–15µm wide, vesicles globose to subglobose (20–45µm), metulae (8–10 x 5–7 µm) covering nearly the entire vesicle in biseriate species. Heads in every colony vary from small with a few chains of conidia to large columnar masses or both mixed in the same area. Small heads with small dome- like vesicles and single series of few phialides up to 10–15×3–5µ.Conidia pyriform to almost globose, colorless to yellow-green, sometimes almost smooth, usually rough, varying from 2×3, 3×4, 4×5µ, or 5×6µ in diameter or even larger.

Aspergillus fumigatus **Fresenius 1803**

Colonies are blue green to grey with a white to tan reverse. Conidiophores short, usually densely crowded, up to 300µm (occasionally 500µm), x 2-8µm in diameter, arising directly from submerged hyphae or as branches from aerial hyphae, septate or

nonseptate, gradually enlarged, upward, with apical flask-shaped vesicles up to 20-30µm in diameter, fertile usually only on the upper half, bearing phialides in one series, usually 6-8 x 2-3µm, crowded, closely packed, with axis roughly parallel to axis of the stalk. Chains of conidia form solid columns up to 400 x 50µm. Colonies of the fungus produce from conidiophores thousands of minute grey-green conidia that readily become airborne. Conidia dark green in mass, globose, 2-3.5µm, mostly 2. 5-3µm.

Aspergillus niger **van Tieghem 1867**

Colonies are initially white, quickly becoming black with conidial production. Reverse is usually without color. Hyphae are septate and hyaline. The species is biseriate. Conidiophores mostly arise from the substratum, smooth, septate or nonseptate, varying greatly in length and diameter, 200–400×7–10µ or several millimeters long and 20µ in diameter. Conidial heads fuscous, blackish-brown, purple brown, in every shade to carbonous black, varying from small, almost columnar masses of a few conidial chains to the more common globose or radiate heads up to 300,500, or 1000µlong; vesicles globose, commonly 20–50µ, up to 100µ in diameter; phialides typically in two series, thickly covering the vesicle. Conidia are brown to black, very rough, globose, and measure 4-5 µm in diameter.

Cladosporium **Link**

Colonies are rather slow growing, mostly olivaceous green to black or brown with a black reverse but also sometimes grey, buff or brown often become powdery due to the production of abundant conidia. It produces a black pigment so that when it grows on a surface it looks black. Conidia are formed in simple or branching chains. They vary greatly in size 5-40 x 3-13 µm. Hyphae creeping, septate, on the surface or in the substrate. Conidiophores almost erect, unbranched or branched only in the apical region with geniculate sympodial elongation in some species, and floccose, often forming a turf, olive-colored. Conidiophores and conidia are equally pigmented. Conidia globose and

ovate, 1-4celled, then usually with a cross-wall, usually greenish, terminal and pressed to the side.

Fusarium Link

Colonies are usually fast growing, pale or brightly colored (depending on the species) and may or may not have a cottony aerial mycelium. The color of the thallus varies from whitish to yellow, brownish, pink, reddish or lilac shades. Species of *Fusarium* typically produce both macro and microconidia from slender phialides. Macroconidia are hyaline, two- to several-celled, fusiform- to sickle-shaped, mostly with an elongated apical cell and pedicellate basal cell. Microconidia are 1- to 2-celled, hyaline, pyriform, fusiform to ovoid, straight or curved. Chlamydoconidia may be present or absent.

Penicillium Link 1809

Colonies are moderate to fast growing and texture is velvety to powdery. Color is green, blue-green, grey-green, white, yellow, or pinkish in the surface and reverse is usually pale to yellowish and sometimes red or brown. Vegetative hyphae creeping, septate, and branched. Conidiophores erect, usually unbranched, septate at the apex with a verticil of erect primary branches, each with a verticil of secondary (metulae) and sometimes tertiary branchlets or with a verticil of conidia-bearing cells (phialides) borne directly on the slightly inflated apex of the conidiophores, sometimes with secondary conidiophores borne on the apex of the main conidiophore. Conidia borne in chains which typically form a brush-like head, not enclosed in slime; well-differentiated foot cells not present. Conidia globose, ovate, or elliptical, smooth or rough.

Rhizopus ehrenberg ex Corda in 1838

The genus *Rhizopus* is characterized by the presence of stolons and pigmented rhizoids, the formation of sporangiophores singly or in groups from nodes directly above the rhizoids, and apophysate, columellate, multi-spored, generally globose sporangia.

Myceliums are of two kinds, one submerged in the substratum and the other aerial, constituting the arching filaments or stolons. These stolons present from place to place the nodes on which occur the rhizoids, which are implanted in the substratum. At these points the sporangiophores arise. Colonies are fast growing and cover an agar surface with a dense cottony growth that is at first white becoming grey or yellowish brown with sporulation. After spore release the apophyses and columella often collapse to form an umbrella-like structure. Spores round or oval, angular, colorless, or colored bluish or brown, with a cuticularized wall, smooth or striate, rarely spinulose. Zygospores naked, formed in the substratum and on the stolons. Suspensors straight, very large and swollen, without appendages. Columellae broadly subjacent, hemispherical, forming after a dehiscence, by collapse, an organ of the shape of the pileus of a mushroom.

5. DISCUSSION

The present investigation deals with the study of seed-borne mycoflora and control of *Pisum sativum* L. (garden pea) of Kathmandu valley. It includes the control of mycoflora *Aspergillus niger* and *Aspergillus flavus* by fungicides Bavistin, Derosal and Dithane M-45 were selected on the basis of their dominance.

The seed mycoflora were isolated by three methods like Standard Blotter Technique, Agar Plate Method and Moist Sand Method. A large number of mycoflora was isolated from all the three methods. They were *Aspergillus niger, Aspergillus flavus, Aspergillus clavatus, Aspergillus fumigatus, Penicillium sp., Rhizopus sp., Cladosporium* and *Fusarium sp.*etc.

In the present investigation, Agar Plate Method was found to be the most suitable because a large number of mycoflora are isolated from this method. The Agar Plate Method was found to be suitable for the saprophytic fungi like *Rhizopus*, *Penicillium* that grow more rapidly than other fungi. The emergence of pathogenic fungi was rather slow.

Kassim *et*. *al*. (1987) found that Agar Plate Method was more suitable for the isolation of mycoflora of barley than Blotter Method in Saudi Arabia.

According to Agarwal, Mathur and Neergaard, 1971, Standard Blotter Technique was found to be the suitable method for the isolation of mycoflora. It was also proved to be the most suitable method for the isolation of seed-borne fungi in rice, wheat, black gram, green gram and soybean and in gram (Suhag, 1973).

All species except, *Aspergillus niger, Aspergillus flavus* were isolated from sterilized as well as unsterilized seeds indicating that they were internally seed-borne as well as externally seed-borne.

Aspergillus niger, Aspergillus flavus, *Penicillium sp.* were the most usual pathogen associated with the leguminous seeds as well as many others (Bal, J.S. and Chohan, 1973).

Fusarium sp. was listed as seed-borne pathogens of broad bean in the list of seed- borne diseases by Mary Noble (1979).

Bilgrami *et.al*. (1976) reported the highest frequency of *Aspergillus flavus* in pulse seeds.

Jain *et.al*. (1982) also reported the presence of *Aspergillus flavus* in moth bean. Similarly, *Aspergillus flavus* was found to be the most dominated species in maize, wheat and beans (Dimitrov *et.al* , 1987).

Fusarium spp. is the most common root rot pathogens isolated from peas in Alberta (Sumar & Howard 1979; Hwang & Chang 1989)**.**

Nair, Neeta and Arora in 1994 isolated nine seed borne fungal pathogens from legume crops, *Alternaria alternata, Verticillium sp. Aspergillus niger, A.flavus, Phoma sp., Rhizoctonia bataticola, Gliocladium viriens*, *Botrydiplodia theobromea* were obtained.

Kaul in 1973 isolated *Colletotrichum lindemuthianum*, *Rhizoctonia solani*, *Rhizopus sp*, *Aspergillus sp, Alternaria sp* from bean (*Phaseolus vulgaris*) seeds.

Trichoderma sp. was also reported as soil fungi such as *Alternaria*, *Fusarium, Verticillium* sp. etc. and itself as a soil inhabitant (Tarr, 1972).

Doubtlessly other spp. Like *Fusarium monoliforme, Aspergillus flavus, Botrytis cinera* are internally seed-borne (Zare *et.al*. 19997, Scrobarova *et.al*. 19997, MacDonald *et.al*. 1996, Burgess *et.al*.1996)

Control:

Fungi are among the chief causes of diseases in plants. The seed is the carrier of some important diseases inciting micro-organisms which causes considerable losses in the yield by producing diseases. Basic and applied research in plant pathology has made a start in the control of some of the most destructive plant diseases.

To control the seed- borne diseases, seed was treated by fungicides before sowing. Seed treatment has been considered as the cheapest and the best method for the direct control of plant disease (Neergaard 1977). Bavistin, Derosal and Dithane M-45 were used for the control of seed mycoflora of pea. For this, 2 methods were as below:

1. Sand method
2. Thread method

1. Sand method:

In this method, well soaked and autoclaved sand was taken in sterilized earthen pot. Surface sterilized seed were rolled on the 7 days old culture of active test pathogens for 2 hours. Then inoculated seeds were treated with 2gm/kg of fungicides separately for 5 minutes (Neergaard, 1977) and sown in sterilized earthen pot filled with sand and 5 seeds on each pot asceptically at equal distance.

In the present observation, Derosal was effective in controlling *Aspergillus niger* of 94.28% similarly Bavistin was effective in controlling *Aspergillus flavus* of 88.57%.

Aspergillus flavus, Alternaria sp., *Chaetomium sp*., *Fusarium sp*., infecting the seeds of sunflower was best controlled by Benlate and Bavistin with 89-93% seedling survival (Chohan and Kaur, 1975).

2. Thread method:

In this method sterilized cotton threads were inoculated with test pathogens *Aspergillus niger* and *Aspergillus flavus* cultured in PDA slants. Then threads were treated with fungicides and incubated on the nutrient slants.

The most effective fungicides are Bavistin and Derosal to prevent the growth of *Aspergillus niger* and *Aspergillus flavus* while Dithane M-45 was not effective.

6. SUMMARY

Pea seeds were collected from the local markets of Kathmandu valley for the purpose of present observation. All the seed samples collected from different places, mixed together to make composite samples from which "working sample" for the actual test was prepared. During the investigation period, three different standard rules as recommended by the International Seed Testing Association, 1950 were performed.

1. Standard Blotter Technique

2. Agar Plate Method

3. Moist Sand Method

Altogether 11 fungal pathogens were isolated by the three methods. Altogether 10 species were isolated by Agar Plate Method so it was the suitable method for the isolation purpose. *Aspergillus niger*, *Aspergillus flavus, Aspergillus fumigatus, Fusarium sp.* were proved as truly seed-borne species. *Aspergillus clavatus, Penicillium sp., Rhizopus sp., Cladosporium sp.* etc. were isolated only in unsterilized seed so they are known as surface contaminant.

Two seed mycoflora *Aspergillus niger* and *Aspergillus flavus* were most dominant and pathogenic in nature.

Three fungicides Bavistin, Derosal and Dithane M-45 were selected as per the availability in the market for the control of two pathogens, *Aspergillus niger* and *Aspergillus flavus*,. These fungicides were used to study the efficiency of fungicides against the test pathogens. Sand method and Thread method were applied for this purpose. The most effective fungicides are Bavistin and Derosal while Dithane M-45 was least effective.

7. REFERENCES

Agarwal, V.K.; S.B.Mathur and P. Neergaard (1971), Some aspects of seed health testing with respect to seed- borne fungi of rice, wheat, black gram, green gram and soybean grown in India, Indian phytopathology, 25;91-100.

Agarwal, V.K. and J.B. Sinclair (1997), Principles of Seed Pathology, 2nd ed. CRC Lewis Publishers, New York. 539 p.

Al-Abdalall, A. H. A, 2010,Pathogenicity of fungi associated with leguminous seeds in the Eastern kingdom of Saudi Arabia. African Journal of Agricultural Research Vol. 5(10), pp. 1117-1126.

Anonymous 1998a. Crop protection with chemicals 1998. Alberta Agriculture, Food and Rural Development. Agdex 606-1.414 pp.

APP (1995), Agricultural Perspective Plan. Ministry of Agriculture and Co-operative, Nepal.

Barnett, H.L and B.B.Hunter (1972), Illustrated genera of Imperfect Fungi, Burgess Publishing Company.

Baron ,G. L (1977), The Genera of Hyphomycetes, Rober K. Krieger Publishing Co.

Bennett, M. A.; V. A. Fritz and N. W. Callan. 1992, Impact of seed treatments on crop stand establishment, HortTechnology, 2 (3): 345-349.

Bhattacharya, A. K. and R. N. Basu. 1990, Retention of vigor and viability of stored pea seed, Indian Agriculturist, 34: 187-193.

Bilgrami, K.S. *et. al*. (1976), Studies on Deterioration of some pulses by fungi, Indian Phytopathology, 29:374-377.

Christensan,C.M and H.M.Kaufman (1965), Deterioration of stored grain by fungi, Annual Review of Phytopathology, (3):69-81.

Crocker, W and Lela V. Barton, (1957), Physiology of seed, Cronica Botanica Co.

Eisa, N.A. and M.A. Barkat (1978), Relative efficiency of fungicides in control of damping off and Stemphyluim leaf spot of broad bean (*Vicia faba*), Plant Dis. Rep., 62(2):114-118.

Ellis, M.A. and E.H. Paschal. 1979. Effect of fungicide seed treatment on internally seedborne fungi, germination and field emergence of pigeon pea (*Cajanus cajan*), Seed Sci. & Technol. 7: 75 – 81.

FAO(1954), Food Composition Tables, Minerals and Vitamins, Rome Italy.

Flentje, N. T. 1964, Pre-emergence rotting of peas in South Australia. I. Factors associated with the seed, Aust. J. Biol. Sci., 17: 643-650.

Forsenberg, J.L. (1949), New method of evaluating fungicides, Phytopathology, 44:133-115.

Halligan, E. A. 1986, The effect of elevated temperatures and their duration on the incidence of hallow heart in pea seeds, Annals of Applied Biology, 109 (3): 619-625.

Haware, M. P. 1971, Fungal mycoflora of seeds of *Pisum sativum* L. and its control, Mycopathol. 43: 343-345.

Hyun, I.H., Heo, N.Y. and Lee, Y. H. 2004, National Plant Quarantine Service, Anyang Korea. 178pp, Illustrated Manual on Identification of Seed-borne Fungi.

Ingold, C.T. (1959), Vastas in Botany, Pergamin Press 345-386.

Jain, S.C. et. al. (1982), Seed mycoflora of moth bean (Vigna acontifolia) its pathogenecity and control, Ind. Jour. Myco. Pl. Patho, 2(12):137-141.

Kassim, M.Y. (1987), A study on seed-borne fungi of barley in Saudi Arabia, Phytopathology. 47(1/2):115-120.

Kaul, J.R., (1973), Comparative effect of long storage after various treatments on the viability and mycoflora of been seeds Indian Journal, Mycology, Plant Pathology 3(1), 17-20.

Kerr, A. 1964, The influence of soil moisture on infection of peas by *Pythium ultimum*, Aust. J. Biol. Sci. 17: 676-685.

Laflamme, P. PAg. (1986), Diseases of field pea *(Pisum sativum* L.) in the Peace River region of Alberta, B.Sc. Agriculture - University of Alberta.

Leviatov, S.; O. Shseyov and S. Wolf. 1993, Roles of different seed components in controlling tomato seed germination at low temperature, Scientia Horticulturae, 56: (3): 197-206.

MacDonald, M.V and R. Chajman (1996), The incidence of *Fusarium monoliforme* on maize during 1992-1995, Plant Pathology (Oxford) 46 (1):112-125.

Mary Noble (1979), Outline of the history of seed pathology, Seed pathology problem and progress, Pg. 3-17.

Mayer, A.M. and A. Poljakoff–Mayber (1982), The Germination of Seeds, Third Edition. P10-p20.

Moussart, A.; B. Tivoli; E. Lemarchand; F. Deneufbourg; S. Roi and G. Sicard. 1998, Role of seed infection by the Ascochyta blight pathogen of dried pea (Mycosphaerella pinodes) in seedling emergence, early disease development and transmission of disease to aerial plant parts, European Journal of Plant Pathology, 104 (1): 93-102

Nair, Neeta and Arora, 1994, Isolation of seed borne fungal pathogens from legume crops, Dep. ot.Gov. Modell Coll. Science Rajpur, Jodhpur 21(12): 105-108 (Bio Abstract No. 52543, Vol. 98 (4) Aug 30, 1994)

Nasim, B; K. Z. Alvi; M. I. Hague, M. U. Raja and S. Chohan (2004), Evaluation of Mycoflora Associated with Pea Seeds and Some Control Measures, Plant Pathology Journal 3 (1): 48-51.

Nayak, B.K and N.Behera (1995), Seed borne fungi and their effect in germination and seedling mortality in black gram, Acta Botanica Indica 23 (1):93-98.

Neergaard, Paul (1977), Seed Pathology, volume-1 Mac Millan Press Ltd.

Neergard, Paul (1975), Some silent points in cooperation within seed pathology, Advance of mycology and plant pathology.

Nehal S. E, and S. A. Rokayah (2009), Inhibitory effects of powdered caraway and peppermint extracts on pea root rot under greenhouse conditions, journal of plant protection research Vol. 49, No. 1.

Nienhuis, J.; R. L. Lower and J. E. Staub. 1983, Selection for improved low temperature germination in cucumber, J. Amer. Soc. Hort. Sci. 108 (6): 1040-1043.

Pereyra GML, Pereyra CM, Ramírez ML, Rosa CAR, Dalcero AM,Cavaglieri LR (2008). Determination of mycobiota and mycotoxins inpig feed in central Argentina. Letters in Appl. Microbiol., 46: 555-561.

Rana Bhat, B. & Ghimire, R (2008), Promotion of Organic Vegetable Production through Farmers' Field School in Chitwan, Nepal.

Rangaswami,G and A. Mahadevan (1999), Disease of crop plants in India fourth edition, Prentice- Hall of India Pvt. Ltd.

Savino, G.; P. Deleo and P. M. High. 1979, Effects of pre-soaking upon seed vigor and viability during storage, Seed Science and Technology, 7: 57-65.

Shah, R. and Jain, J. P. 1993, Seed mycoflora of mustard and its control, Indian J. Mycol. 23: 291-295.

Sharma, P and H.N. Gour, Location of seed-borne mycoflora in pea (*Pisum sativum* L) and efficacy of fungicides on germination and seedling vigour.

Sharma P and Gour HN, Effect of seed dressing fungicides on microflora incidence during storage of pea (*Pisum sativum*).

Shrestha, K. (1986), List of Seed Borne Organism in Nepal, Nep, J. of Agric, Vol 15, Supplement, 167-171.

Singh, K, J. C. Frisrad, U. Thrane and S.B.Mathur, An Illustrated Manual on Identification of Some Seed- borne Apergilli, Fusaria, Penicillia and their Mycotoxins.

Skoric, V, 1927, Bacterial blight of pea: overwintering, dissemination and pathological histology, *Phytopathology* 17:611-628.

Suhag, L.S. (1973), Mycoflora of Gram seeds, Pathology and Control, Indian Journal of Mycology and Plant Pathology 3(1):40-43.

Suhag, L.S. (1975) Fungalflora of Moong (*Phaseolus aureus*) seeds, Pathology and Control, Indian Journal of Mycology and Plant Pathology 5(2):165-168.

Suryanarayana, D., Seed Pathology (1978), Seed- borne diseases of some important crop plants, their identification and control, Vikas Publishing House Pvt. Ltd. Pp.2, 3, 63:66.

Suryanarayanan, T.S. and C.S. Suryanarayanan (1990), Dept. of Botany, Ramakrishna Mission Vivekananda College- Fungi associated with stored sunflower seeds (Bio. Abstract No. 80578, Vol. 92 (7) Oct. 1991).

Susuri, L.; D.J. Hagedorn & R.E. Rand, 1982. Alternaria blight of pea. Plant Dis., 66:328-330.

Tarr, S.A.J., (1972), Principles of Plant Pathology, Mc. Millan.

Tu J.C. 1987a. Integrated control of pea root rot disease complex in Ontario. Plant Disease. 7 l:9-13.

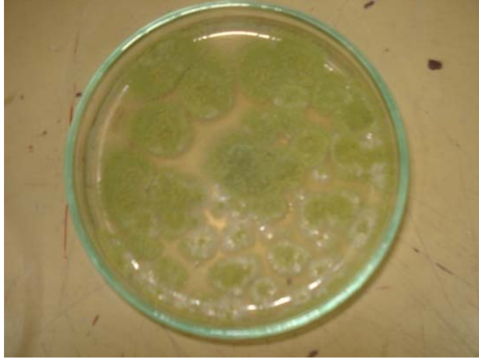
Verma, S (2004), Seed mycoflora of *Pisum sativum* in Himachal Pradesh, Plant Disease Research (Ludhiana), 2004 Vol. 19 No. 2 pp. 189.

Zaghdani, A.S. (2002), Effect of pre-sowing seed treatments for quality of cucumber, pepper, tomato and pea seed, PhD Dissertation, Budapest, Hungary.

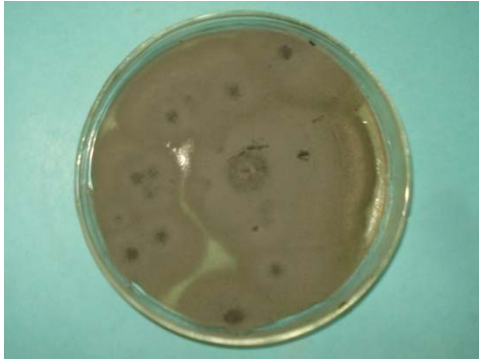
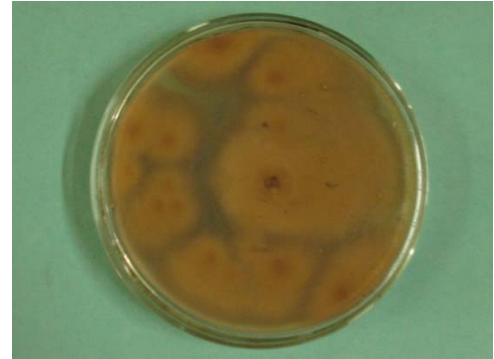
8. PHOTOGRAPHS

Blotter method of *Pisum sativum* L. (USS)

Blotter method of *Pisum sativum* L. (SS)

Pure culture surface view	Pure culture reverse view
	

Aspergillus flavus

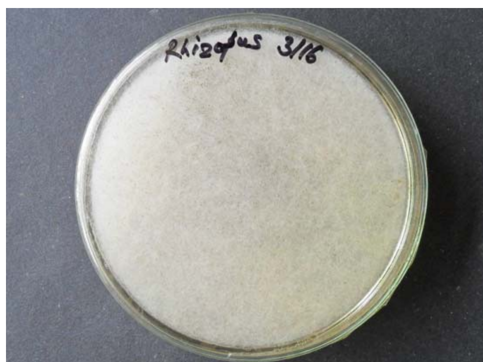
Aspergillus fumigatus

Aspergillus niger

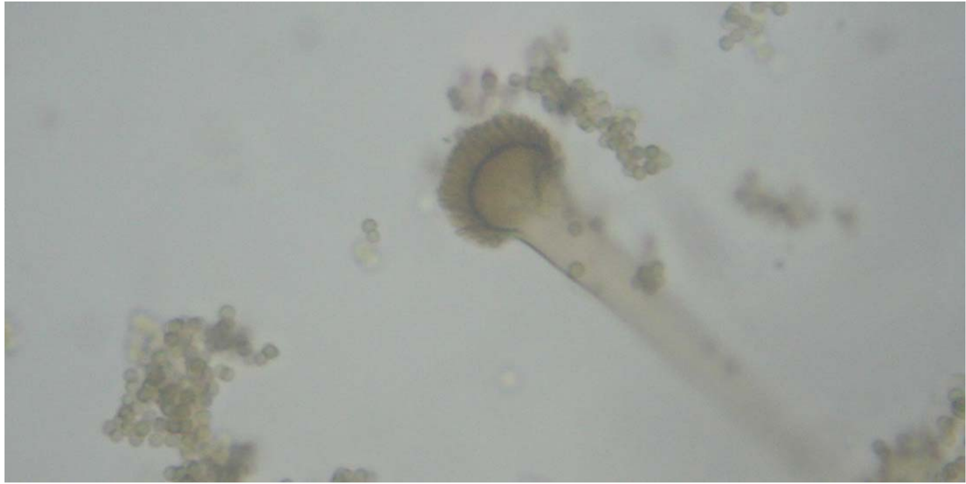
Pure culture surface view

Pure culture reverse view

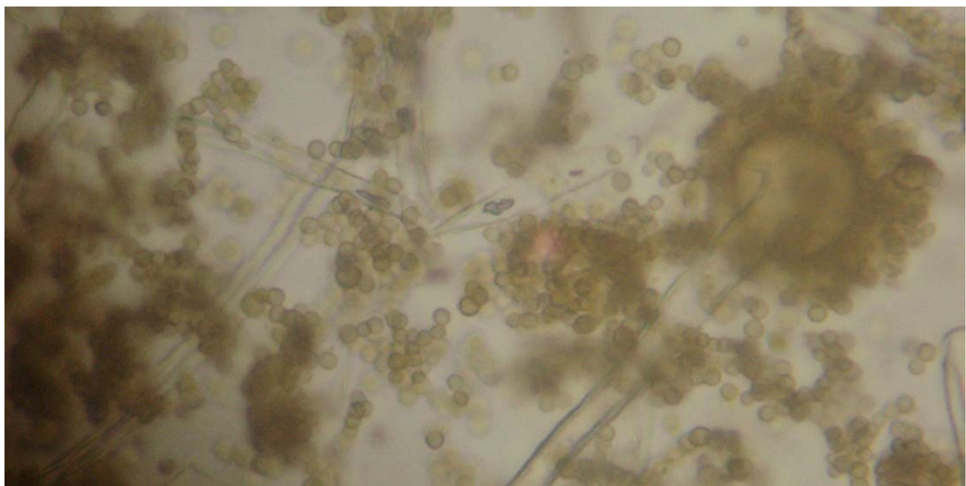
Fusarium sp.



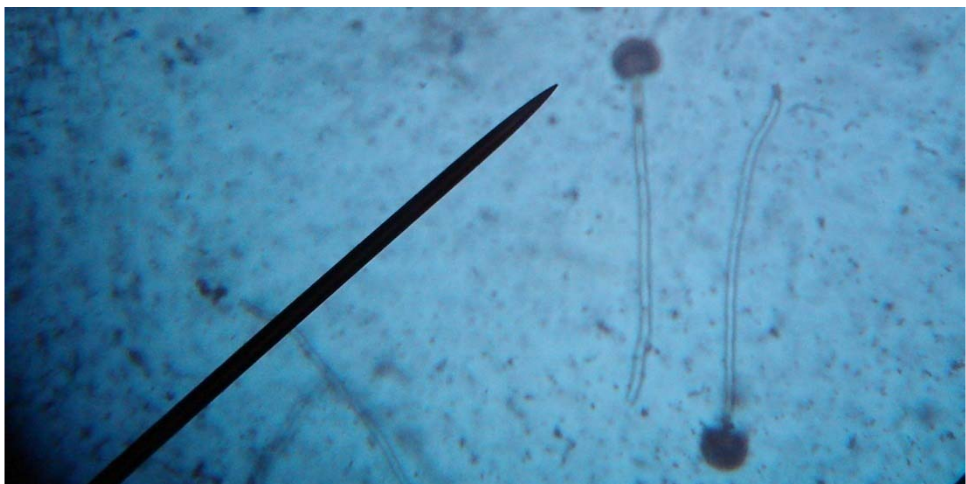
Rhizopus sp.



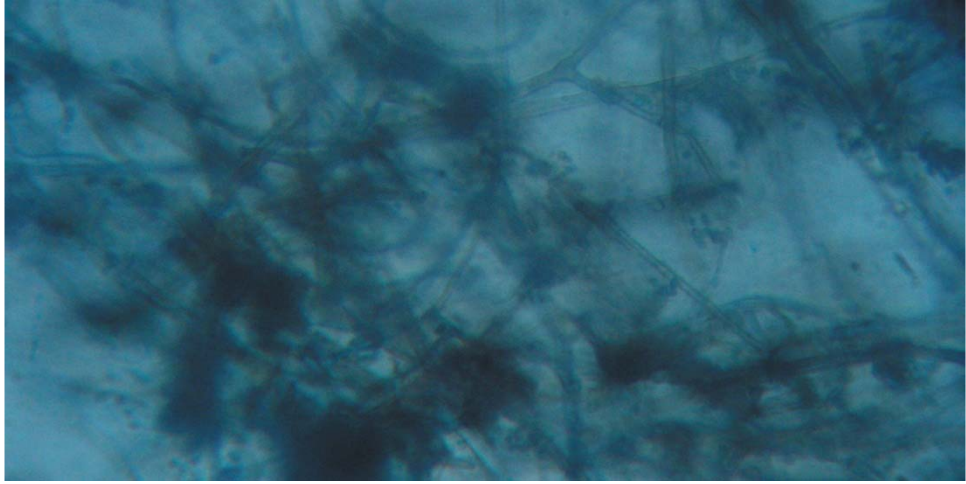
Aspergillus fumigatus



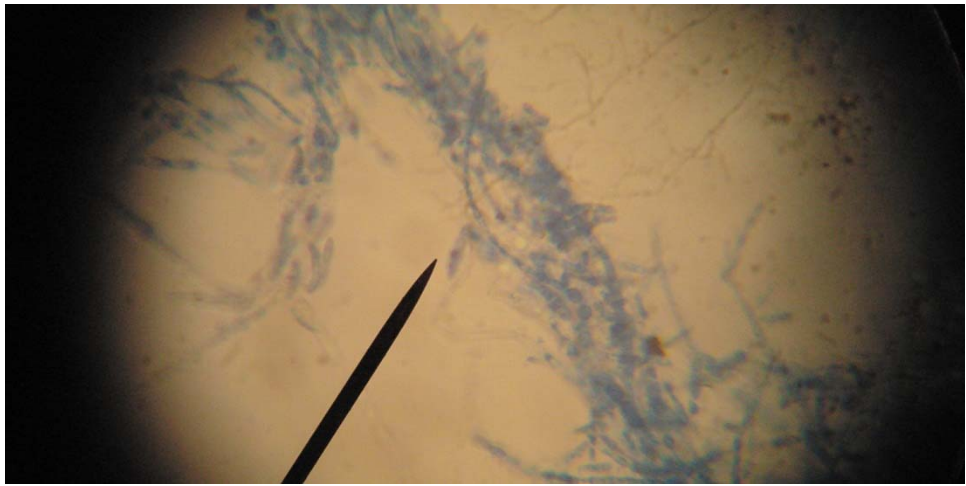
Aspergillus flavus



Aspergillus niger



Cladosporium sp.



Fusarium sp.

Rhizopus sp.

<u>Pathogenecity test of *Aspergillus niger*</u>